

Supplementary Information for

New globally distributed bacterial phyla within the FCB superphylum

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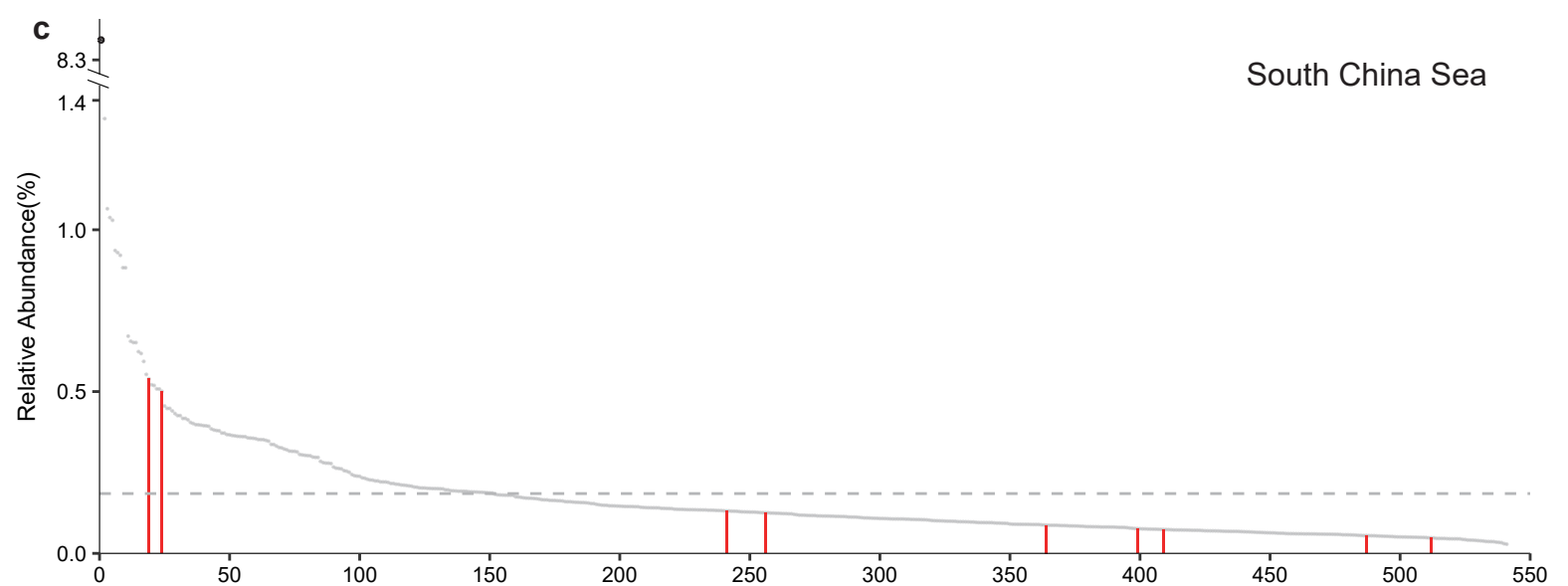
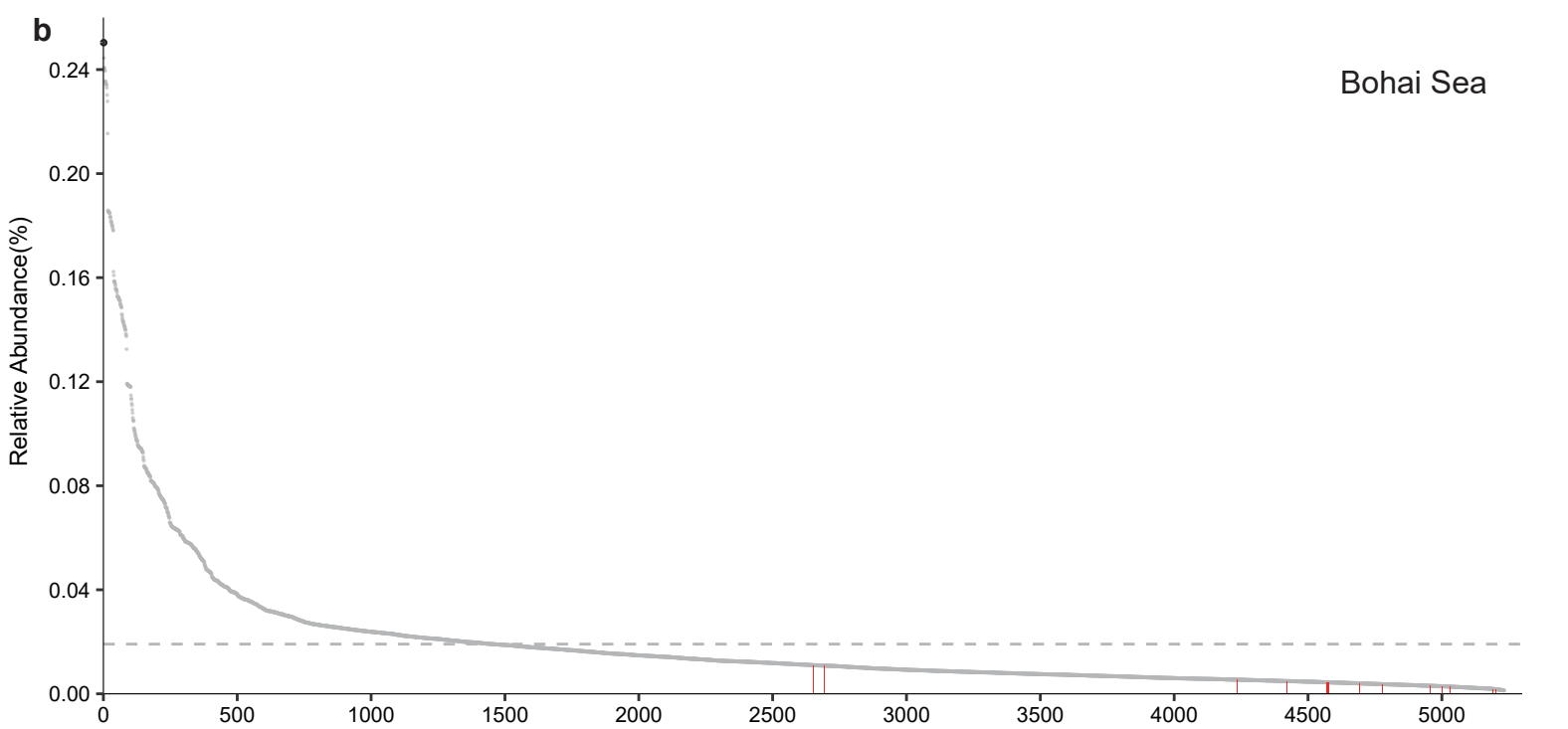
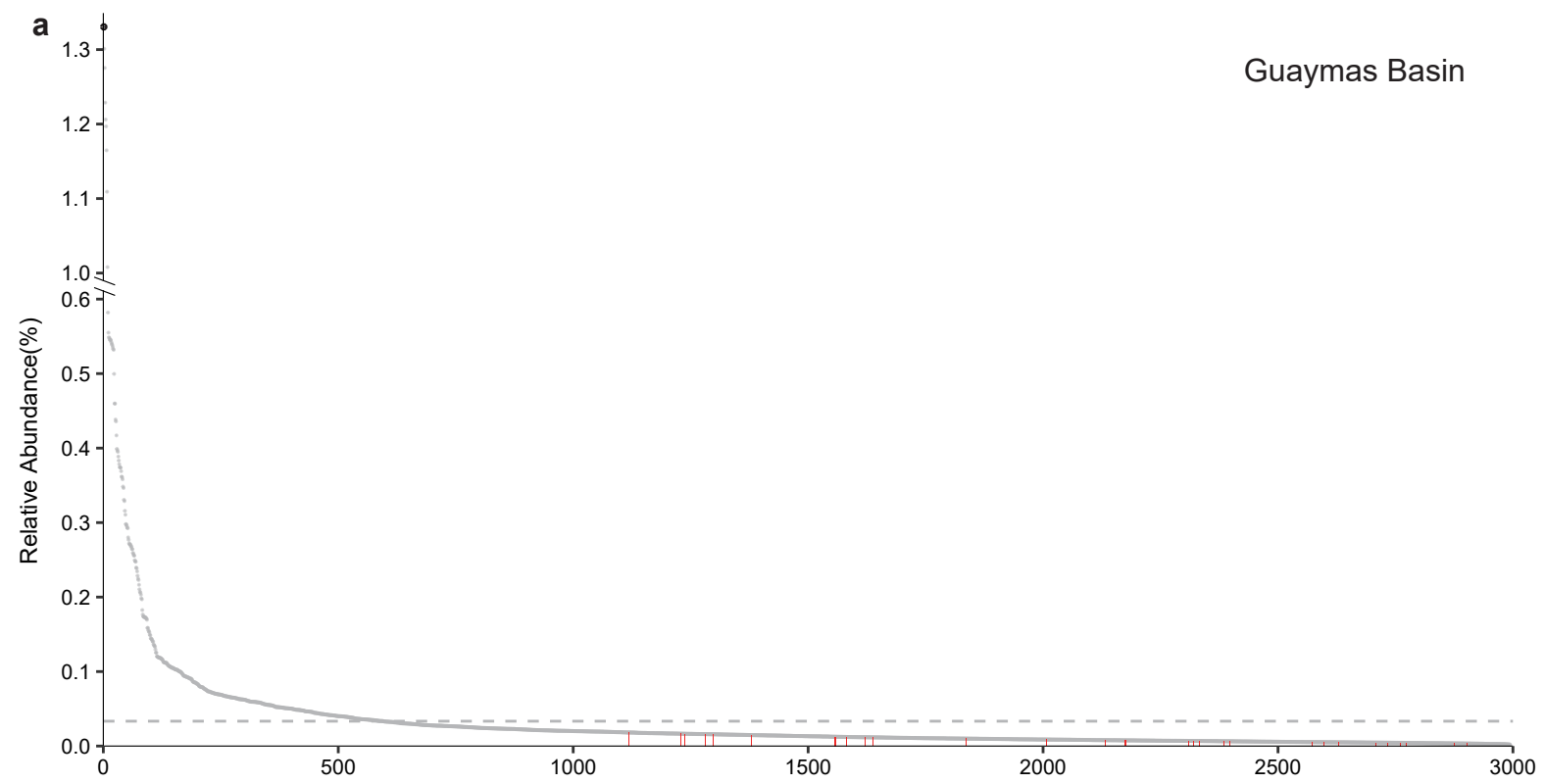
Supplementary Figs. 1-9

Supplementary Notes

Other Supplementary Information for this manuscript includes the following:

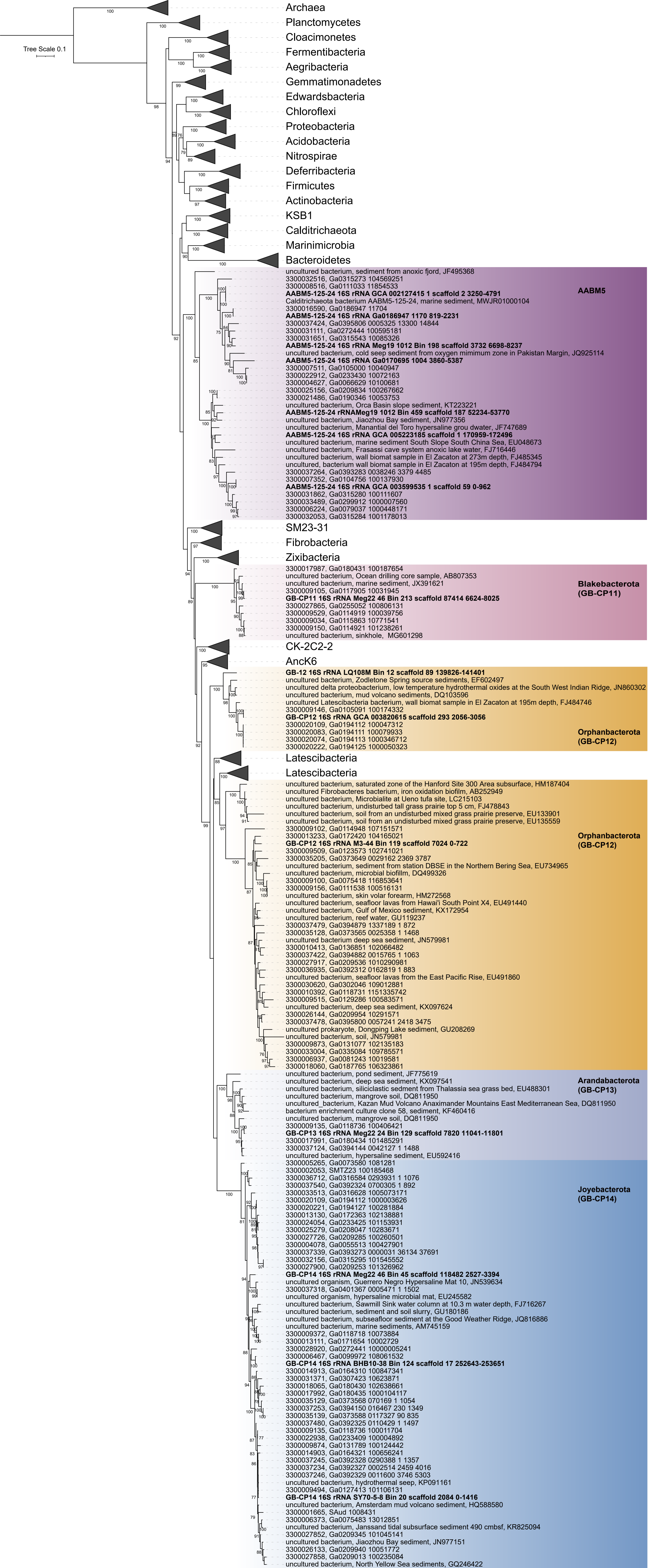
Description of Supplementary Data 1-15

Supplementary References



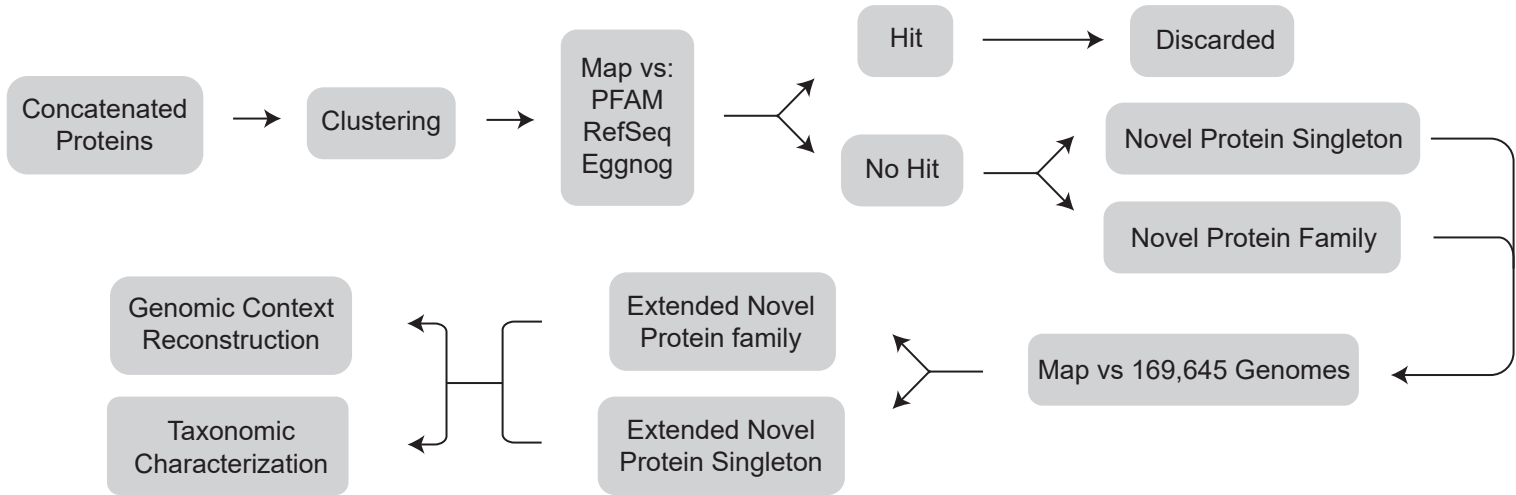
Supplementary Fig. 1 Normalized relative abundance of each metagenome assembled genome (MAG) recovered from three different sites. (a) Normalized relative abundance of 2,994 MAGs recovered from Guaymas Basin samples. (b) Normalized relative abundance of 5,233 MAGs recovered from Bohai Sea samples. (c) Normalized relative abundance of 541 MAGs recovered from cold seep samples in South China Sea. Red lines are the normalized relative abundance of 30, 14, and 9 MAGs in this study. Dash line is the average relative abundance of all MAGs. Black dot represents the highest MAG abundance in the sampling site. Source data are provided as a Source Data file.

Supplementary Fig. 2 Average amino acid identity (AAI) of MAGs including these novel bacteria. Heatmap using pheatmap package in R based on AAI for each MAG pair. The neighboring phyla in the phylogenetic tree (as shown in Fig. 1) based on ribosomal protein marker genes were used as reference genomes in the heatmap to show the distinct AAI of five phyla compared to other genomes. Genome self-comparisons are presented in blue. Source data are provided as a Source Data file.

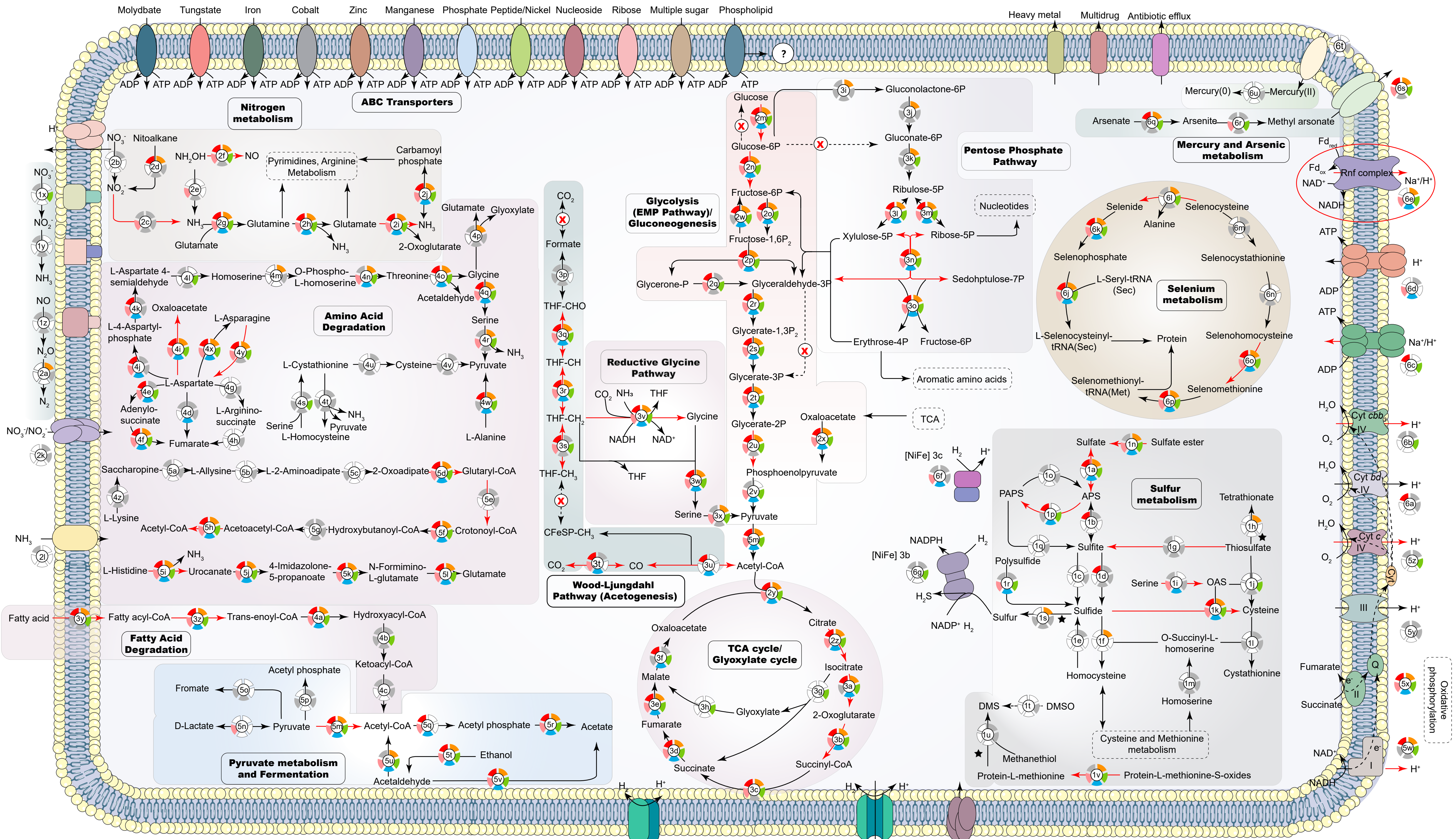


Supplementary Fig. 3 Maximum likelihood phylogenetic tree of 16S rRNA genes in these novel bacteria.

Sequences recovered from the MAGs in this study are shown in bold. The tree was generated using IQ_TREE v1.6.12. Source data are provided as a Source Data file.



Supplementary Fig. 4 Outline of in-house pipeline used to characterize novel protein families. The protein contents of 55 novel MAGs in this study were clustered based on similarity (30% as threshold). These proteins were then mapped against public databases to identify novel protein singletons/families. The identified novel protein singletons/families were mapped to the curated prokaryotic genomes to reconstruct genomic context and characterize the taxonomy.

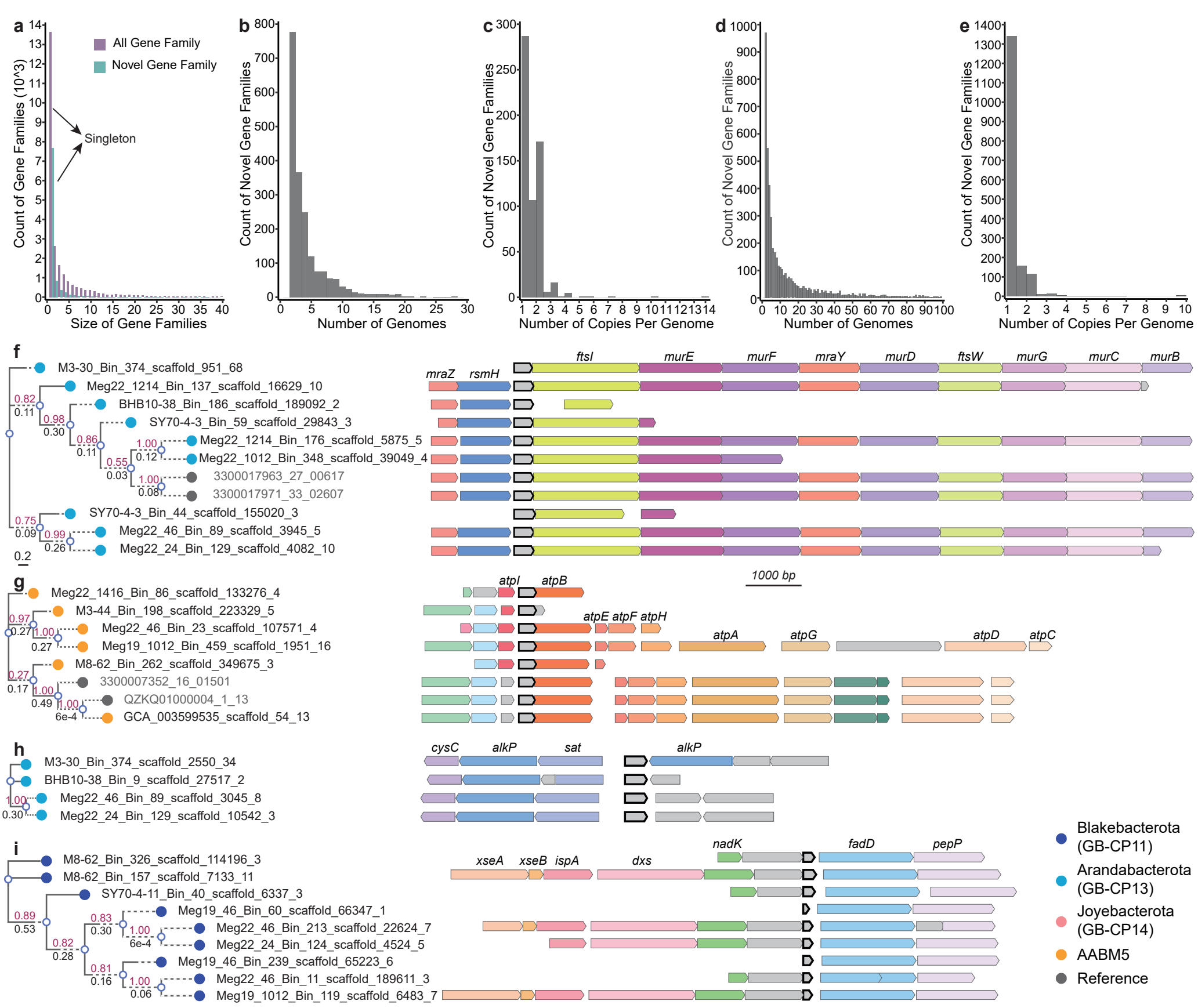


■ AABM5-125-24
 ■ Blakebacterota (GB-CP11)
 ■ Orphanbacterota (GB-CP12)
 ■ Arandabacterota (GB-CP13)
 ■ Joyebacterota (GB-CP14)

[FeFe] A3 (6h) [NiFe] 4g (6i) Sulfate (1w)
 [NiFe] 3c (6f) [NiFe] 3b (6g)
 [FeFe] A3 (6h) [NiFe] 4g (6i) Sulfate (1w)

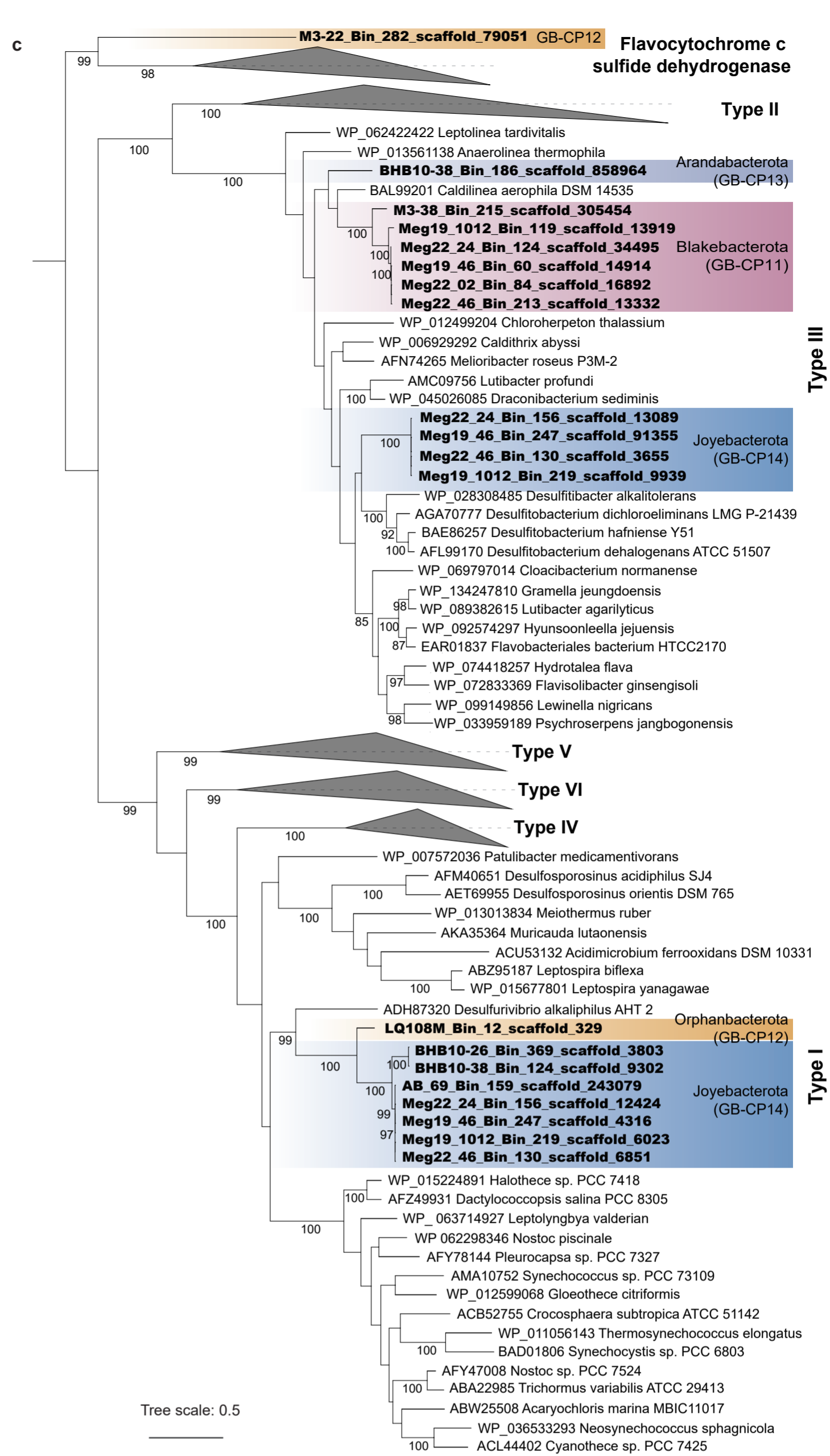
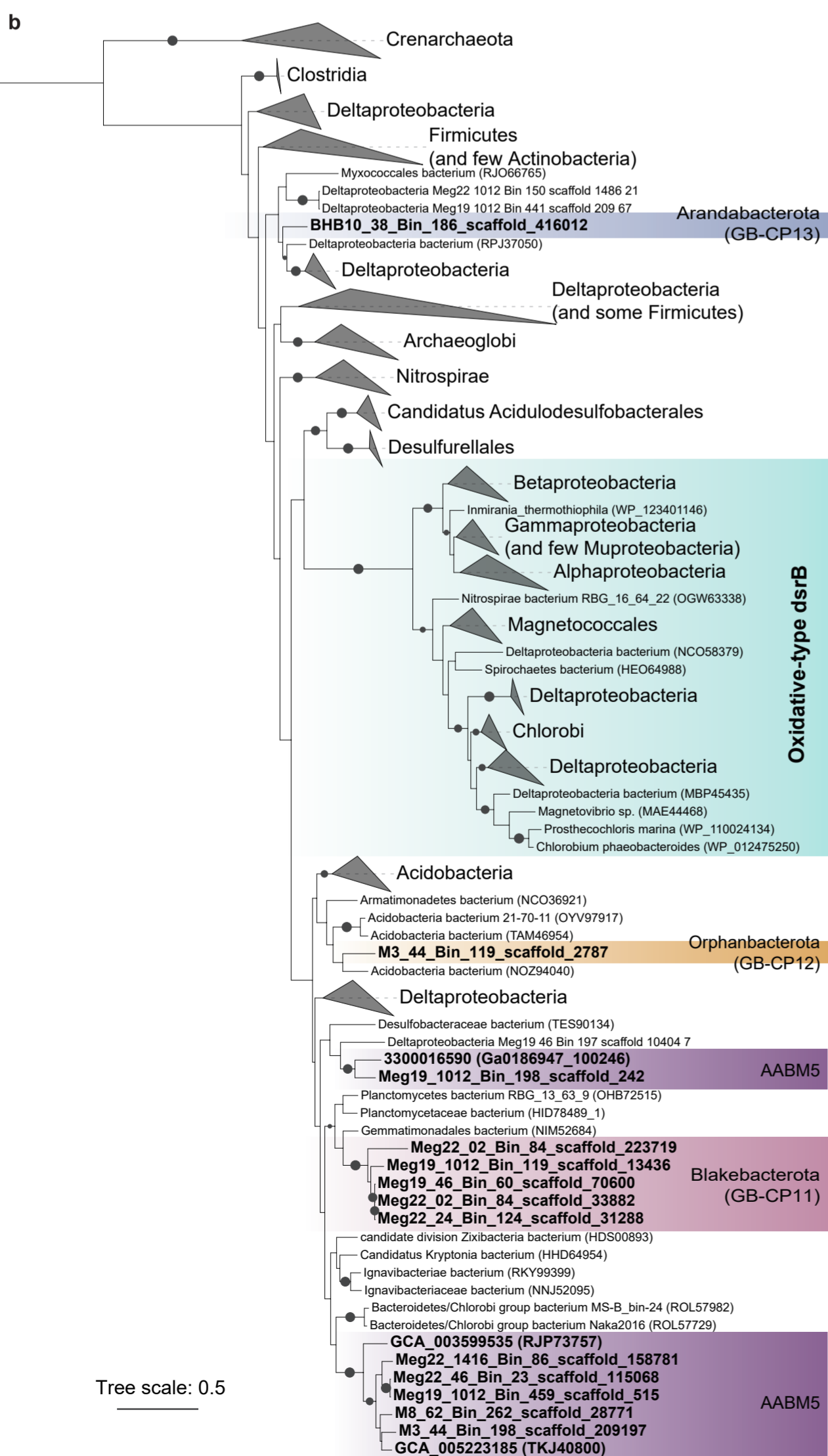
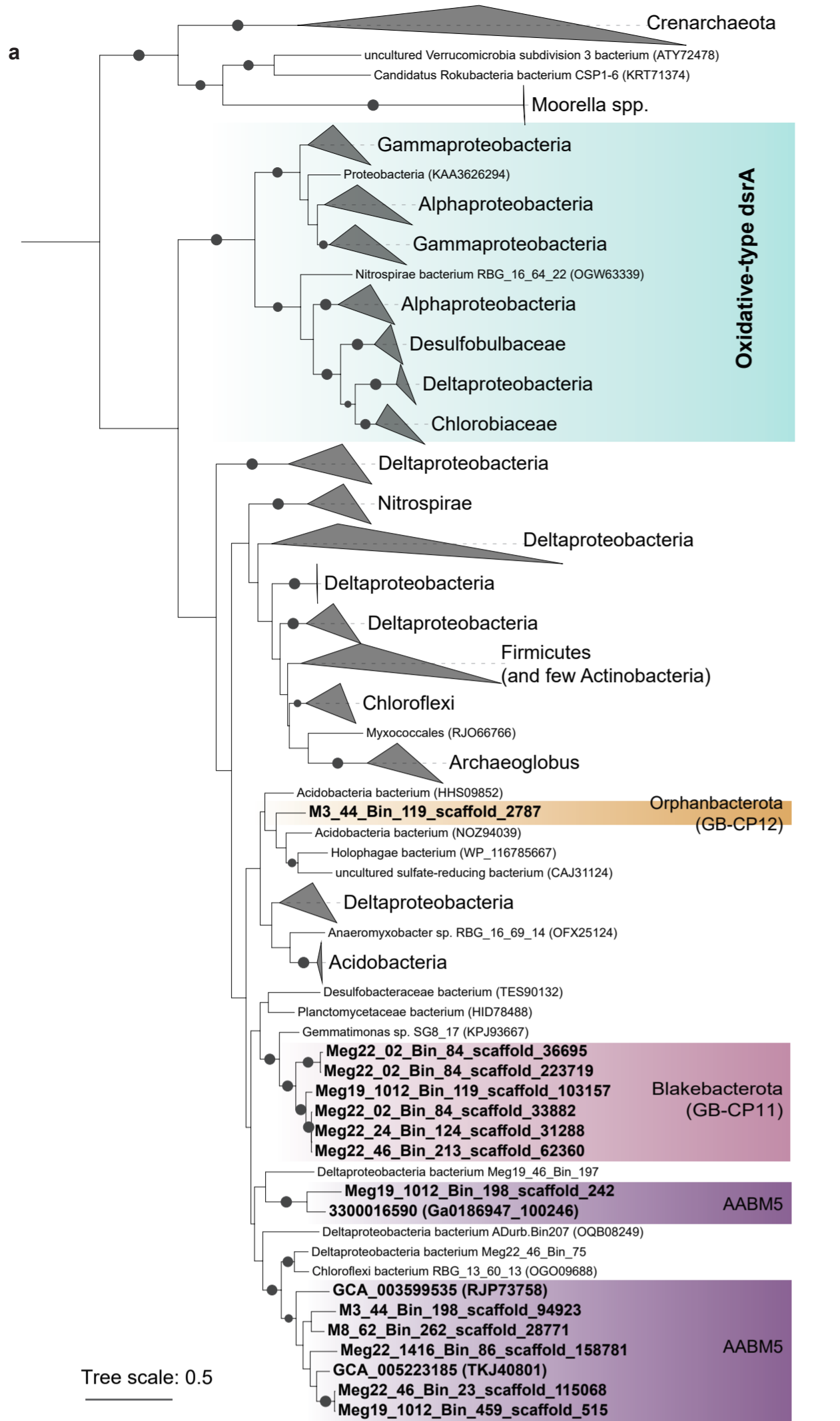
Supplementary Fig. 5 Overview of the metabolic potential of the five newly described bacterial phyla.

Within each color wheel, colored segments shown in grey and blank represent gene presence in over 50%, less than 50%, and gene absence, respectively, within a phylum. Red arrows indicate the enzymes/subunits that neighbor novel proteins (proteins without any homologues in current databases). The Rnf complex is highlighted in the red circle on the right side of the diagram to underscore the genomic co-localization of this complex with novel protein families. TCA: tricarboxylic acid. DMS: dimethyl sulfide. DMSO: dimethyl sulfoxide. OAS: O-acetyl-L-serine.



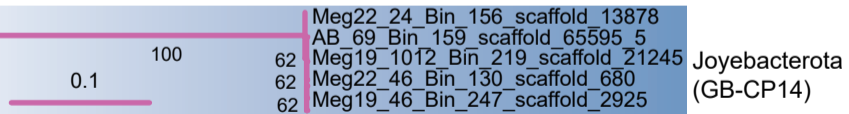
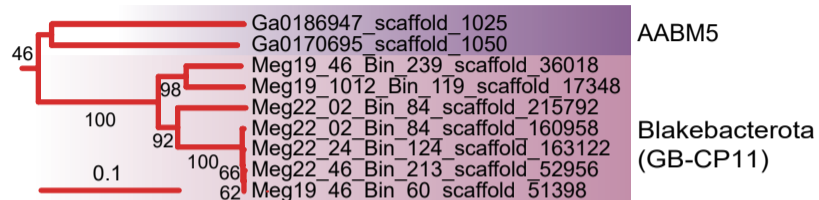
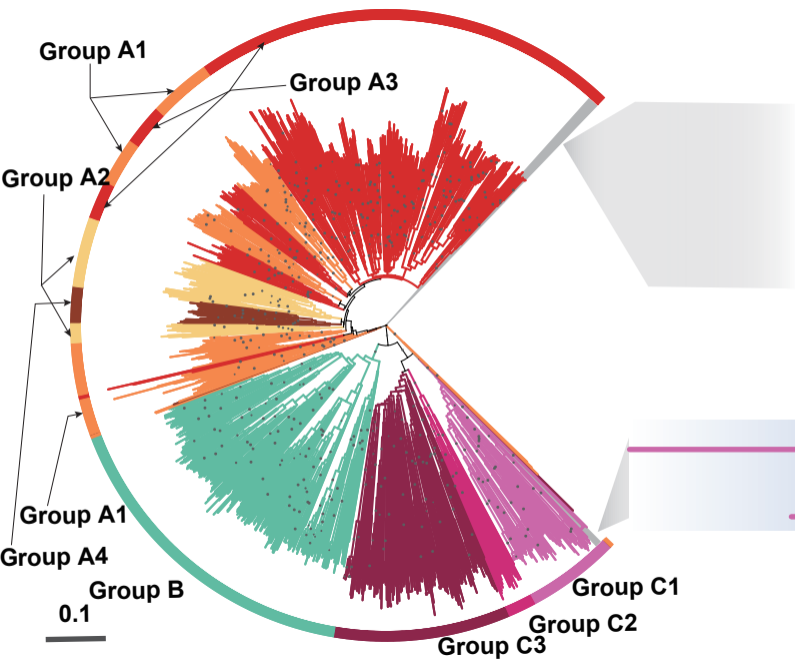
Supplementary Fig. 6 Characteristics of gene families and novel gene family clusters (marked in grey with a black outline) neighboring conserved known proteins. (a) Most of the clustered gene families are singleton in these five phyla. Novel families represent an important fraction of the gene clusters but show a limited number of genes. (b) Distribution of the number of genomes in which the novel gene families are detected shows most of the gene families are shared by less than 10 genomes, but could be up to 33 genomes. Singletons are not shown for data visualization. (c) Average number of copies of the novel gene families in the 61 bacterial genomes shows most of the gene families had less than three copies per genome, and the singletons are excluded for data visualization. (d) Among the collection of 169,642 genomes, most of the novel families are shared in less than 50 genomes, but could be over 4,000 genomes. Singletons are not shown for data visualization. (e) Average number of copies of the novel gene families in the 169,642 bacterial genomes shows most of the gene families had less than three copies per genome, and the singletons are excluded for data visualization. (f) A novel gene family mainly detected in Arandabacterota and with conserved 16S rRNA methyltransferase (*rsmH*; K03438) and transcriptional regulator (*mraZ*; K03925) downstream, and a gene cluster related to peptidoglycan biosynthesis (*ftsI*; K03587, *murE*; K01928, *murF*; K01929, *mraY*; K01000, *murD*; K01925, *ftsW*; K03588, *murG*; K02563, *murC*; K01924, and *murB*; K00075) upstream. (g) A novel gene family mainly detected in AABM5 associated with F-type ATPase (*atpI*; K02116, *atpB*; K02108, *atpE*; K02110, *atpF*; K02109, *atpH*; K02113, *atpA*; K02111, *atpG*; K02115, *atpD*; K02112, and *atpC*; K02114). (h) A novel gene family only detected in Arandabacterota and associated with proteins related to sulfur assimilation (*sat*; K00958, *alkP*; COG3379, and *cysC*; K00860). (i) A novel gene cluster only detected in Blakebacterota, neighboring another novel gene, a NAD⁺ kinase (*nadK*; K00858), *dxs* (K01662), *ispA* (K13789), *xseB* (K03602), and *xseA* (K03601) downstream, and genes for fatty acid degradation (*fadD*; K01897) and *pepP* (K01262) upstream. Grey color denotes the protein without homologues in the current database. The phylogeny was constructed using FastTree2 and numbers on the top and bottom of the branch represent the bootstrap and branch length, respectively. Source data are provided as a Source Data file.

Supplementary Fig. 7 Carbohydrate-active enzymes (CAZyme) and peptidase encoded by MAGs in this study. (a) CAZymes, including carbohydrate esterase (CE), glycoside hydrolase (GH), and polysaccharide lyase (PL), identified in the five phyla. Substrates for each family are stacked on top. (b) Peptidases, classified as family aspartic (A), cysteine (C), unassigned inhibitors (I), metallo (M), asparagine (N), mixed (P), serine (S), threonine (T), and unknown (U) by the MEROPS database identified in five phyla. Colors filled in the circle denote the percentage of genomes within one phylum encode the gene. Sizes of the circle denote the average number of gene copies identified in the MAG within one phylum. Asterisk denotes the family identified with potential secretion signal, with a number on top of the circle representing the number of sequences identified with potential secretion signal using PSORTb v3.0. Numbers in brackets denote the total number of MAGs in each phylum. The cluster using pheatmap package in R is based on the normalized data considering the equal contribution by the percentage of MAG containing the enzyme and the average copy number per MAG in the phylum. Source data are provided as a Source Data file.



Supplementary Fig. 8 Maximum likelihood phylogenetic tree of genes associated with sulfur cycling.

(a) A phylogenetic tree of the gene encoding for alpha subunit of dissimilatory sulfite reductase (DsrA). (b) A phylogenetic tree of the gene encoding for beta subunit of dissimilatory sulfite reductase (DsrB). (c) A phylogenetic tree of the gene encoding for sulfide-quinone reductase (SQR). One sequence recovered from genome M3-22_Bin_282 was annotated as sulfide-quinone reductase by Hidden Markov Model (HMM) profile-based annotation, but is phylogenetically close to flavocytochrome *c* sulfide dehydrogenase. Sequences recovered from the MAGs in this study are in bold. Source data are provided as a Source Data file.



Supplementary Fig. 9 Maximum likelihood phylogenetic tree of FeFe hydrogenases. FeFe hydrogenases identified in this study belong to types A3 and C1 as highlighted with a grey background. Bootstrap values ≥ 80 are shown in circles. Source data are provided as a Source Data file.

Supplementary Notes

Phylogeny of five phyla and novel protein identification

Based on the 37 concatenated marker genes, the 55 metagenome assembled genomes (MAGs) recovered in this study formed five groups. We included all the publicly available genomes, belonging to the phyla identified by GTDB-Tk, from NCBI by May, 2020. We further included the genomes which were reported previously. Our phylogenetic results indicate that the 55 newly reconstructed MAGs together with six MAGs from NCBI and IMG/M databases belong to four new phyla, which are designated Guaymas Basin Candidate Phylum (GB-CP) GB-CP11, GB-CP12, GB-CP13, and GB-CP14, and one poorly described phylum, Candidate division AABM5-125-24 (AABM5 hereafter) (Fig. 1). We propose these four new phyla GB-CP11, GB-CP12, GB-CP13, and GB-CP14 be named “Blakebacterota”, “Orphanbacterota”, “Arandabacterota”, and “Joyebacterota”, respectively. The metabolic analyses were based on the 61 MAGs. When we analyzed the genomic context to search for novel proteins, we only used the 55 MAGs we recovered in this study. When we expanded the search against the collection of 169,642 bacterial and archaeal genomes, we further identified 19 genomes phylogenetically related to AABM5. Thus, the two novel protein families which are widely present in more than 70% of the AABM5 genomes (31 curated AABM5 genomes) and rarely detected in the collection of 169,642 prokaryotic genomes.

Carbohydrate-active enzymes (CAZymes)

All genomes within these five phyla have genes encoding for potential diverse CAZymes (Supplementary Fig. 7), enzymes related to breaking down different carbohydrates, which are further divided into five categories: Carbohydrate Esterases (CEs), Glycoside Hydrolases (GHs), Polysaccharide Lyases (PLs), Glycoside Transferases (GTs), and Carbohydrate-binding Modules (CBMs). These CAZymes are significant for the degradation of different types of carbohydrates, e.g., agar, xylan, starch, mannan, cellulose, chitin, laminarin, and pectin. Among ~5,400 genes classified as CAZymes in these five phyla, over 3,200 are classified as GT and CBM, 11, 91, and 8 are identified as CE, GH, and PL families/subfamilies, respectively (Supplementary Data 11 and 12). AABM5 has a wider range of diverse CAZymes with 59 families/subfamilies in CE, PL, and GH, while Joyebacterota has the least diversity of CAZymes with only 41 families/subfamilies. These five phyla share three and eight families of CE and GH, respectively. The types of CAZymes that are present in > 50% MAGs within a single phylum are similar across the five phyla, and mostly appear to be in more than one copy in the MAGs. All five phyla have phylum-specific CAZyme families/subfamilies, comprising 49 out of 110. However, most of those phylum-specific families/subfamilies are only present in less than half of MAGs within that phylum, and with few copies. Specifically, for those types of CAZymes widely distributed only in the single group (> 50% MAGs), GH113 (β -mannanase) is only found in Orphanbacterota; GH127 (β -L-arabinofuranosidase) and GH142 (β -L-arabinofuranosidase) are only present in

Blakebacterota; and PL1_2 (pectate and pectin lyase) is only found in Joyebacterota. Notably, Blakebacterota does not have genes encoding for PL.

Based on the predicted localization of enzymes, 15 families/subfamily of CE, GH, and PL are predicted to be extracellular within the five phyla suggesting that complex substrates are degraded outside the cell and later taken up for consumption. The generated monosaccharide, e.g., glucose, could further support the metabolism of the cell through glycolysis and the tricarboxylic acid (TCA) cycle. These secreted CAZymes might contribute to the degradation of complex substrates, e.g., pectin, laminarin, agar, chitin, and xylan in the surrounding environments. The types of CAZymes with secretion signals in each group are different, even for the same substrate. Interestingly, none of the five phyla encodes the entire set of enzymes for the complete degradation of the complex substrate (Supplementary Data 10 and 11) suggesting handoffs of the degradation of complex substrates outside of the cell. However, only 36 sequences have the signal of extracellular potential among those identified ~2,200 CE, GH, and PL genes.

Detrital proteins

There are ~4,600 different peptidase-related sequences, including ~4,250 classified peptidase-like genes, ~250 peptidase inhibitor genes, and ~100 unclassified genes identified within these five phyla (Supplementary Data 13 and 14). Metallo and serine peptidases are the most two diverse families in all the five phyla. Among the 117 classified families/subfamilies, 31 of them are found in all phyla, and many of them are present in more than 75% of MAGs in all the five phyla. Examples of conserved peptidases are: C26 (gamma-glutamyl hydrolase), that acts as an endopeptidase for substrates with gamma-linked glutamate bonds, such as folyl penta-gamma-glutamate; C44 (amidophosphoribosyltransferase precursor), of which mature enzymes catalyzing the formation of phosphoribosylamine from phosphoribosylpyrophosphate (PRPP) and glutamine; M23B (lysostaphin), an endopeptidase that lyse bacterial cell wall peptidoglycans; M38 (isoaspartyl dipeptidase); M41 (FtsH peptidase) and S16 (Lon-A peptidase), mostly are ATP-dependent endopeptidases; I87, inhibiting FtsH and modulating the degradation of mistranslation products that disrupt membranes¹, is encoded in more than four copies per MAG in all these five phyla (Supplementary Fig. 7). Orphanbacterota has 87 families of peptidases as the most diverse phylum, while Joyebacterota has 71 families which is the least diverse phylum. 24 out of 122 classified families/subfamilies were exclusively found in a single phylum, and those unique families/subfamilies are mostly present in less than 50% MAGs in that phylum with fewer gene copy number than the rest of types of peptidases. In addition, none of genes in those unique families/subfamilies has the signal of secretion.

A total of 185 genes, distributed in 14 families/subfamilies, appear to be potentially extracellular. The only extracellular family/subfamily consistently found in the five phyla is the serine endopeptidase subtilisin (S08A), widely distributed in eukaryotes and prokaryotes^{2,3}. Blakebacterota, Orphanbacterota, and Arandabacterota have many potential genes identified as

extracellular peptidases belonging to family M28 (subfamilies M28C, M28E, and M28F). These three subfamilies are mainly *Streptomyces*-type aminopeptidases and carboxypeptidases, releasing basic amino acids and C-terminal glutamates, respectively. Joyebacterota has different types of potential extracellular peptidase compared with other phyla. Joyebacterota has six sequences with signals of potential secretion belonging to family C01A (papain), a heat-resistant enzyme with an optimal temperature range of 60 to 70 °C⁴. This may highlight the significant role of Joyebacterota in the extracellular degradation of proteins in the hydrothermal vent area.

Central metabolism

Glycolysis: MAGs from Orphanbacterota, Arandabacterota, and Joyebacterota encode most of the key genes for glycolysis (Supplementary Fig. 5). Interestingly, the gene encoding for pyruvate kinase was not found in AABM5 and Blakebacterota, suggesting that these two phyla may not have the complete pathway of glycolysis for energy production. In addition, less than half of MAGs in Blakebacterota and Arandabacterota have genes encoding for triosephosphate isomerase, transforming dihydroxyacetone phosphate to glyceraldehyde 3-phosphate (GADP) for further glycolysis. None of the MAGs has genes encoding for glyceraldehyde-3-phosphate dehydrogenase, which suggests that the transformation of glyceraldehyde 3-phosphate to 3-phosphoglycerate has to be a two-step process via 1,3-bisphosphoglycerate. Moreover, all phyla have the fructose-1,6-bisphosphatase and the phosphoenolpyruvate carboxykinase, a rate-limiting enzyme in gluconeogenesis and catalyzing the oxydecarboxylation and phosphorylation of oxaloacetate⁵. (Supplementary Data 8 and 9).

Pyruvate metabolism: Pyruvate could be converted to acetyl-CoA by different pathways (Supplementary Fig. 5). AABM5, Blakebacterota, and Orphanbacterota encode the pyruvate dehydrogenase complex. Except for Blakebacterota, the other four phyla have pyruvate ferredoxin oxidoreductase, catalyzing the oxidative decarboxylation of pyruvate to acetyl-CoA and CO₂⁶. In addition, all MAGs encode the gene for 2-oxoglutarate/2-oxoacid ferredoxin oxidoreductase, which exhibits a broad substrate specificity toward 2-oxoacids, and accepts multiple substrates, including 2-oxoglutarate, 2-oxobutanoate, and pyruvate to form acetyl-CoA^{7,8}, which could be further utilized for the oxidative TCA cycle. Joyebacterota MAGs encode genes for the cytochrome D-lactate dehydrogenase converting lactate to pyruvate. The direct formation of acetate from pyruvate through pyruvate dehydrogenase is missing in all the five phyla. Acetate still could be produced through acetyl-CoA or acetyl-phosphate. They have genes encoding alcohol dehydrogenase and acetaldehyde dehydrogenase (Supplementary Data 8 and 9), suggesting they oxidize ethanol to acetaldehyde, and further convert acetaldehyde to acetyl-CoA.

Pentose phosphate pathway (PPP) and Galactose pathway: This pathway generates NADH, pentose, and ribose 5-phosphate for nucleotide and amino acid biosynthesis, and reduced molecules for anabolism (Supplementary Fig. 5). MAGs in Blakebacterota, and few MAGs in AABM5, Orphanbacterota, Arandabacterota, and Joyebacterota display both oxidative and non-oxidative PPP, while the rest of MAGs only encodes the non-oxidative PPP. Few MAGs have the gene encoding for ribokinase, catalyzing the phosphorylation of ribose, while they are not those MAGs with the ribose ABC transporter. None of the MAGs has the entire pathway for galactose metabolism for galactose degradation, or ribulose monophosphate pathway for formaldehyde fixation and detoxification.

Wood-Ljungdahl pathway (WLP): All five phyla have the partial WLP (Supplementary Fig. 5), one of the most ancient carbon fixation pathways⁹. Genes encoding formate dehydrogenase, transforming CO₂ to formate, are not present in any of the five phyla MAGs. One MAG from each phylum (AABM5, Blakebacterota, Arandabacterota, and Joyebacterota) encodes formyl-tetrahydrofolate (HCO-THF) synthase activating formate to formyl-H4F. All the five phyla are capable of further reducing formyl-THF to methyl-THF by the presence of *fold* and *metF* genes. Joyebacterota MAGs encode *ccoF* gene which is potentially involved in anaerobic CO oxidation^{10,11}. AABM5 and Arandabacterota have most of the CO-dehydrogenase–acetyl-CoA-synthase complex CODH/ACS, the key enzyme for the carbonyl branch for the WLP. However, all MAGs lack the enzyme CODH/ACS methyltransferase subunit, which is responsible for the transfer of the methyl moiety from methyl-THF to the corrinoid iron–sulfur protein (CFeSP) for acetyl-CoA synthesis.

The synthetic pathway for formate assimilation¹², the reductive glycine pathway (rGlyP)¹² is found across MAGs from the five phyla. The glycine cleavage system (GCS), catalyzing the reversible conversion of CO₂, methenyl-THF, and ammonia to glycine and tetrahydrofolate (THF), is annotated in all the five phyla. We explore the possibility of CO oxidation in these bacterial genomes, yet none of the MAG encode the three subunits of the coxLMS complex and only one MAG in Blakebacterota has the large subunit of aerobic CO dehydrogenase (*coxL*).

TCA cycle: All the five phyla have the complete set of genes for the TCA cycle (Supplementary Fig. 5), though some MAGs lack few genes (Supplementary Data 8 and 9). Interestingly, none of the MAGs encode ATP-citrate lyase, and only very few genes encoding for fumarate reductase are annotated in the MAG suggesting that they could not fix carbon through the reductive TCA cycle. Previously described 2-oxoglutarate synthase, one of the three key enzymes for reductive direction of the TCA cycle¹³, in *Helicobacteraceae*¹⁴ participated in the oxidative TCA cycle. Thus the 2-oxoglutarate synthase in these five phyla may also participate in the oxidative TCA cycle as well.

Electron transport chain (ETC): All the five phyla encode most of the components of the ETC (Supplementary Data 8 and 9). They all have the subunits for complex I and II. AABM5 and Orphanbacterota have F-type ATPase, while Arandabacterota and Joyebacterota have the V/A-type ATPase. Notably, Blakebacterota only has some subunit for either F-type ATPase or V/A-type ATPase. Arandabacterota and Joyebacterota appear to lack cytochrome oxidase suggesting an anaerobic lifestyle. Orphanbacterota, some Blakebacterota and AABM5 MAGs encode more than one type of cytochrome oxidase with different affinities for oxygen, e.g., cytochrome *c* oxidase, cytochrome *cbb₃* type oxidase and cytochrome *bd* ubiquinol oxidase, which indicates that they are aerobes and able to utilize oxygen at different concentrations efficiently. AABM5 and most MAGs in Blakebacterota only have the oxygen high-affinity ones, mainly cytochrome *bd* ubiquinol oxidase, suggesting that they may only use the oxygen as the electron acceptor efficiently when the oxygen concentration is low to scavenge oxygen as a way of protection rather than for energy production.

Additionally, we identified the acetogenic type Rhodobacter nitrogen fixation (Rnf) electron transport complex in four among these five phyla (Blakebacterota, Orphanbacterota, Arandabacterota, and Joyebacterota) (Supplementary Fig. 5). This complex could serve as a respiratory enzyme that couples the reduction of NAD⁺ to oxidize reduced ferredoxin. The free energy of this exergonic reaction could be used to pump sodium ions or protons out of cells, thereby generating a potential gradient, which is further used for ATP synthesis. Those MAGs with Rnf complex genes only have partial WLP (see details in the WLP section), yet they all have the reductive glycine pathway (rGlyP) for formate assimilation.

Lipids: Genes encoding for enzymes which are responsible for the transport, activation, and cleavage of fatty acids through beta oxidation¹⁵ are found in all phyla (e.g., genes encoding for acyl-CoA dehydrogenase and enoyl-CoA hydratase are commonly found in these five phyla). However, only two Orphanbacterota MAGs appear to have the complete pathway for beta oxidation (i.e., they encode for the 3-hydroxyacyl-CoA dehydrogenase and acetyl-CoA acyltransferase). This suggests a limited ability of fatty acid degradation in these five phyla.

Amino acids: Different types of amino acids, including alanine, serine, asparagine, histidine, and lysine could be degraded via central metabolism (i.e., TCA cycle and pyruvate metabolism) which are also the key pathways for energy production in most phyla (Supplementary Fig. 5). In addition, genes encoding for glutamine synthetase, glutamate synthase, and glutamate dehydrogenase are commonly annotated in these five phyla suggesting active anabolic and catabolic metabolisms through glutamate, glutamine, and ammonium. However, all phyla lack the genes encoding key enzymes for the degradation of branched-chain amino acids and aromatic amino acids, e.g., valine, leucine, isoleucine, and tyrosine.

Sulfur metabolism

Marine sediments are active sites for sulfur and nitrogen cycling. Detailed genome-specific metabolic potential reveals that all these five phyla are capable of using different sulfur and nitrogen compounds as energy sources (Fig. 4). Most AABM5 MAGs and one Blakebacterota MAG encode sulfate adenylyltransferase (SAT), reversibly reducing sulfate to adenylyl sulfate (APS), and adenylylsulfate reductase (AprAB), reversibly reducing APS to sulfite. In addition, they also encode the quinone-modifying oxidoreductase gene (QmoABC) for the efficient electron delivery to adenylylsulfate reductase¹⁶. Most of the MAGs from Blakebacterota, Orphanbacterota, and Arandabacterota only have one copy of the *sat* gene, which reversibly reduces sulfate to APS. Most AABM5 MAGs, and some Blakebacterota, Orphanbacterota, and Arandabacterota MAGs encode dissimilatory sulfite reductase (DsrAB) (Supplementary Data 15). Based on their phylogenetic position, they are reductive type sulfite reductase (Supplementary Fig. 8). Based on their metabolic repertoire, only AABM5 MAGs encode the entire pathway for dissimilatory sulfate reduction to H₂S. Most MAGs in Blakebacterota, and only few Orphanbacterota and Arandabacterota MAGs display a partial pathway for dissimilatory sulfate reduction. Interestingly, it seems that these bacteria from Guaymas Basin have more tendency to reduce sulfate than those from the Bohai Sea, e.g., MAGs in Blakebacterota and AABM5, though the sulfate concentration in the Bohai Sea sediment could be over 1,000 mg/kg¹⁷. There are frequent horizontal gene transfer events (HGT) for *dsrAB* genes¹⁸. The closely related DsrAB sequences to Blakebacterota and AABM5 belong to different taxonomic groups, suggesting they were transferred. Moreover, the acquisition of *dsrAB* genes in these five phyla is more fundamental in the hydrothermal sediment than the coastal sediment, despite that the sulfate-reducing bacteria, e.g., *Desulfobulbus* and *Desulfotignum*, were abundant in the coastal sediment¹⁷. MAGs encoding sulfate-reduction associated genes also encode hydrogenases, e.g., MvhADG complex in AABM5 MAGs and HndD, the catalytic subunit of NADP-reducing hydrogenase, in few Blakebacterota MAGs, suggesting the coupling of hydrogen oxidation with sulfite reduction.

Many genomes contain sulfate assimilation genes. For example, AABM5, Orphanbacterota, Arandabacterota, and Joyebacterota MAGs encode adenylylsulfate kinase (CysC), converting APS to 3'-phosphoadenylyl sulfate (PAPS). AABM5 and Orphanbacterota also have 3'(2'),5'-bisphosphate nucleotidase (SAL) to hydrolyse PAPS to APS. Two Orphanbacterota MAGs (BHB10-2_Bin_362 and LQ108M_Bin_12) encode phosphoadenosine 5'-phosphosulfate reductase (CysH) and sulfite reductase (ferredoxin) (SIR) which produces sulfite from PAPS using thioredoxin as an electron donor¹⁹ and reduces sulfite to H₂S for assimilation²⁰, respectively.

Less than half of the MAGs in each phylum encode partial thiosulfate reductase (PhsABC), tetrathionate reductase (TtrABC), and anaerobic sulfite reductase (AsrABC), yet none of MAGs encode the complete known metabolic pathways (Supplementary Data 8 and 9). More than 50% AABM5, Orphanbacterota, and Arandabacterota MAGs, and close to 50% Joyebacterota MAGs have homologs to eukaryotic thiosulfate/3-mercaptopyruvate sulfurtransferase (TST)²¹, which

could transfer thiosulfate and cyanide to sulfite and thiocyanate. One MAG from AABM5, Blakebacterota, and two Orphanbacterota MAGs display genes encoding for sulfhydrogenase I complex (HydADGB)²², along with NADPH-dependent hydrogen-evolving hydrogenase (HydAD) with sulfur reducing activity (HydGB). Most Arandabacterota and Joyebacterota MAGs only have the genes encoding for the two subunits with the activity of sulfur reductase (HydGB), reducing sulfur or polysulfide to H₂S.

Genes encoding for sulfide:quinone oxidoreductase (SQR), are observed in Blakebacterota, Orphanbacterota, Arandabacterota, and Joyebacterota. SQR is present in more than 50% Blakebacterota MAGs suggesting a chemolithoheterotrophic lifestyle by harvesting energy from sulfide oxidation. Four Joyebacterota MAGs have two *sqr* genes belonging to Type III and I (Supplementary Fig. 8), which are common for sulfur bacteria²³. SQR in Arandabacterota and Blakebacterota MAGs belong to type III, and only one Orphanbacterota MAG encodes one Type I SQR. Most MAGs encoding SQR were from Guaymas Basin samples. Additionally, one annotated SQR from the Orphanbacterota MAG is proved to be flavocytochrome *c* sulfide dehydrogenase (FCSD), oxidizing H₂S through cytochrome *c*, based on the phylogenetic tree (Supplementary Fig. 8). Blakebacterota is probably capable of oxidizing thiosulfate to tetrathionate since most of the MAGs encode thiosulfate dehydrogenase (DoxD). Only two Arandabacterota MAGs have *soxB* genes, yet lacking the rest of the components of the SOX pathway. Furthermore, all the groups lack the complete set of genes encoding for sulfite dehydrogenase (SoeABC/SorAB) and sulfite oxidase (SUOX).

Exploring the assimilation of organic sulfur compounds in these four groups, we found that all of them lack the full set of *dmsABC* genes reducing dimethyl sulfoxide (DMSO) to dimethyl sulfide (DMS). One MAG from Blakebacterota and Orphanbacterota could methylate L-methionine or methanethiol (MeSH) to yield DMS with methanethiol S-methyltransferase (via *MddA*) under oxic conditions. Interestingly, two Blakebacterota MAGs display *ssuE* gene encoding for FMN reductase, but the missing for *ssuD*, encoding for alkanesulfonate monooxygenase, and sulfonate transport system (SsuABC) indicating that they probably are not capable of reducing alkane sulfonate. Except for AABM5, most MAGs have genes encoding for peptide methionine sulfoxide reductases (*MsrA/MsrB/MsrP*). These enzymes play an essential role in preventing oxidative-stress damage caused by reactive oxygen species by reducing the oxidized form of methionine thereby reactivating damaged peptides²⁴. Except for AABM5, most MAGs encode sulfatase, capable of cleaving sulfate ester bonds from a wide variety of substrates, as a source of sulfur and carbon via organic sulfate degradation²⁵. These sulfatasases are involved in the regulation of the sulfation states that provide different functions, e.g., osmoprotection²⁶ and pathogenic processes²⁷.

Most AABM5 MAGs encode the sulfate permease, while it is observed in only a few Orphanbacterota, Arandabacterota MAGs. In addition, only one AABM5 MAG has most genes

encoding for sulfate/thiosulfate transport system (CysAUWP), except that the subunit CysP is missing, which is required for sulfate/thiosulfate ABC transporter²⁸.

Nitrogen metabolism

These five phyla encode both the oxidative and reductive pathways of the nitrogen cycle (Supplementary Fig. 5). Most of the MAGs in these five phyla encode genes for glutamine synthetase and glutamate dehydrogenase for ammonia assimilation. The gene encoding for glutaminase, converting glutamine to glutamate, is annotated in all five phyla. With exception of two Arandabacterota MAGs, all five phyla display *hao* genes encoding for hydroxylamine dehydrogenase oxidizing hydroxylamine to nitric oxide. However, the genes encoding for ammonia monooxygenase, oxidizing ammonia to hydroxylamine, are missing in all MAGs. Genes encoding for carbamate kinase (ArcC), which catalyze the reversible formation of ATP and carbamate with carbamoyl phosphate and ADP, are found in most MAGs. However, none of them display genes encoding for carbamoyl phosphate synthetase, catalyzing the ATP-dependent synthesis of carbamoyl phosphate from glutamine or ammonia and bicarbonate.

Only few genes encoding for nitrate/nitrite or ammonium transporter are observed in some MAGs from AABM5, Blakebacterota, and Orphanbacterota (Supplementary Data 8 and 9). The cytoplasmic assimilatory nitrate reductase genes are not observed in any MAG. Few MAGs in Blakebacterota and Orphanbacterota have genes encoding for the major components of membrane-bound nitrate reductase, which could contribute to the generation of ATP by proton motive force. In addition, more than half of the Orphanbacterota MAGs have genes for periplasmic nitrate reductase, which would not create a proton gradient. Over 50% Blakebacterota MAGs encode the nitrous oxide reductase complex (NosZDFYLR), including the catalytic subunit, accessory protein, and transporters. Blakebacterota may be capable of oxidizing nitrous oxide, a product of incomplete denitrification or nitrification in sediments to nitrogen gas (Supplementary Data 8 and 9). Few AABM5 and Arandabacterota MAGs have genes for periplasmic dissimilatory nitrite reduction (NrfAH). Half of Joyebacterota MAGs, and less than half in the other four phyla have genes encoding for the large subunit of the assimilatory nitrite reductase. MAGs in AABM5 and Blakebacterota have genes encoding for nitronate monooxygenase, oxidizing alkyl nitronate with oxygen to nitrite.

Hydrogen metabolism

Hydrogen production or consumption through hydrogenase is thought to be crucial in energy cycling in both coastal and hydrothermal environments^{29,30}. All of these five phyla have different types of hydrogenases indicating the diverse hydrogen metabolism which could supply intracellular reducing equivalents to further couple different metabolic pathways in these bacteria^{31,32}. Most of the hydrogenases are found from hydrothermal sediments and cold seep sediments, especially that [FeFe] hydrogenases are only found in the Guaymas Basin samples in

this study. The phylogenetic position of each type of hydrogenase is consistent with the phylogeny of these five phyla (Fig. 3 and Supplementary Fig. 9).

F₄₂₀-non-reducing hydrogenase (MvhADG), belonging to type 3c [NiFe] hydrogenase, is distributed in all these five phyla, especially that in more than half MAGs in Arandabacterota and close to half MAGs in Joyebacterota. This F₄₂₀-non-reducing hydrogenase provides reducing equivalents to the heterodisulfide reductase without reacting with F₄₂₀, i.e., transporting electrons using H₂ as electron donor³³. The bidirectional and oxygen-tolerant NADPH-dependent hydrogen-evolving hydrogenase within sulfhydrogenase complex I (see Sulfur metabolism section), belongs to type 3b [NiFe] hydrogenase, and is distributed mainly in Orphanbacterota and one MAG in Blakebacterota and AABM5 (Supplementary Data 8 and 9). They could reduce polysulfide to hydrogen sulfide²², oxidize H₂ using NADP⁺ as the electron acceptor³⁴, and could also produce H₂ with NADPH as the electron donor when the S₀ is absent during carbohydrate fermentation³⁵. The members of this hydrogenase in this study are phylogenetically distant with each other (Fig. 3) despite four of the six MAGs belonging to Orphanbacterota, indicating the horizontal gene transfer of this type 3b [NiFe] hydrogenase may be more frequent than the other types of hydrogenase.

None of the type 4g [NiFe] hydrogenase have been biochemically characterized, but they are generally described as to membrane-bound hydrogenases coupling the formation of H₂ from reduced ferredoxin or CO, and H⁺ or Na⁺ translocation that allow for energy-generation by establishing ion gradients over the membrane³⁶⁻³⁸. Three sub-types of catalytic subunit of hydrogenase are classified as type 4g [NiFe] hydrogenase based on the annotation within phyla AABM5, Arandabacterota, and Joyebacterota: (i) membrane-bound hydrogenase (MbhL) is present in over 50% Arandabacterota MAGs, close to 50% Joyebacterota MAGs, and one AABM5 MAG. This hydrogenase is a redox driven ion pump that generates a proton motive force from H⁺ reduction coupled to electrons derived from ferredoxin oxidation³⁹; (ii) the hydrogenase component (HycE) in membrane-bound formate hydrogenlyase complex is present in close to 50% Joyebacterota MAGs and one Orphanbacterota MAG. This hydrogenase uses electrons derived from formate oxidation during glucose fermentation to reduce protons and release CO₂ and H₂⁴⁰; (iii) the catalytic subunit of ech hydrogenase complex (EchE) is present in three Joyebacterota MAGs and one Arandabacterota MAG. This ech hydrogenase is the potential respiratory enzyme oxidizing ferredoxin or CO, during which H₂ and a transmembrane electrochemical ion gradient are formed for energy conservation in methanogenesis and carbon fixation^{38,41}. It may also reversely serve as the source of reduced ferredoxin using H₂ as the electron donor⁴². Overall, these sequences of type 4g [NiFe] hydrogenase formed three monophyletic groups consistent with the phylogenetic position, and close to a hydrogenase from phylum *Thermotogae*. However, these three sub-types of type 4g [NiFe] hydrogenase could not be distinguished based on the phylogenetic position (Fig. 3).

Type A3 [FeFe] hydrogenase (HndD), that reversibly bifurcates electrons from H₂ to ferredoxin and NAD³⁷, was found in AABM5 and Blakebacterota. Type C1 [FeFe] ferredoxin hydrogenase, which serves as H₂-sensors, was found to be present in Joyebacterota.

Mercury, selenium, and arsenic metabolism

Submarine hydrothermal systems are important natural sources of mercury (Hg)⁴³. Hg resisting microorganisms are widely distributed in deep sea hydrothermal vents and in terrestrial geothermal springs^{30,44}. Longqi Hydrothermal vent, in the Southwest Indian Ridge, is commonly associated with massive sulfides and Hg⁴⁵. Metabolic reconstructions suggest that a MAG in Orphanbacterota (LQ108M_Bin_12) is capable of transporting mercury into cytoplasm, and reducing extremely toxic Hg²⁺ to metallic Hg⁰⁴⁶ with mercuric transport protein (MerT), MerR family transcriptional regulator (MerR), and mercuric reductase (MerA), which indicate that Orphanbacterota is potentially involved in mercury detoxification⁴⁷.

Selenium could exist in both organic and inorganic with different oxidation states in marine sediments⁴⁸, and selenocysteine is the 21st amino acid⁴⁹. MAGs from both coastal sediments and hydrothermal vent sediments have genes for mobilization of organic selenium. Some AABM5, Blakebacterota, and Orphanbacterota MAGs have the gene encoding for the putative selenate reductase (YgfK)⁵⁰, yet the entire complex (YgfKM and XdhD) was found to be incomplete in all the MAGs (Supplementary Data 8 and 9). All groups have the gene encoding for selenide water dikinase (SelD) catalyzing selenide with ATP to selenophosphate⁵¹, the selenium donor for the selenocysteinyl-tRNA (Sec) with L-seryl-tRNA (Ser) selenium transferase (SelA)⁵², which is further utilized for protein biosynthesis. The selenocysteine could be decomposed into selenide and alanine with cysteine desulfurase/selenocysteine lyase (SufS)⁵³, and this pathway is mainly observed in Blakebacterota and Orphanbacterota. The cystathionine gamma-synthase (MetB), which is also important for sulfur cycling, could also use selenocysteine as the substrate to form selenocystathionine. The transaminase (CCBL), rarely annotated in Arandabacterota and Joyebacterota could transform the selenocystathionine into selenohomocysteine. Most MAGs in all five phyla have the gene encoding for 5-methyltetrahydrofolate homocysteine methyltransferase (MetH), which also transfers a methyl to selenohomocysteine to form selenomethionine. The formed selenomethionine could be further transformed into methaneselenol with methionine-gamma lyase in AABM5, Blakebacterota, and Orphanbacterota MAGs. The selenomethionine could be further incorporated into selenomethionyl-tRNA (Met) with methionyl-tRNA synthetase (MetG)⁵⁴, which is further utilized for protein biosynthesis. These MAGs seem to have the pathway to synthesize selenium protein from selenide through the selenocysteinyl-tRNA (Sec) or the selenomethionyl-tRNA (Met). Meanwhile, selenium could also be released into selenide from selenocysteine during the biosynthesis.

Arsenate and arsenite are the two dominant formats of inorganic arsenic in marine environments⁵⁵. They induce toxicity by blocking general cell metabolism⁵⁶. All these five phyla have genes for the arsenic detoxification system. MAGs in all five phyla, though less than half MAGs in Arandabacterota, and Joyebacterota, are capable of reducing arsenate to arsenite via arsenate reductase (ArsC) through thioredoxin⁵⁷. Even though arsenite is more toxic than arsenate, arsenite could be extruded from the cell by arsenite transporter (ArsAB) or transformed to methyl arsonate, less toxic than the inorganic format⁵⁸ by arsenite methyltransferase (AS3MT). The ArsC could also be a potential pathway for energy production by using arsenate as the terminal electron acceptor with sulfide or lactate as the electron donor^{59,60}, rather than merely a way of detoxification.

Active interaction with environments

All the five phyla have genes encoding for diverse transport systems including the importers for different substrates from small ions to large proteins for energy conservation. They also display exporters for detoxification or resistance of antibiotics, indicating an active exchange with the surrounding environments. A putative ATP-binding cassette (ABC) multiple sugar transport system is annotated in all five phyla (Supplementary Fig. 5), especially in most MAGs in Orphanbacterota, Arandabacterota, and Joyebacterota. Interestingly, the ABC transporter for specific oligosaccharide is rare, for example the specific transporter for the maltose, cellobiose, lactose, xylobiose, and etc., are missing in all phyla. The general nucleoside transport system (NupABC), transporting all common nucleosides across the membrane⁶¹, is annotated in most MAGs in Orphanbacterota, Arandabacterota, and Joyebacterota. In addition, the ribose transporter is found in a few MAGs in AABM5 and Blakebacterota. Interestingly, the transporter for the rest monosaccharide, e.g., glucose, arabinose, galactose, xylose, and fucose are missing in all MAGs within the five phyla. Furthermore, very few genes related to the phosphotransferase system are annotated. Thus, we suggest the uptake of carbohydrates from the environment in these phyla is through the putative ABC multiple sugar transport system. The peptide could also be transported into the cell through the peptide/nickel transport system as the additional source of organic carbon.

All the five phyla have the genes encoding for the lipopolysaccharide biosynthesis and phospholipid transport system (Supplementary Data 8 and 9), which preserves outer membrane lipid asymmetry by the reverse transport of phospholipids from the outer membrane to the inner membrane⁶², suggesting they are gram-negative bacteria. Transporters for the other important elements for life, including molybdate, tungstate, phosphate, iron, manganese, cobalt, sodium, and zinc are identified in MAGs. Besides those ABC transporters, several MAGs have genes encoding for the multidrug resistance protein, heavy metal efflux system, and several antibiotic resistance proteins, indicating these bacteria may have the ability to resist antibiotics or heavy metals in the environment.

Description of novel taxa

Candidatus Blakebacterota (Bla.ke. N.L. neut. bacteria; N.L. neut. n. Blakebacterota a bacteria named after Dr. Ruth Blake). Type species: *Candidatus Blakebacterum guaymasense*.

Candidatus Blakebacterum guaymasense (gu.a.y.ma.sen'se N.L. neut. adj. pertaining to Guaymas Basin, Mexico). This uncultured lineage is represented by the genome "Meg22_46_Bin_213" consisting of 3.09 Mbps in 527 contigs with an estimated completeness of 89.0%, an estimated contamination of 6.6%. The MAG was recovered from Guaymas Basin sediment from 4-6 cm at station Meg22.

Candidatus Blakebacteraceae (Bla.ke.bac.te.ra.ce'ae. N.L. neut. n. Blakebacterum, *Candidatus* generic name; -aceae ending to denote the family; N.L. fem. pl. n. Blakebacteraceae, the Blakebacterum family).

The family is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Blakebacterum*.

Candidatus Blakebacterales (Bla.ke.bac.te.ra'les. N.L. neut. n. Blakebacterum, *Candidatus* generic name; -ales ending to denote the order; N.L. fem. pl. n. Blakebacterales, the Blakebacterum order).

The order is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Blakebacterum*.

Candidatus Blakebacteria (Bla.ke.bac.te'ri.a. N.L. neut. n. Blakebacterum, *Candidatus* generic name; -ia ending to denote the class; N.L. fem. pl. n. Blakebacteria, the Blakebacterum class).

The class is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Blakebacterum*.

Candidatus Blakebacterota (Bla.ke.bac.te.ro'ta. N.L. neut. n. Blakebacterum, *Candidatus* generic name; -ota ending to denote the phylum; N.L. fem. pl. n. Blakebacterota, the Blakebacterum phylum).

The phylum is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Blakebacterum*.

Candidatus Orphanbacterota (Or.phan. N.L. neut. bacteria; N.L. neut. n. Orphanbacterota a bacteria named after Dr. Victoria Orphan). Type species: *Candidatus Orphanbacterum longqiense*.

Candidatus Orphanbacterum longqiense (lon.gqi.en'se N.L. neut. adj. pertaining to Longqi hydrothermal vent area in Southwest Indian Ocean). This uncultured lineage is represented by the genome "LQ108M_Bin_12" consisting of 4.88 Mbps in 49 contigs with an estimated completeness of 97.8%, an estimated contamination of 1.1%. The MAG was recovered from microbiome in Longqi hydrothermal vent area in Southwest Indian Ocean.

Candidatus Orphanbacteraceae (Or.phan.bac.te.ra.ce'ae. N.L. neut. n. Orphanbacterum, Candidatus generic name; -aceae ending to denote the family; N.L. fem. pl. n. Orphanbacteraceae, the Orphanbacterum family).

The family is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Orphanbacterum*.

Candidatus Orphanbacterales (Or.phan.bac.te.ra'les. N.L. neut. n. Orphanbacterum, Candidatus generic name; -ales ending to denote the order; N.L. fem. pl. n. Orphanbacterales, the Orphanbacterum order).

The order is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Orphanbacterum*.

Candidatus Orphanbacteria (Or.phan.bac.te'ri.a. N.L. neut. n. Orphanbacterum, Candidatus generic name; -ia ending to denote the class; N.L. fem. pl. n. Orphanbacteria, the Orphanbacterum class).

The class is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Orphanbacterum*.

Candidatus Orphanbacterota (Or.phan.bac.te.ro'ta. N.L. neut. n. Orphanbacterum, Candidatus generic name; -ota ending to denote the phylum; N.L. fem. pl. n. Orphanbacterota, the Orphanbacterum phylum).

The phylum is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Orphanbacterum*.

Candidatus Arandabacterota (A.ran.da. N.L. neut. bacteria; N.L. neut. n. Arandabacterota a bacteria named after Dr. Raquel Negrete-Aranda). Type species: *Candidatus Arandabacterum bohaiense*.

Candidatus Arandabacterum bohaiense (bo.ha.i.en'se N.L. neut. adj. pertaining to Bohai Sea, China). This uncultured lineage is represented by the genome "M3_30_Bin_374" consisting of 3.32 Mbps in 198 contigs with an estimated completeness of 92.2%, an estimated contamination of 2.3%. The MAG was recovered from Bohai Sea sediment from 28-30 cm at station M3.

Candidatus Arandabacteraceae (A.ran.da.bac.te.ra.ce'ae. N.L. neut. n. Arandabacterum, Candidatus generic name; -aceae ending to denote the family; N.L. fem. pl. n. Arandabacteraceae, the Arandabacterum family).

The family is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Arandabacterum*.

Candidatus Arandabacterales (A.ran.da.bac.te.ra'les. N.L. neut. n. Arandabacterum, Candidatus generic name; -ales ending to denote the order; N.L. fem. pl. n. Arandabacterales, the Arandabacterum order).

The order is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Arandabacterum*.

Candidatus Arandabacteria (A.ran.da.bac.te'ri.a. N.L. neut. n. Arandabacterum, *Candidatus* generic name; -ia ending to denote the class; N.L. fem. pl. n. Arandabacteria, the Arandabacterum class).

The class is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Arandabacterum*.

Candidatus Arandabacterota (A.ran.da.bac.te.ro'ta. N.L. neut. n. Arandabacterum, *Candidatus* generic name; -ota ending to denote the phylum; N.L. fem. pl. n. Arandabacterota, the Arandabacterum phylum).

The phylum is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Arandabacterum*.

Candidatus Joyebacterota (Jo.y.e. N.L. neut. bacteria; N.L. neut. n. Joyebacterota a bacteria named after Dr. Samantha Joye). Type species: *Candidatus Joyebacterum haimaense*.

Candidatus Joyebacterum haimaense (ha.i.maen'se N.L. neut. adj. pertaining to Haima cold seep in South China Sea, China). This uncultured lineage is represented by the genome "SY70-5-12_Bin_1" consisting of 2.91 Mbps in 231 contigs with an estimated completeness of 98.9%, an estimated contamination of 4.9%. The MAG was recovered from South China Sea sediment from 22-24 cm at station SY70.

Candidatus Joyebacteraceae (Jo.y.e.bac.te.ra.ce'ae. N.L. neut. n. Joyebacterum, *Candidatus* generic name; -aceae ending to denote the family; N.L. fem. pl. n. Joyebacteraceae, the Joyebacterum family).

The family is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Joyebacterum*.

Candidatus Joyebacterales (Jo.y.e.bac.te.ra'les. N.L. neut. n. Joyebacterum, *Candidatus* generic name; -ales ending to denote the order; N.L. fem. pl. n. Joyebacterales, the Joyebacterum order).

The order is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Joyebacterum*.

Candidatus Joyebacteria (Jo.y.e.bac.te'ri.a. N.L. neut. n. Joyebacterum, *Candidatus* generic name; -ia ending to denote the class; N.L. fem. pl. n. Joyebacteria, the Joyebacterum class).

The class is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Joyebacterum*.

Candidatus Joyebacterota (Jo.y.e.bac.te.ro'ta. N.L. neut. n. Joyebacterum, *Candidatus* generic name; -ota ending to denote the phylum; N.L. fem. pl. n. Joyebacterota, the Joyebacterum phylum).

The phylum is described based on a 37 concatenated conserved marker gene phylogeny. Type genus is *Candidatus Joyebacterum*.

Supplementary References:

1. Zhou, Z., Tran, P. Q., Kieft, K. & Anantharaman, K. Genome diversification in globally distributed novel marine Proteobacteria is linked to environmental adaptation. *ISME J.* **14**, 2060–2077 (2020).
2. Armijos Jaramillo, V. D., Vargas, W. A., Sukno, S. A. & Thon, M. R. New insights into the evolution and structure of Colletotrichum plant-like subtilisins (CPLSs). *Commun. Integr. Biol.* **6**, e25727 (2013).
3. Iqbal, M. *et al.* Comparative evolutionary histories of fungal proteases reveal gene gains in the mycoparasitic and nematode-parasitic fungus *Clonostachys rosea*. *BMC Evol. Biol.* **18**, 171 (2018).
4. Winnick, T., Davis, A. R. & Greenberg, D. M. PHYSICOCHEMICAL PROPERTIES OF THE PROTEOLYTIC ENZYME FROM THE LATEX OF THE MILKWEED, ASCLEPIAS SPECIOSA TORR. SOME COMPARISONS WITH OTHER PROTEASES : I. CHEMICAL PROPERTIES, ACTIVATION-INHIBITION, pH-ACTIVITY, AND TEMPERATURE-ACTIVITY CURVES. *J. Gen. Physiol.* **23**, 275–288 (1940).
5. Latorre-Muro, P. *et al.* Dynamic Acetylation of Phosphoenolpyruvate Carboxykinase Toggles Enzyme Activity between Gluconeogenic and Anaplerotic Reactions. *Mol. Cell* **71**, 718–732.e9 (2018).
6. Pieulle, L. *et al.* Isolation and characterization of the pyruvate-ferredoxin oxidoreductase from the sulfate-reducing bacterium *Desulfovibrio africanus*. *Biochim. Biophys. Acta* **1250**, 49–59 (1995).
7. Zhang, Q., Iwasaki, T., Wakagi, T. & Oshima, T. 2-oxoacid:ferredoxin oxidoreductase from the thermoacidophilic archaeon, *Sulfolobus* sp. strain 7. *J. Biochem.* **120**, 587–599 (1996).
8. Fukuda, E. & Wakagi, T. Substrate recognition by 2-oxoacid:ferredoxin oxidoreductase from *Sulfolobus* sp. strain 7. *Biochim. Biophys. Acta* **1597**, 74–80 (2002).
9. Adam, P. S., Borrel, G. & Gribaldo, S. Evolutionary history of carbon monoxide dehydrogenase/acetyl-CoA synthase, one of the oldest enzymatic complexes. *Proc. Natl. Acad. Sci. U. S. A.* **115**, E1166–E1173 (2018).
10. Whitham, J. M., Tirado-Acevedo, O., Chinn, M. S., Pawlak, J. J. & Grunden, A. M. Metabolic response of *Clostridium ljungdahlii* to oxygen exposure. *Appl. Environ. Microbiol.* **81**, 8379–8391 (2015).

11. Geelhoed, J. S., Henstra, A. M. & Stams, A. J. M. Carboxydrotrophic growth of *Geobacter sulfurreducens*. *Appl. Microbiol. Biotechnol.* **100**, 997–1007 (2016).
12. Claassens, N. J. Reductive Glycine Pathway: A Versatile Route for One-Carbon Biotech. *Trends Biotechnol.* **39**, 327–329 (2021).
13. Schauder, R., Widdel, F. & Fuchs, G. Carbon assimilation pathways in sulfate-reducing bacteria II. Enzymes of a reductive citric acid cycle in the autotrophic *Desulfobacter hydrogenophilus*. *Arch. Microbiol.* **148**, 218–225 (1987).
14. Hughes, N. J., Clayton, C. L., Chalk, P. A. & Kelly, D. J. *Helicobacter pylori* porCDAB and oorDABC genes encode distinct pyruvate:flavodoxin and 2-oxoglutarate:acceptor oxidoreductases which mediate electron transport to NADP. *J. Bacteriol.* **180**, 1119–1128 (1998).
15. Fujita, Y., Matsuoka, H. & Hirooka, K. Regulation of fatty acid metabolism in bacteria. *Mol. Microbiol.* **66**, 829–839 (2007).
16. Duarte, A. G., Santos, A. A. & Pereira, I. A. C. Electron transfer between the QmoABC membrane complex and adenosine 5'-phosphosulfate reductase. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* **1857**, 380–386 (2016).
17. Niu, Z.-S. *et al.* Human activities can drive sulfate-reducing bacteria community in Chinese intertidal sediments by affecting metal distribution. *Sci. Total Environ.* **786**, 147490 (2021).
18. Anantharaman, K. *et al.* Expanded diversity of microbial groups that shape the dissimilatory sulfur cycle. *ISME J.* **12**, 1715–1728 (2018).
19. Berendt, U., Haverkamp, T., Prior, A. & Schwenn, J. D. Reaction mechanism of thioredoxin: 3'-phospho-adenylylsulfate reductase investigated by site-directed mutagenesis. *Eur. J. Biochem.* **233**, 347–356 (1995).
20. Gisselmann, G., Klausmeier, P. & Schwenn, J. D. The ferredoxin:sulphite reductase gene from *Synechococcus* PCC7942. *Biochim. Biophys. Acta* **1144**, 102–106 (1993).

21. Papenbrock, J. & Schmidt, A. Characterization of a sulfurtransferase from *Arabidopsis thaliana*. *Eur. J. Biochem.* **267**, 145–154 (2000).
22. Ma, K., Schicho, R. N., Kelly, R. M. & Adams, M. W. Hydrogenase of the hyperthermophile *Pyrococcus furiosus* is an elemental sulfur reductase or sulfhydrogenase: evidence for a sulfur-reducing hydrogenase ancestor. *Proc. Natl. Acad. Sci. U. S. A.* **90**, 5341–5344 (1993).
23. Sousa, F. M., Pereira, J. G., Marreiros, B. C. & Pereira, M. M. Taxonomic distribution, structure/function relationship and metabolic context of the two families of sulfide dehydrogenases: SQR and FCSD. *Biochim. Biophys. Acta Bioenerg.* **1859**, 742–753 (2018).
24. Gennaris, A. *et al.* Repairing oxidized proteins in the bacterial envelope using respiratory chain electrons. *Nature* **528**, 409–412 (2015).
25. Lucas, J. J., Burchiel, S. W. & Segel, I. H. Choline sulfatase of *Pseudomonas aeruginosa*. *Arch. Biochem. Biophys.* **153**, 664–672 (1972).
26. Osterås, M., Boncompagni, E., Vincent, N., Poggi, M. C. & Le Rudulier, D. Presence of a gene encoding choline sulfatase in *Sinorhizobium meliloti* bet operon: choline-O-sulfate is metabolized into glycine betaine. *Proc. Natl. Acad. Sci. U. S. A.* **95**, 11394–11399 (1998).
27. Mougous, J. D., Green, R. E., Williams, S. J., Brenner, S. E. & Bertozzi, C. R. Sulfotransferases and sulfatases in mycobacteria. *Chem. Biol.* **9**, 767–776 (2002).
28. Sirko, A., Zatyka, M., Sadowy, E. & Hulanicka, D. Sulfate and thiosulfate transport in *Escherichia coli* K-12: evidence for a functional overlapping of sulfate- and thiosulfate-binding proteins. *J. Bacteriol.* **177**, 4134–4136 (1995).
29. Kessler, A. J. *et al.* Bacterial fermentation and respiration processes are uncoupled in anoxic permeable sediments. *Nat Microbiol* **4**, 1014–1023 (2019).
30. De Anda, V. *et al.* Brockarchaeota, a novel archaeal phylum with unique and versatile carbon cycling pathways. *Nat. Commun.* **12**, 2404 (2021).

31. Cramm, R. Genomic view of energy metabolism in *Ralstonia eutropha* H16. *J. Mol. Microbiol. Biotechnol.* **16**, 38–52 (2009).
32. Peters, J. W. *et al.* [FeFe]- and [NiFe]-hydrogenase diversity, mechanism, and maturation. *Biochim. Biophys. Acta* **1853**, 1350–1369 (2015).
33. Stojanowic, A., Mander, G. J., Duin, E. C. & Hedderich, R. Physiological role of the F420-non-reducing hydrogenase (Mvh) from *Methanothermobacter marburgensis*. *Arch. Microbiol.* **180**, 194–203 (2003).
34. van Haaster, D. J., Silva, P. J., Hagedoorn, P.-L., Jongejan, J. A. & Hagen, W. R. Reinvestigation of the steady-state kinetics and physiological function of the soluble NiFe-hydrogenase I of *Pyrococcus furiosus*. *J. Bacteriol.* **190**, 1584–1587 (2008).
35. Ma, K., Weiss, R. & Adams, M. W. Characterization of hydrogenase II from the hyperthermophilic archaeon *Pyrococcus furiosus* and assessment of its role in sulfur reduction. *J. Bacteriol.* **182**, 1864–1871 (2000).
36. McTernan, P. M. *et al.* Intact functional fourteen-subunit respiratory membrane-bound [NiFe]-hydrogenase complex of the hyperthermophilic archaeon *Pyrococcus furiosus*. *J. Biol. Chem.* **289**, 19364–19372 (2014).
37. Greening, C. *et al.* Genomic and metagenomic surveys of hydrogenase distribution indicate H₂ is a widely utilised energy source for microbial growth and survival. *ISME J.* **10**, 761–777 (2016).
38. Schoelmerich, M. C. & Müller, V. Energy-converting hydrogenases: the link between H₂ metabolism and energy conservation. *Cell. Mol. Life Sci.* **77**, 1461–1481 (2020).
39. Vignais, P. M. & Colbeau, A. Molecular biology of microbial hydrogenases. *Curr. Issues Mol. Biol.* **6**, 159–188 (2004).
40. McDowall, J. S. *et al.* Bacterial formate hydrogenlyase complex. *Proc. Natl. Acad. Sci. U. S. A.* **111**, E3948–56 (2014).

41. Meuer, J., Kuettner, H. C., Zhang, J. K., Hedderich, R. & Metcalf, W. W. Genetic analysis of the archaeon *Methanosarcina barkeri* Fusaro reveals a central role for Ech hydrogenase and ferredoxin in methanogenesis and carbon fixation. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 5632–5637 (2002).
42. Hedderich, R. & Forzi, L. Energy-converting [NiFe] hydrogenases: more than just H₂ activation. *J. Mol. Microbiol. Biotechnol.* **10**, 92–104 (2005).
43. Lamborg, C. H., Von Damm, K. L., Fitzgerald, W. F., Hammerschmidt, C. R. & Zierenberg, R. Mercury and monomethylmercury in fluids from Sea Cliff submarine hydrothermal field, Gorda Ridge. *Geophys. Res. Lett.* **33**, (2006).
44. Crepo-Medina, M. *et al.* Adaptation of chemosynthetic microorganisms to elevated mercury concentrations in deep-sea hydrothermal vents. *Limnol. Oceanogr.* **54**, 41–49 (2009).
45. Zhu, C. *et al.* Seawater versus mantle sources of mercury in sulfide-rich seafloor hydrothermal systems, Southwest Indian Ridge. *Geochim. Cosmochim. Acta* **281**, 91–101 (2020).
46. Fox, B. S. & Walsh, C. T. Mercuric reductase: homology to glutathione reductase and lipoamide dehydrogenase. Iodoacetamide alkylation and sequence of the active site peptide. *Biochemistry* **22**, 4082–4088 (1983).
47. Schue, M., Glendinning, K. J., Hobman, J. L. & Brown, N. L. Evidence for direct interactions between the mercuric ion transporter (MerT) and mercuric reductase (MerA) from the Tn501 mer operon. *Biometals* **21**, 107–116 (2008).
48. Meseck, S. & Cutter, G. Selenium Behavior in San Francisco Bay Sediments. *Estuaries Coasts* **35**, 646–657 (2012).
49. Böck, A. *et al.* Selenocysteine: the 21st amino acid. *Mol. Microbiol.* **5**, 515–520 (1991).
50. Bébien, M., Kirsch, J., Méjean, V. & Verméglio, A. Involvement of a putative molybdenum enzyme in the reduction of selenate by *Escherichia coli*. *Microbiology* **148**, 3865–3872 (2002).
51. Leinfelder, W., Forchhammer, K., Veprek, B., Zehelein, E. & Böck, A. In vitro synthesis of

- selenocysteinyI-tRNA(UCA) from seryl-tRNA(UCA): involvement and characterization of the selD gene product. *Proc. Natl. Acad. Sci. U. S. A.* **87**, 543–547 (1990).
52. Forchhammer, K., Leinfelder, W., Boesmiller, K., Veprek, B. & Böck, A. Selenocysteine synthase from *Escherichia coli*. Nucleotide sequence of the gene (selA) and purification of the protein. *J. Biol. Chem.* **266**, 6318–6323 (1991).
 53. Mihara, H., Kurihara, T., Yoshimura, T., Soda, K. & Esaki, N. Cysteine sulfinatase, a NIFS-like protein of *Escherichia coli* with selenocysteine lyase and cysteine desulfurase activities. Gene cloning, purification, and characterization of a novel pyridoxal enzyme. *J. Biol. Chem.* **272**, 22417–22424 (1997).
 54. Dardel, F., Fayat, G. & Blanquet, S. Molecular cloning and primary structure of the *Escherichia coli* methionyl-tRNA synthetase gene. *J. Bacteriol.* **160**, 1115–1122 (1984).
 55. Neff, J. M. Ecotoxicology of arsenic in the marine environment. *Environ. Toxicol. Chem.* **16**, 917–927 (1997).
 56. Mukhopadhyay, R., Rosen, B. P., Phung, L. T. & Silver, S. Microbial arsenic: from geocycles to genes and enzymes. *FEMS Microbiol. Rev.* **26**, 311–325 (2002).
 57. Messens, J., Hayburn, G., Desmyter, A., Laus, G. & Wyns, L. The essential catalytic redox couple in arsenate reductase from *Staphylococcus aureus*. *Biochemistry* **38**, 16857–16865 (1999).
 58. Ben Fekih, I. *et al.* Distribution of Arsenic Resistance Genes in Prokaryotes. *Front. Microbiol.* **9**, 2473 (2018).
 59. Macy, J. M., Santini, J. M., Pauling, B. V., O'Neill, A. H. & Sly, L. I. Two new arsenate/sulfate-reducing bacteria: mechanisms of arsenate reduction. *Arch. Microbiol.* **173**, 49–57 (2000).
 60. Hoelt, S. E., Kulp, T. R., Stolz, J. F., Hollibaugh, J. T. & Oremland, R. S. Dissimilatory arsenate reduction with sulfide as electron donor: experiments with mono lake water and Isolation of strain MLMS-1, a chemoautotrophic arsenate respirer. *Appl. Environ. Microbiol.* **70**, 2741–2747 (2004).

61. Martinussen, J., Sørensen, C., Jendresen, C. B. & Kilstrup, M. Two nucleoside transporters in *Lactococcus lactis* with different substrate specificities. *Microbiology* **156**, 3148–3157 (2010).
62. Malinverni, J. C. & Silhavy, T. J. An ABC transport system that maintains lipid asymmetry in the gram-negative outer membrane. *Proc. Natl. Acad. Sci. U. S. A.* **106**, 8009–8014 (2009).