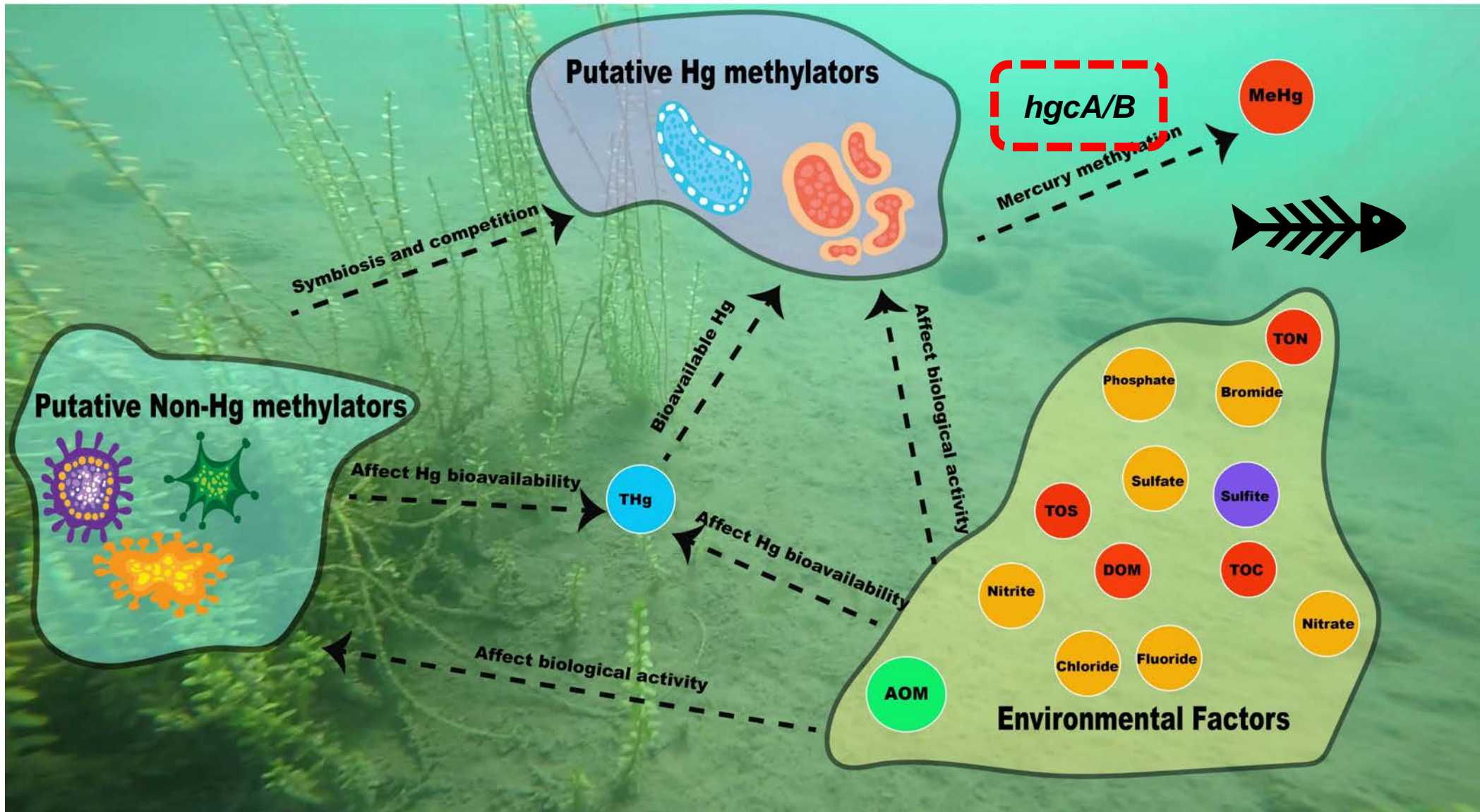


Global survey of *hgcA*-carrying genomes in marine and freshwater sediments: Insights into mercury methylation processes

Yong-Li Wang^a, Kaoru Ikuma^b, Amanda M.V. Brown^c, Amrika Deonarine^{a, *}

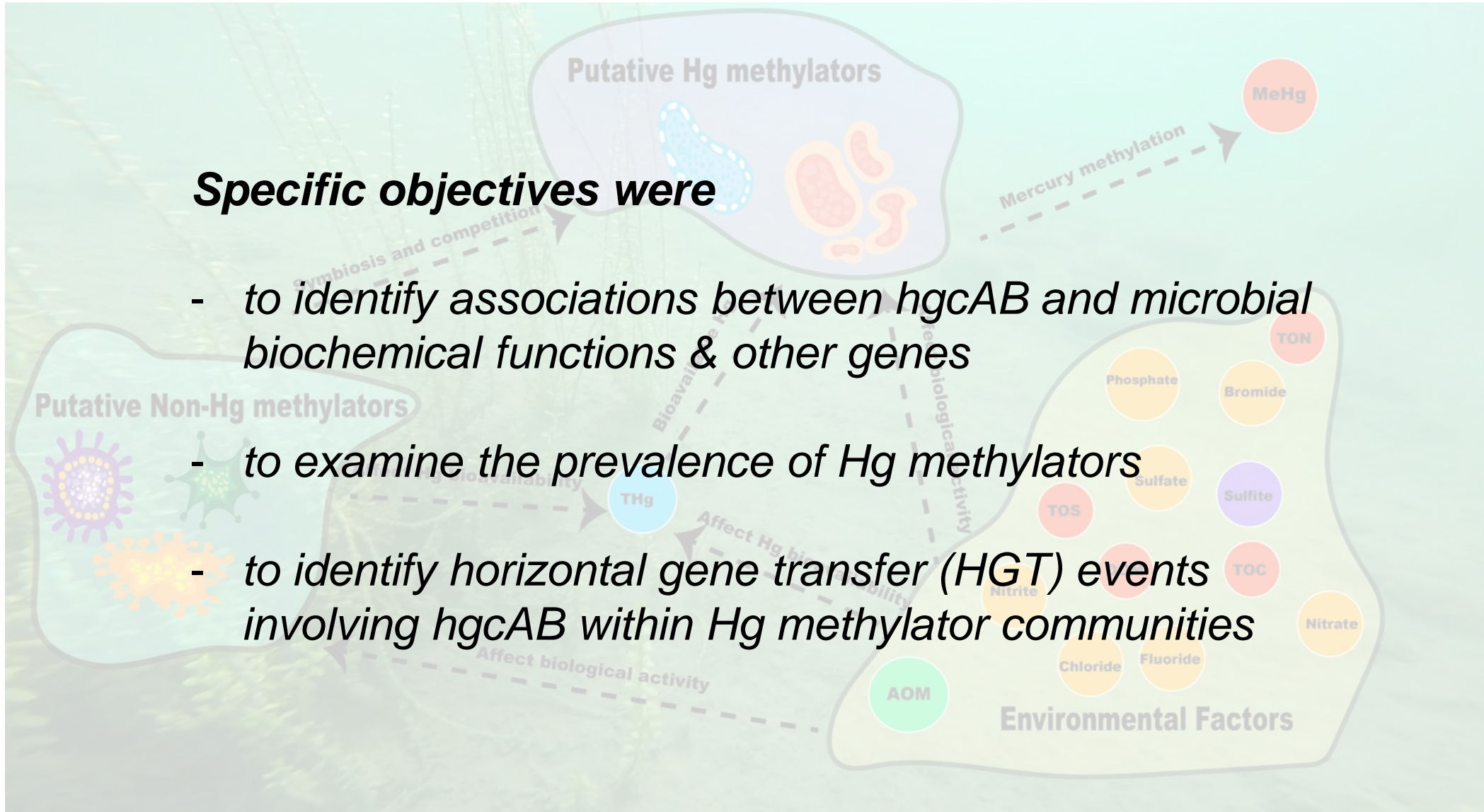


Wang, Y. L., Ikuma, K., Brown, A. M., & Deonarine, A. (2024). Global survey of *hgcA*-carrying genomes in marine and freshwater sediments: Insights into mercury methylation processes. *Environmental Pollution*, 124117.



Specific objectives were

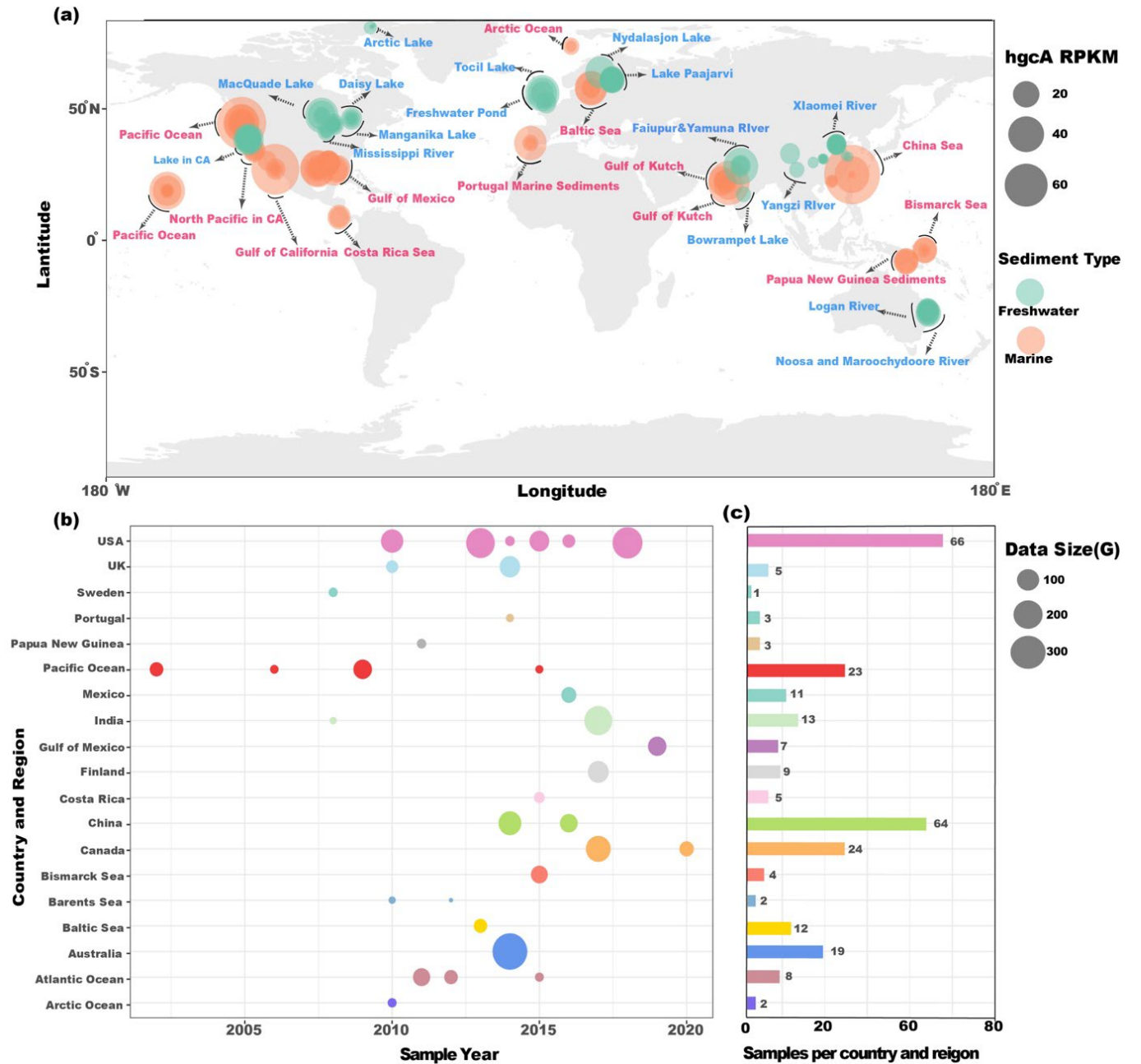
- *to identify associations between hgcAB and microbial biochemical functions & other genes*
- *to examine the prevalence of Hg methylators*
- *to identify horizontal gene transfer (HGT) events involving hgcAB within Hg methylator communities*



Methods

Database queries

A total of 281 sediments (freshwater (129) and seawater (152)) samples from 46 bio-projects were queried using NCBI databases through accession numbers using the Prefetch in the SRA Toolkit (<https://hpc.nih.gov/apps/sratoolkit.html>).



Metagenomic data processing

Samples from the same studies and areas were co-assembled with MEGAHIT v1.2.9 with the default settings and only contigs of length 2000 were kept for subsequent analysis.

Binning was performed with metaWRAP (v1.2) which using metabat, metabat (v2) and maxbin (v2).

All metagenomic assembled genomes (MAGs) produced were refined with MetaWRAP (v1.2) and MAGs with integrity greater than 70% and contamination less than 10% were combined and picked out.

To further improved the quality of MAGs, metagenomic reads were remapped to each MAG then reassembled by SPAdes (3.13.0)

Identification of *hgcA/B*

hgcA and *hgcB* identification first used gene-specific HMMs (hidden Markov models) to identify and extract sequences from all metagenomic bins with an E-value threshold of 10^{-5} .

Then sequences from Hg-MATE-Db were also used to search for *hgcA* and *hgcB* encoding genes in MAGs by BLASTp with an E-value threshold of 10^{-5} .

BLAST results were further confirmed by examining the existence of conserved motifs [N(V/I) WCA(A/G) (A/G) (K/G)] in *hgcA* and [CX₂CX₂CX₃C] in *hgcB*, respectively.

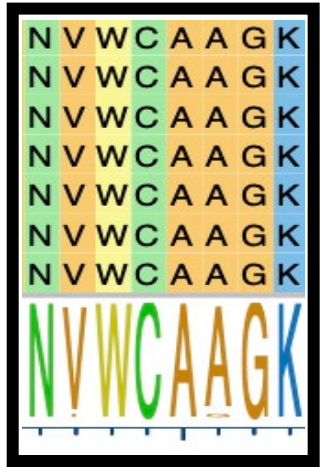
Elemental cycling genes profiles of metagenomic samples

The HUMAnN 3.0 pipeline (<https://huttenhower.sph.harvard.edu/humann/>) was used to obtain the carbon, nitrogen, and sulfur functional profiles for each metagenomic sample based on reads. Kyoto Encyclopedia of Genes and Genomes Orthology (KEGG) pathways of MAGs of interest were predicted with the BlastKOALA (<https://www.kegg.jp/blastkoala>).

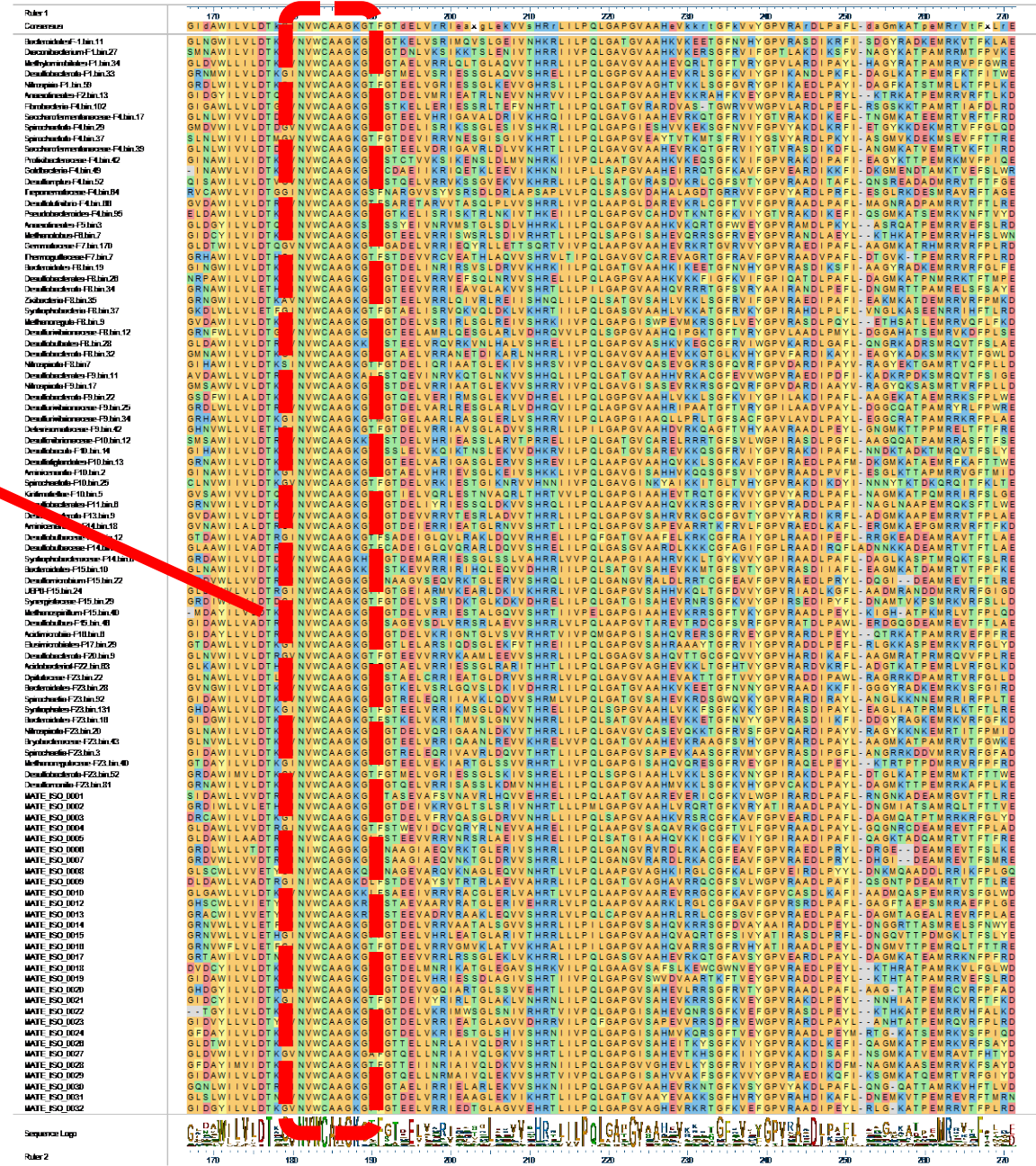
Multiple sequence alignment and phylogenetic analysis

The 143 *hgcA* sequences and 135 *hgcB* sequences, which confirmed Hg methylating capability (32 *hgcA* and *hgcB* reference sequences), were aligned by Mafft (v7.475) [45] and visualized by the DNASTar software. Maximum likelihood trees were constructed based on the alignment with 1000 ultrafast bootstrap replicates by using iqtree2 (v2.1.2, tree-building parameters: -m LG+F+R9 -B 1000 -alrt 1000) and visualized in Figtree (v1.4.4) [46].

Conserved motifs of *hgcA* from *hgcA*-carrying MAGs



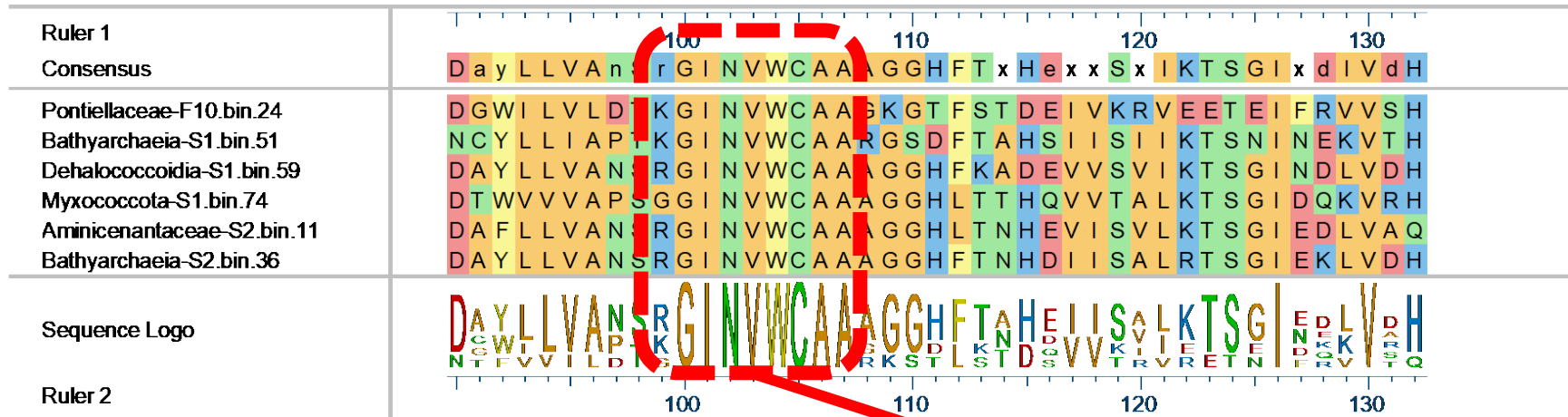
Conserved motifs of *hgcA*



A total 143 *hgcA* sequences and 135 *hgcB* sequences from were identified in this study.

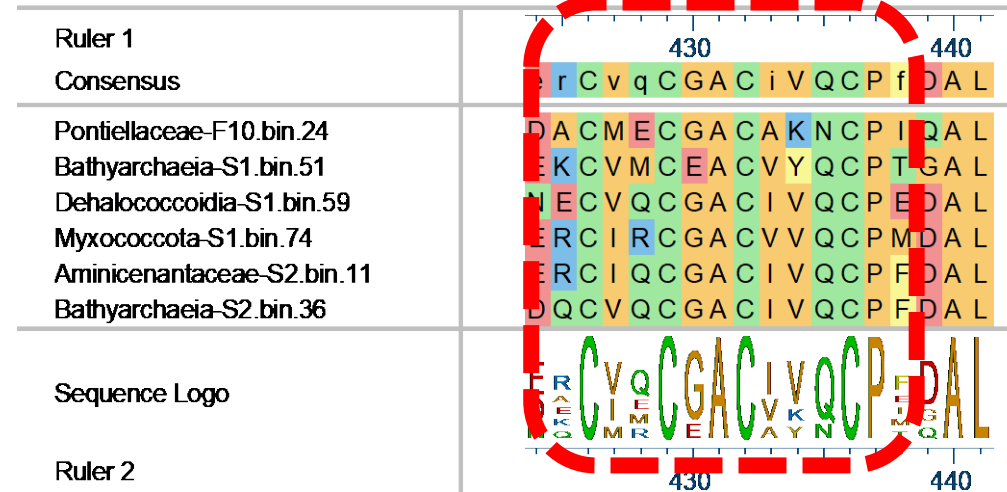
8 MAGs carrying only *hgcA* without *hgcB*, from five phyla *Desulfobacterota*, *Bacteroidetes*, *Bacteroidetes*, *Planctomycetota*, and *KSB1*

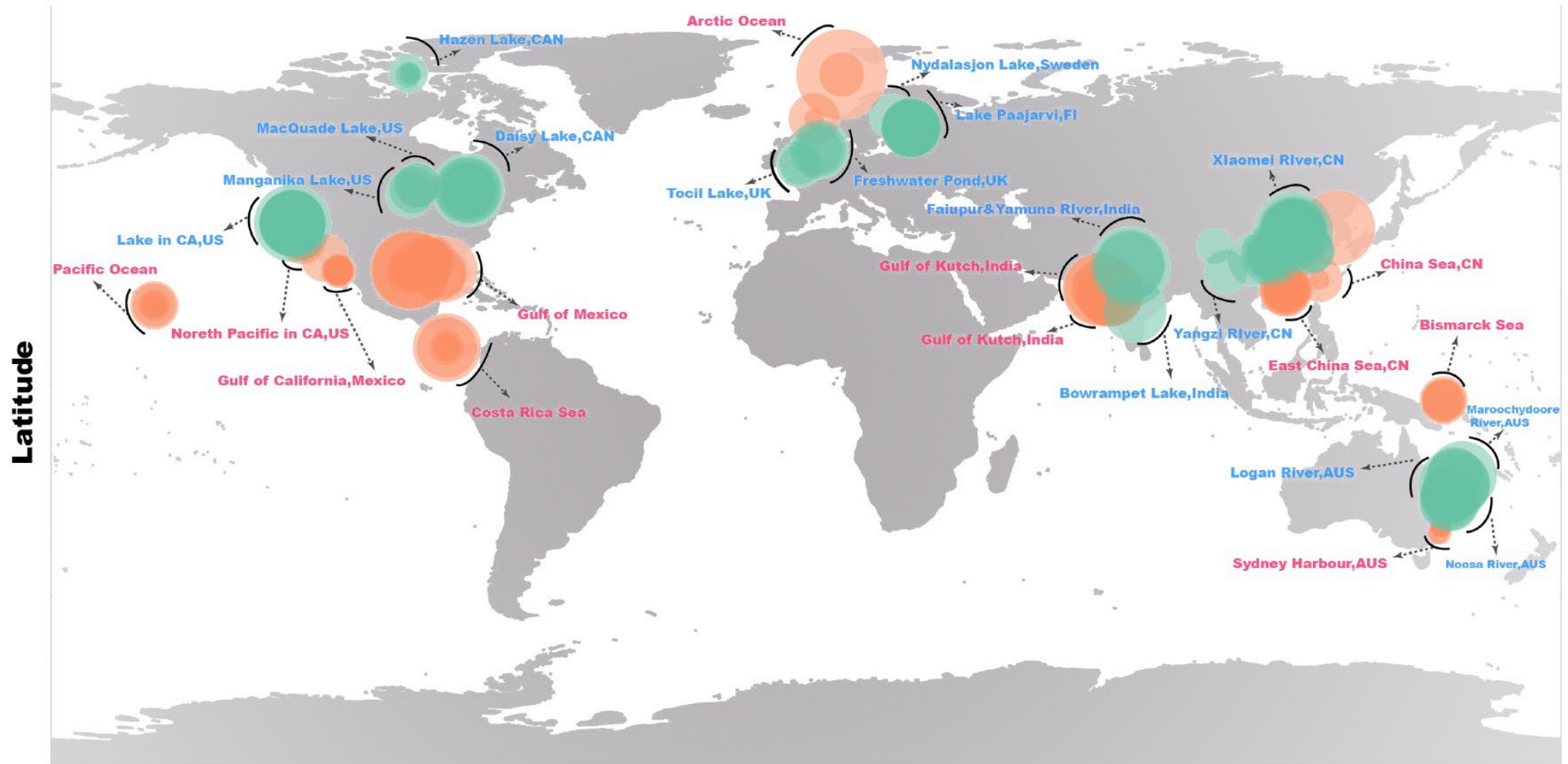
Conserved motifs of fused *hgcA/B*



Conserved motifs

These six MAGs encoded for tandem **[GINWCAA]** conservative motifs, which were different from the motifs of *hgcA* **[NVWCAAAGK]**. However, both *hgcB* and fused *hgcAB* had conservative motifs **[CX2CX2CX3C]**.





Type

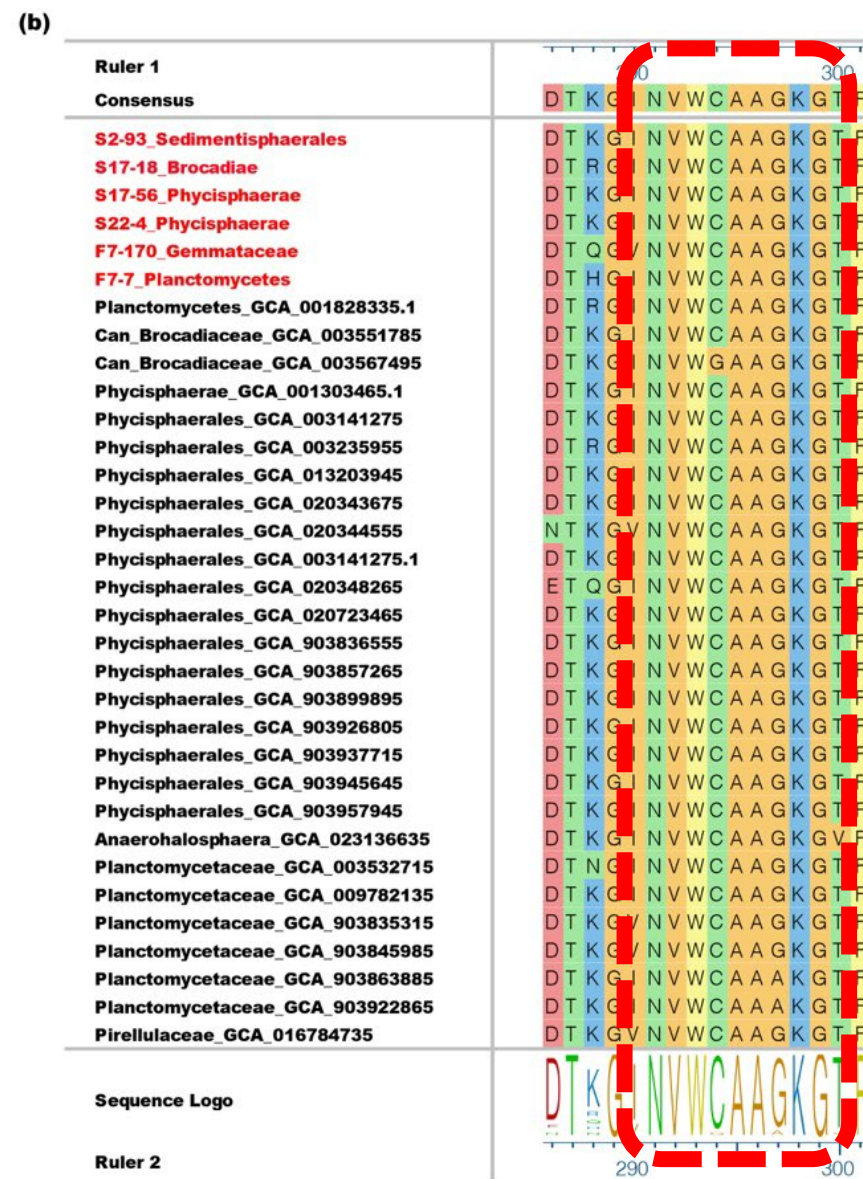
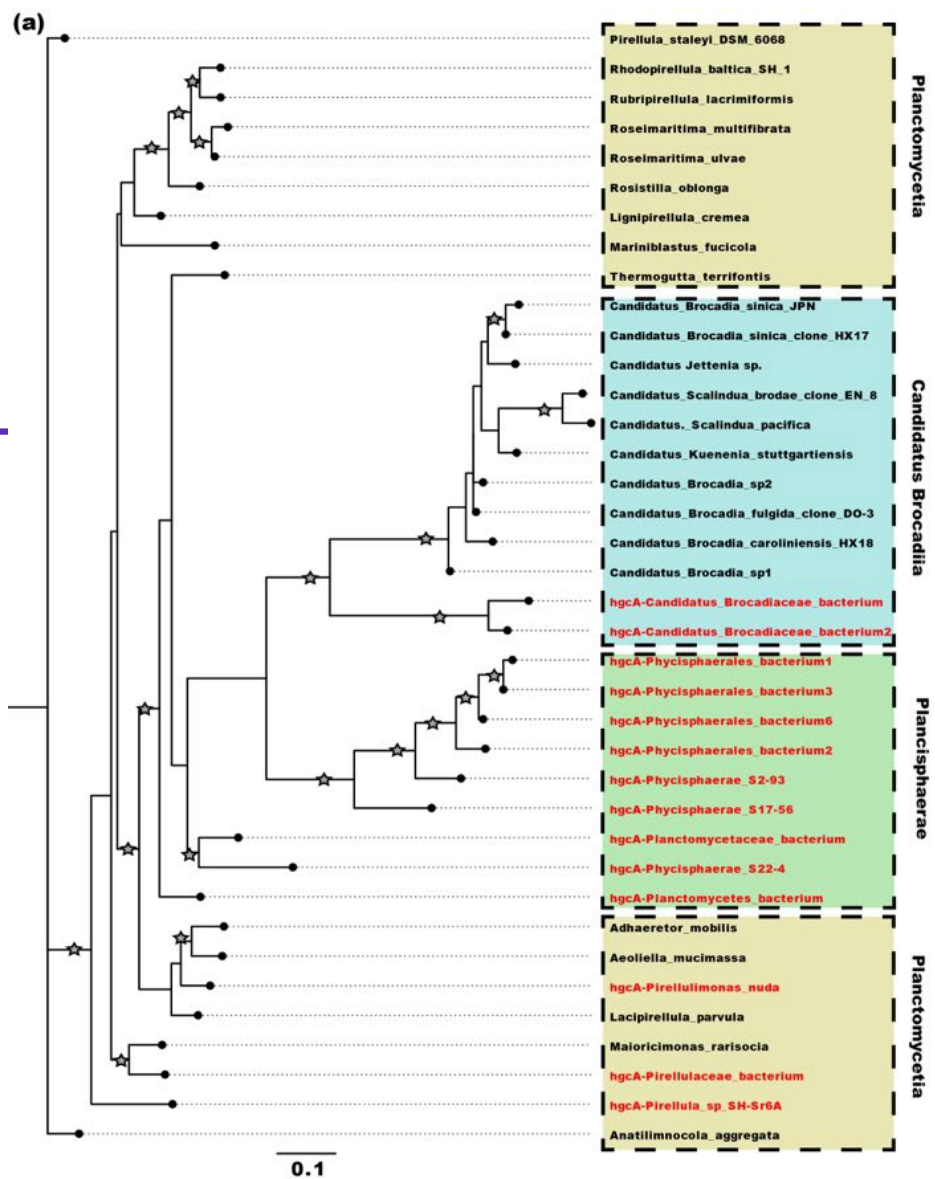
- Freshwater sediment
- Seawater sediment

hgCA

- 0.0000
- 0.0025
- 0.0050
- 0.0075

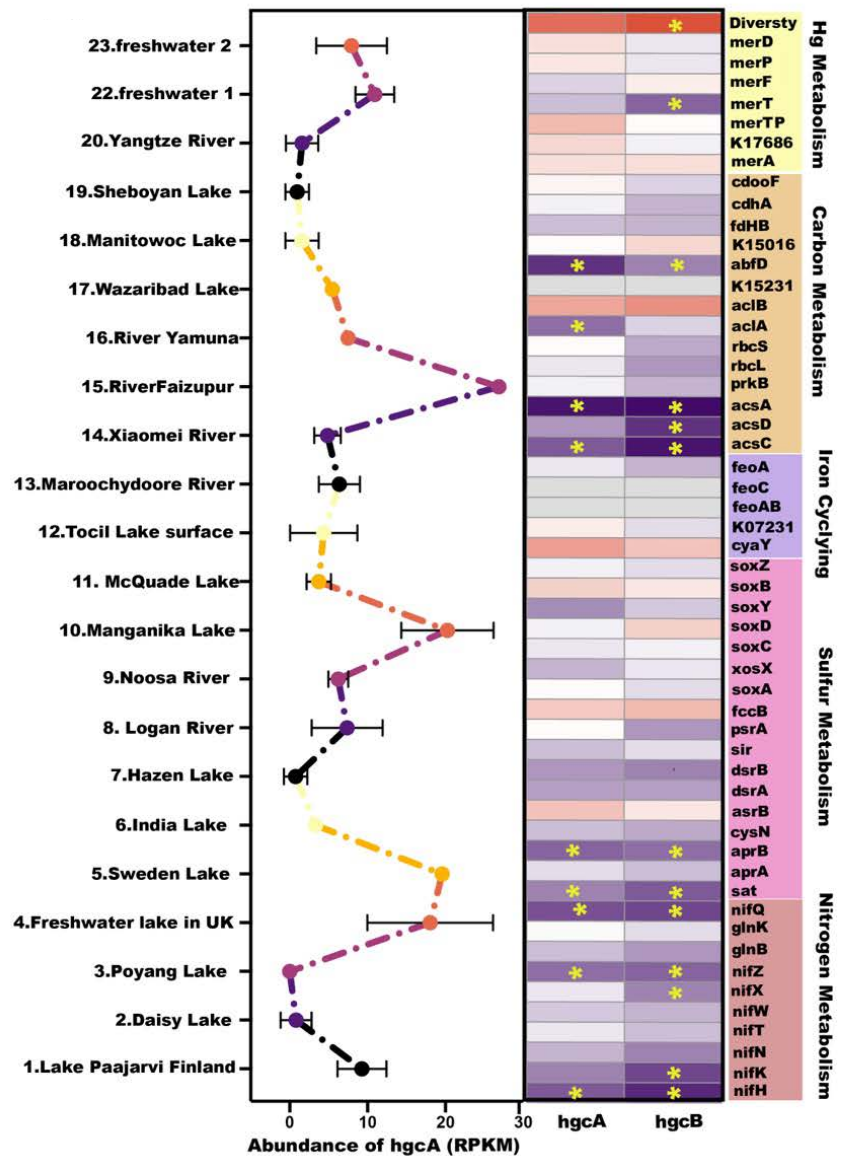
Hg methylators from Planctomycetota are widespread in both seawater and freshwater sediment

Over 3000 whole genomes from the phylum Planctomycetota in NCBI were queried, and **27 *hgcA*-carrying MAGs** from the Planctomycetota were identified

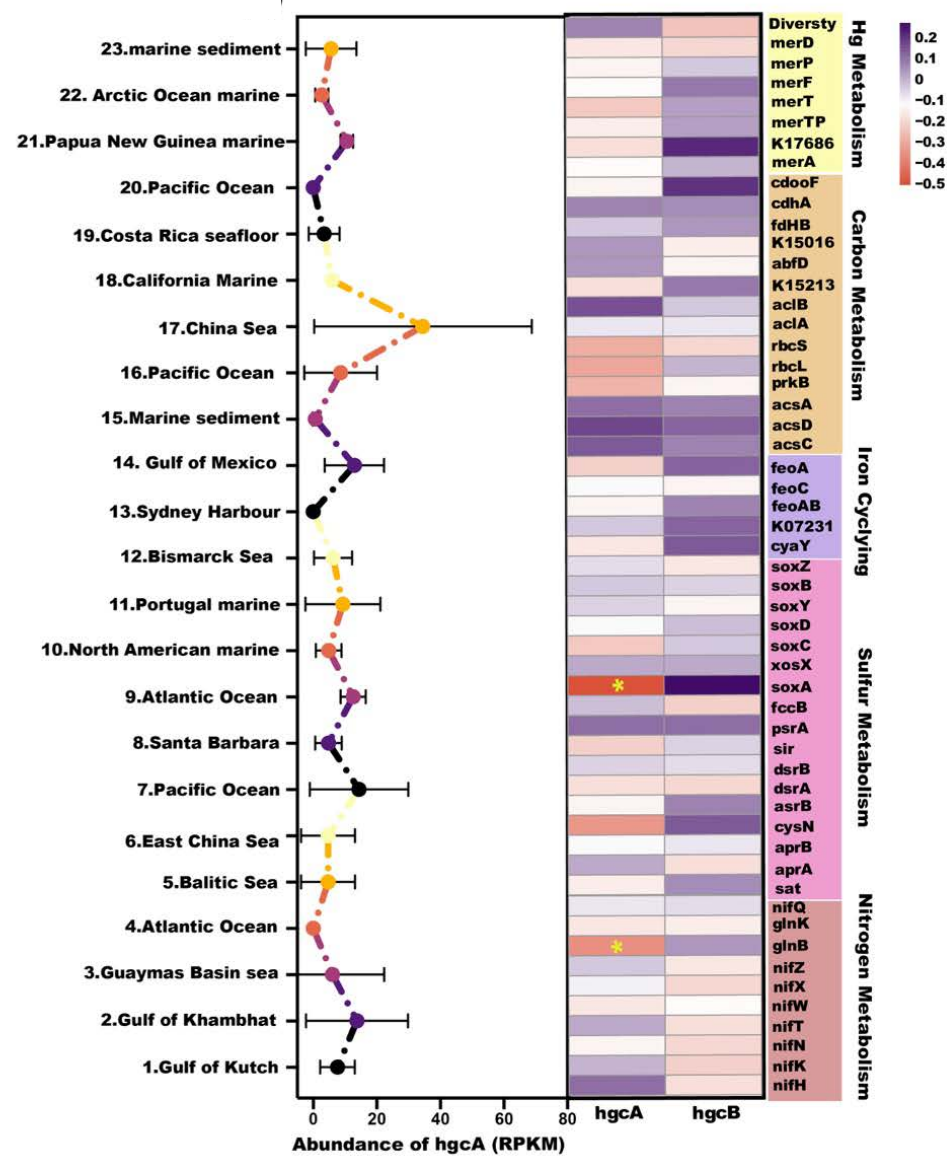


Spearman's correlation analysis between *hgcA*, *hgcB* and other elemental cycling genes

Freshwater



Seawater

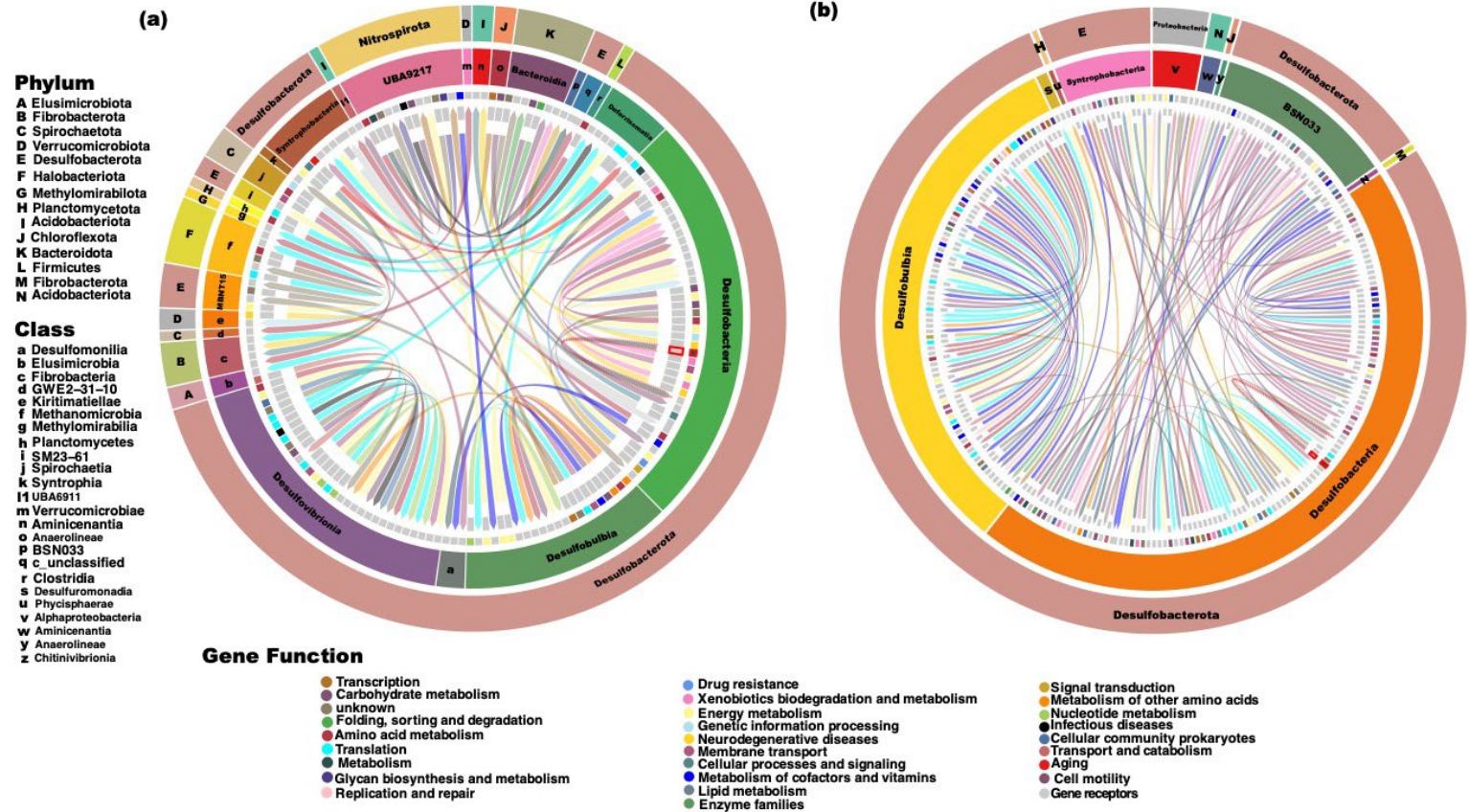


reads per kilobase

Prediction of Horizontal Gene Transfer Between *hgcA*-Carrying MAGs

SRB was a major contributor to HGTs in the Hg methylators community accounting for **97.5%** and **71%** of HGTs in seawater and freshwater sediments

Gene gain and loss mediated through HGTs were previously suggested to underpin divergent phylogenetic distribution of *hgcA/B*

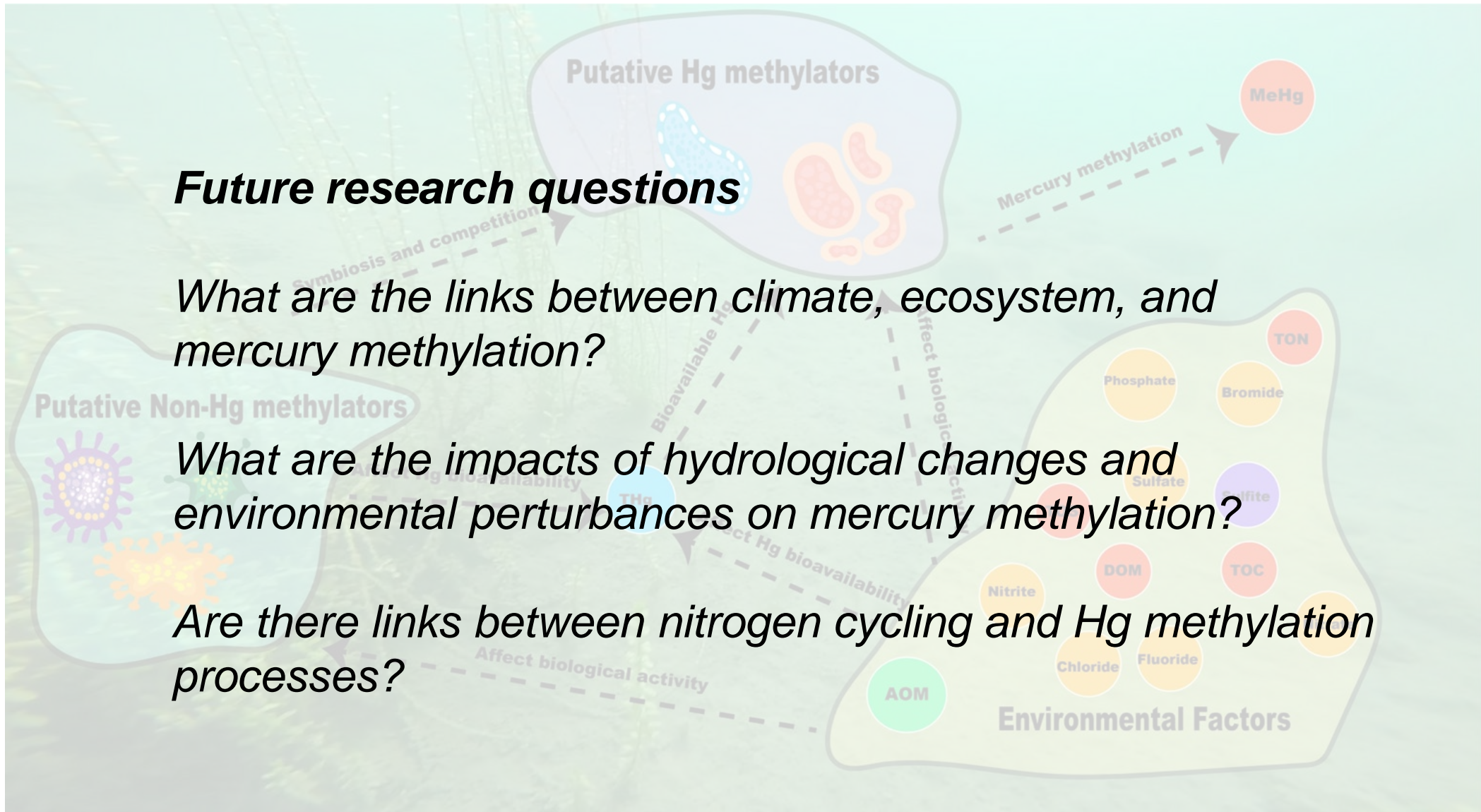


Future research questions

What are the links between climate, ecosystem, and mercury methylation?

What are the impacts of hydrological changes and environmental perturbances on mercury methylation?

Are there links between nitrogen cycling and Hg methylation processes?

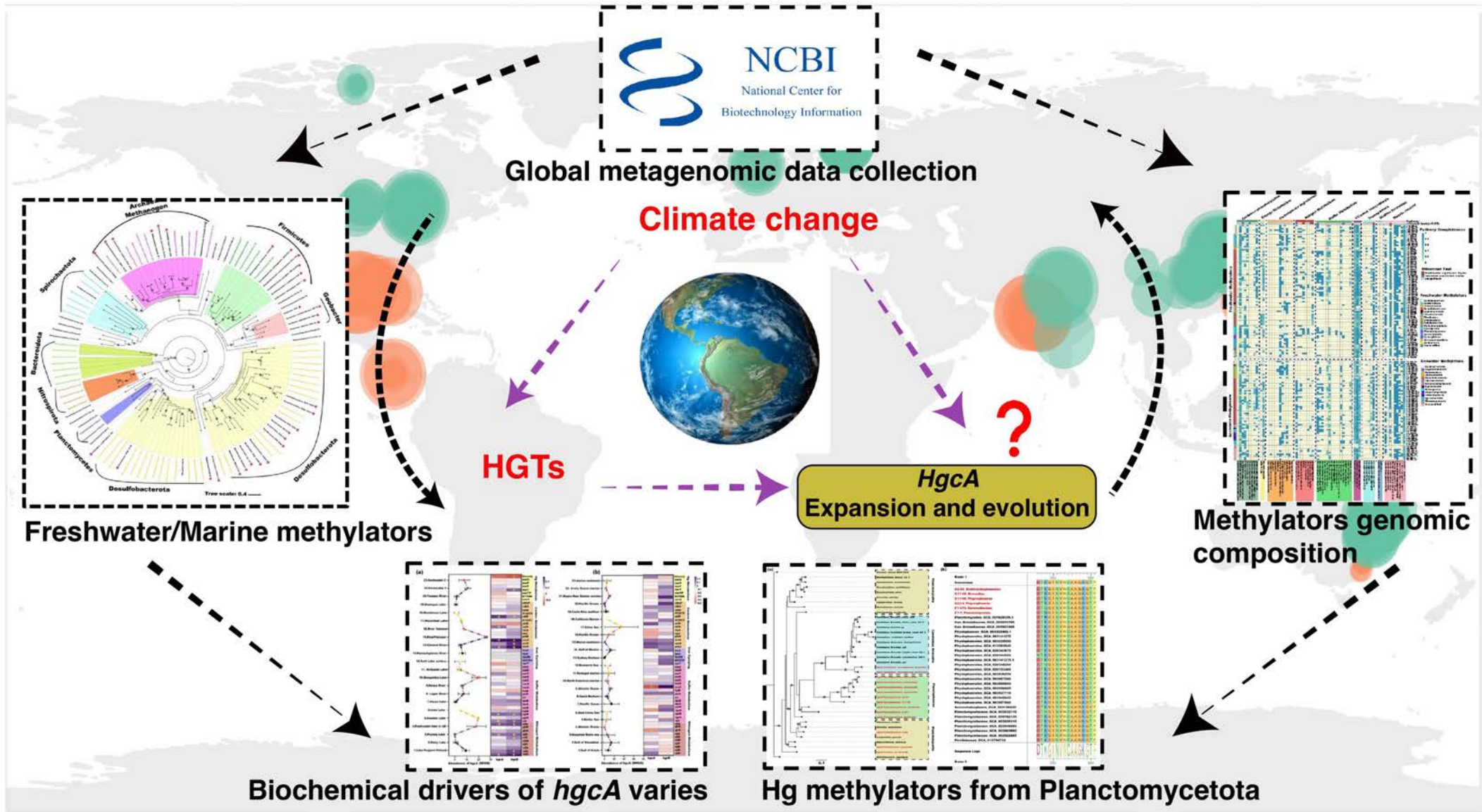


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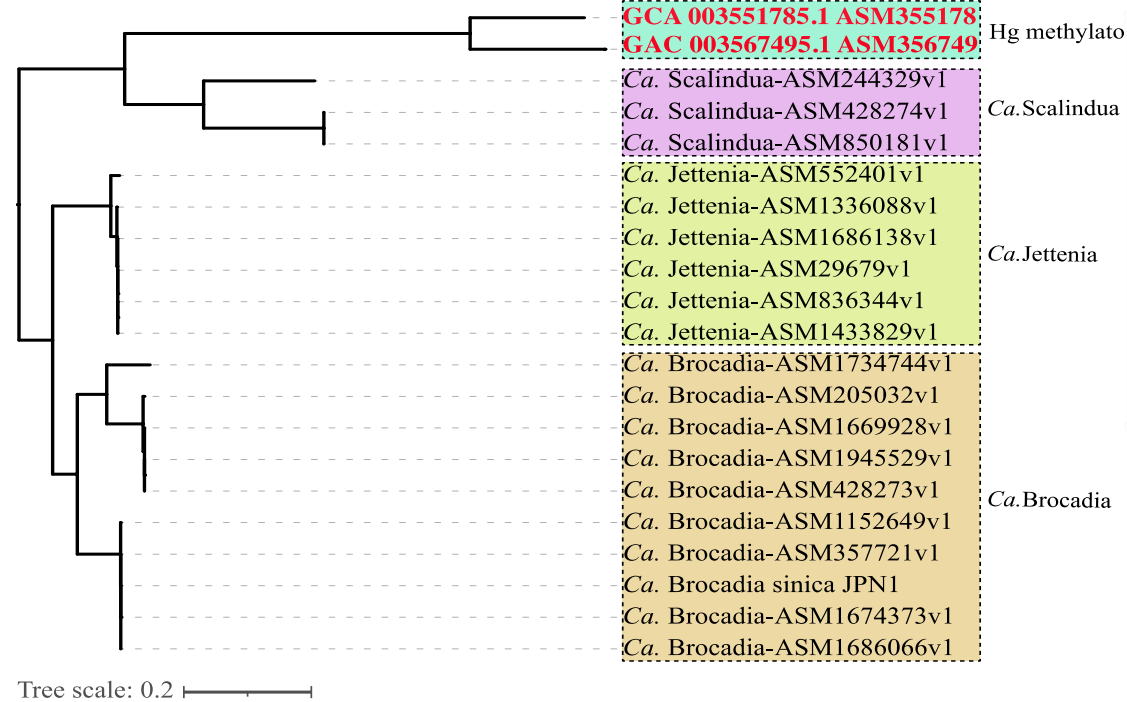




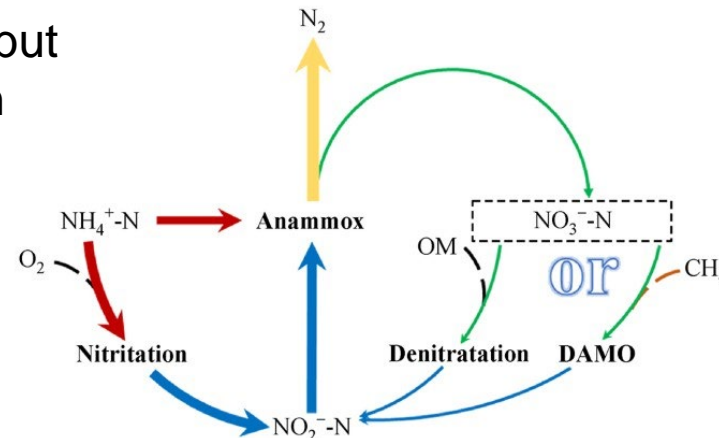
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Potential anammox (anaerobic ammonium oxidation) *hgcA*-carrying strains

The whole-genome phylogenetic tree of Planctomycetota *hgcA*-carrying MAGs and reference anammox genomes, two anammox MAGs ***Candidatus Brocadiaceae bacterium*** (GCA_003551785.1 and GCA_003567495.1) which were closed to ***Candidatus Scalindua***

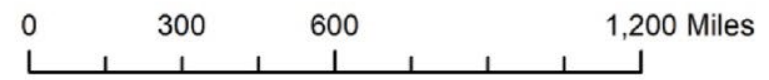
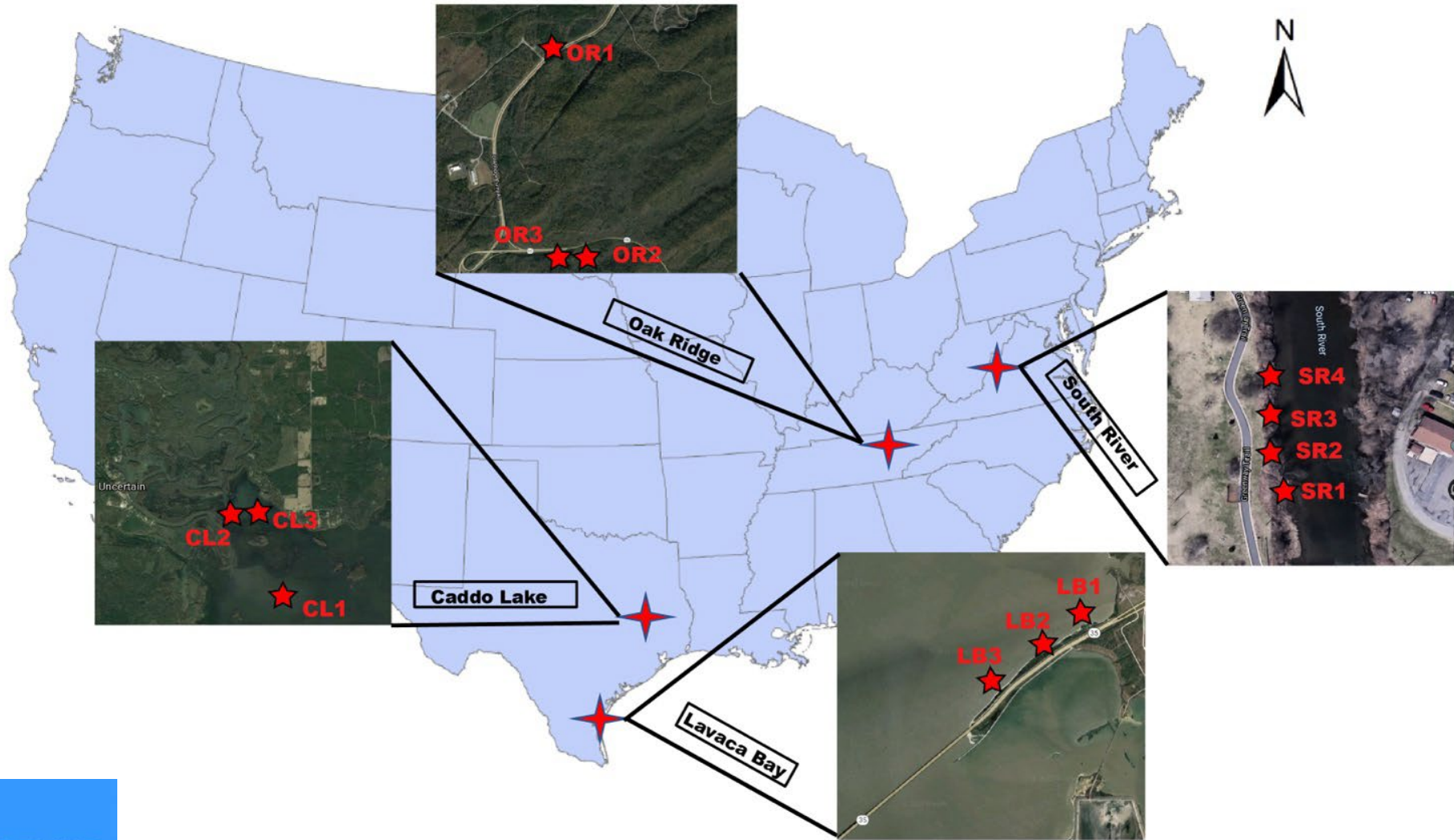


The *hgcA*-carrying anammox strains may be capable of Hg methylation, but this needs to be further confirmed in the laboratory.

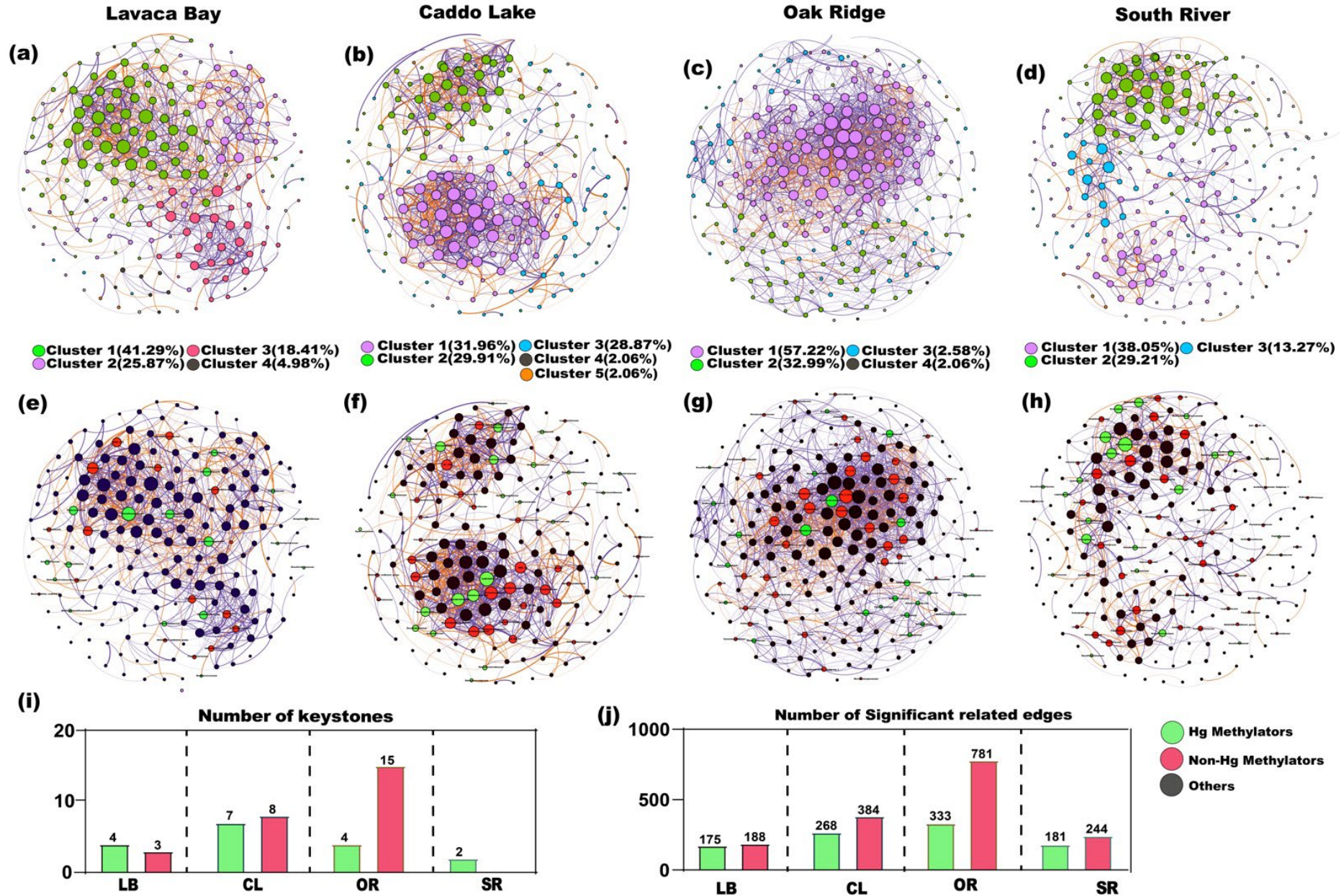


Anammox technology in low temperature wastewater at WWTP Holland

Sediment Collection



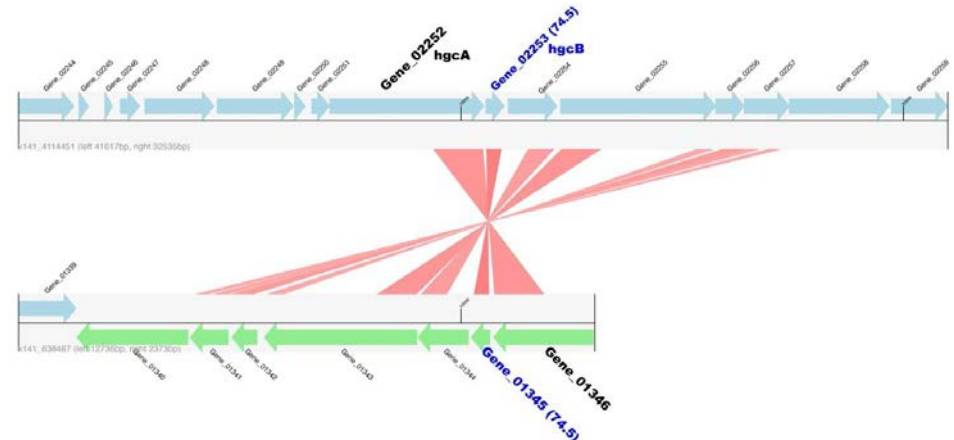
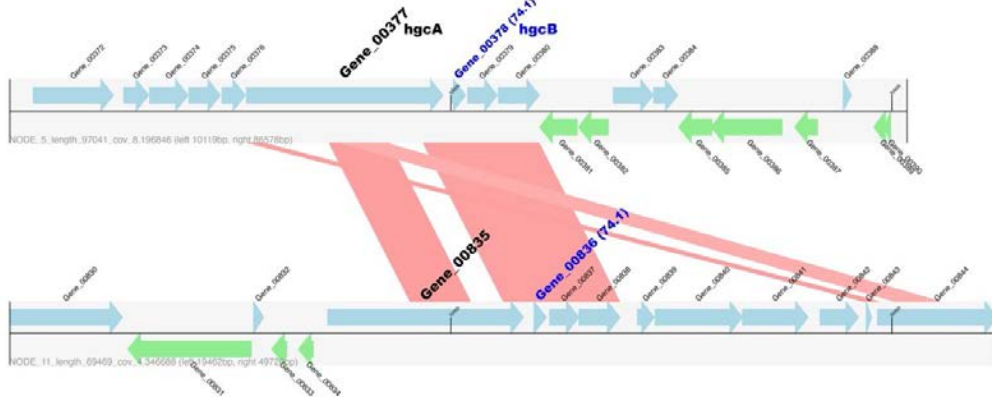
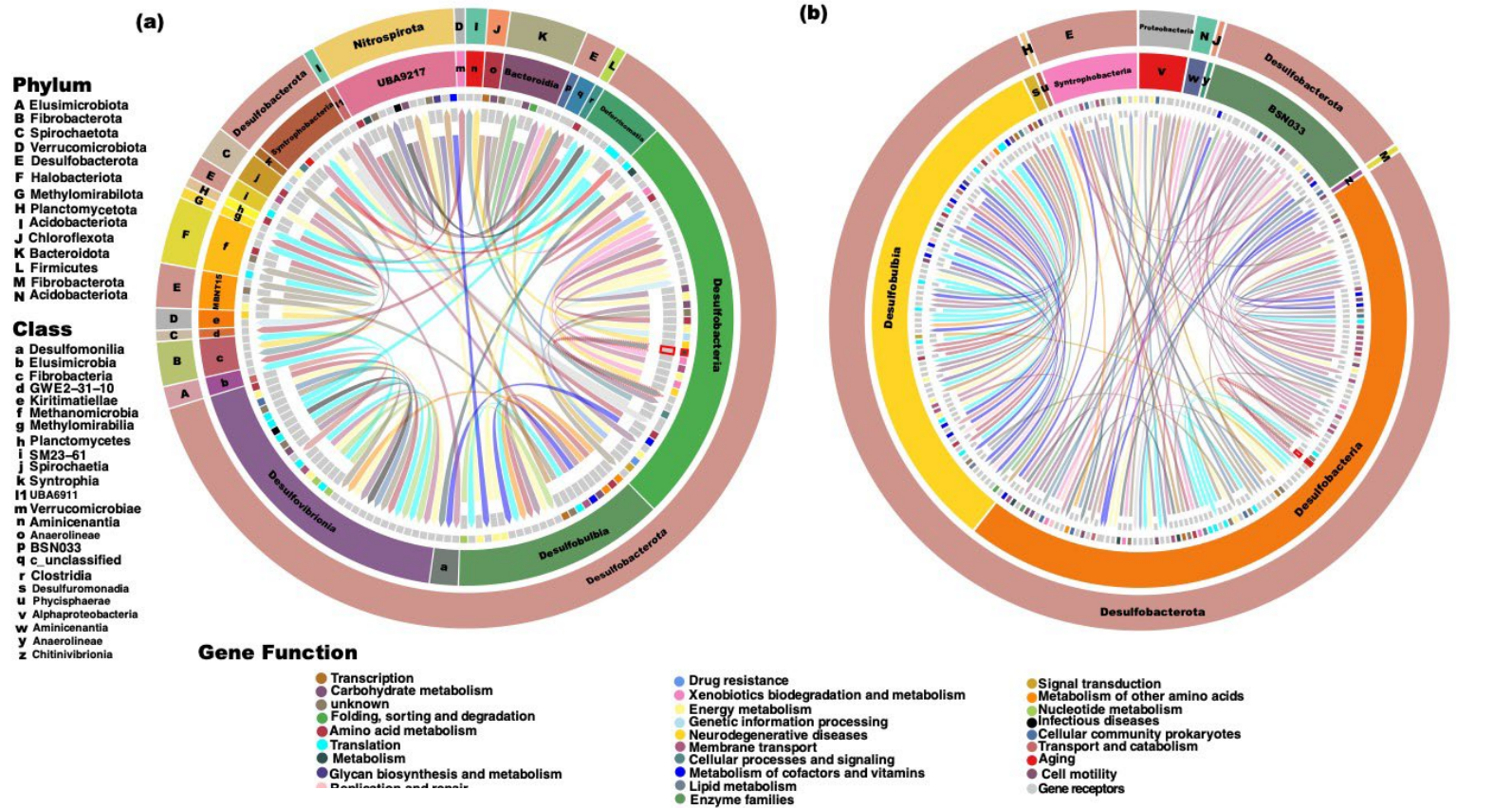
Co-occurrence network analysis between putative methylators and putative non-mercury methylators



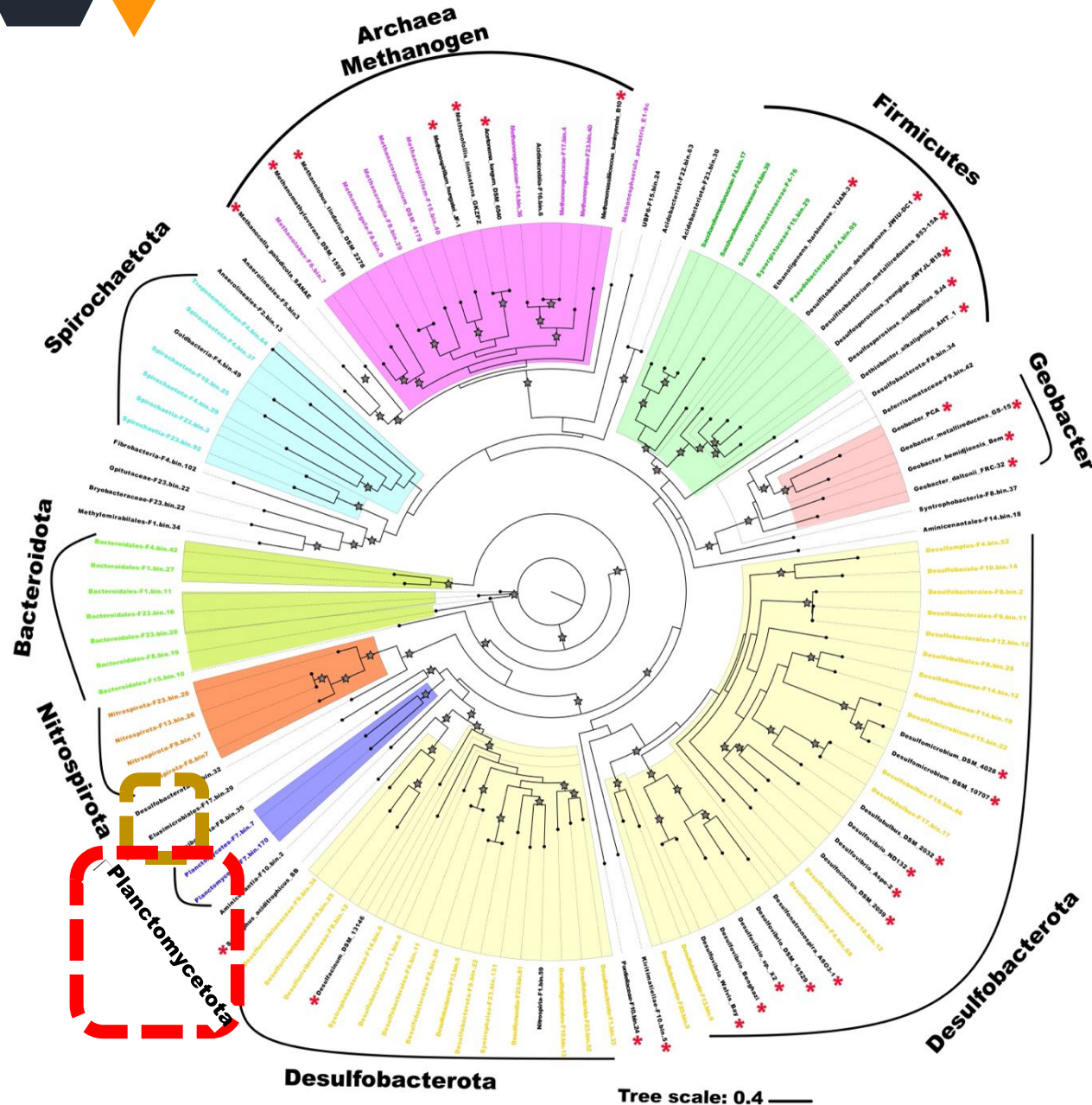
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Gene gain and loss mediated through HGTs were previously suggested to underpin divergent phylogenetic distribution of *hgcA/B*



hgcA-carrying MAGs from freshwater sediments



82 MAGs from the 17 Phyla were identified as having genes homologous with *hgcA* with the average completeness **85.59%** and contamination **3.23%**.

The Hg methylators from these 9 Phyla were only found in freshwater sediments:
Methylomirabilota, **Firmicutes**, Goldbacteria, **Halobacteriota**, Zixibacteria, Verrucomicrobiota, Synergistota, Actinobacteriota, Elusimicrobiota

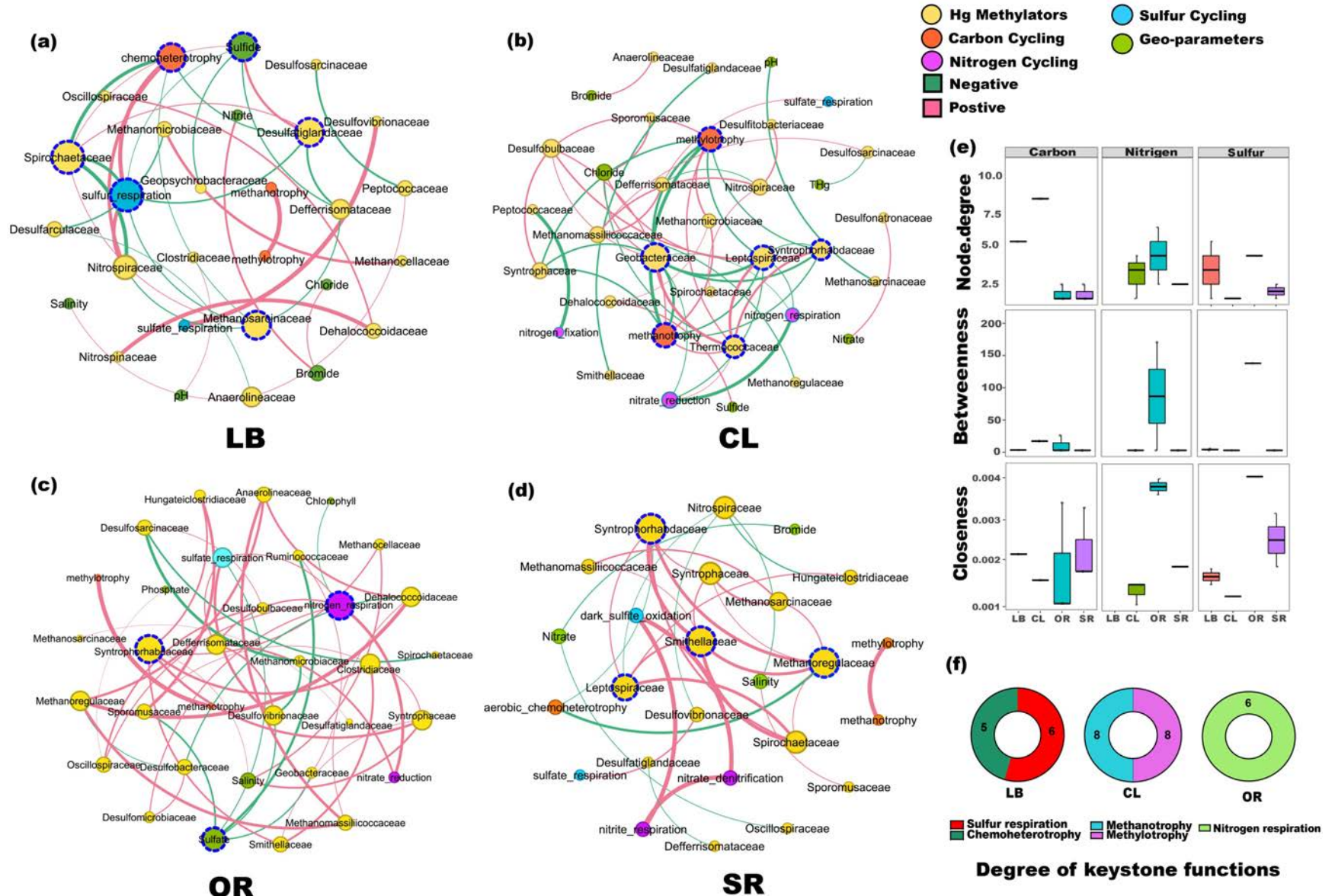
The Hg methylators from the Phyla **Desulfobacterota** and **Bacteroidota** were dominant and widespread in both freshwater (**47.62%**) and seawater (**54.1%**) sediments.

Co-occurrence network analysis between putative methylators and functions from non-mercury methylators

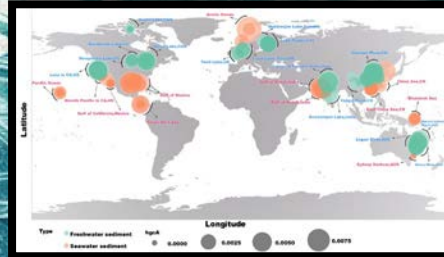
Our analysis of functional networks indicated that microbial keystones varied across sediment samples.

In LB, keystone functions included sulfur respiration and chemoheterotrophy

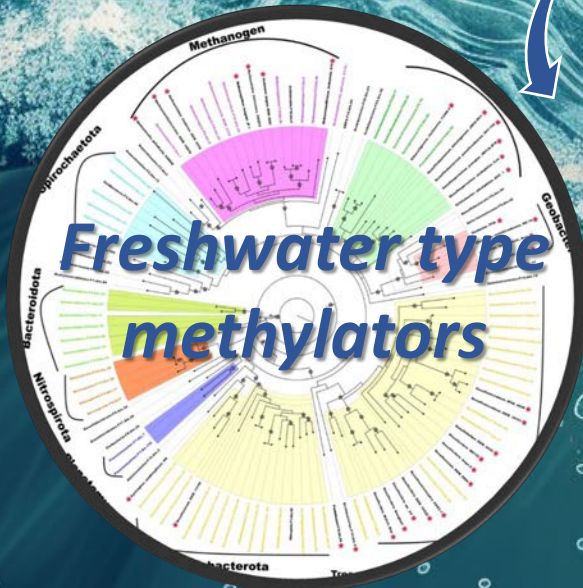
In CL, the only keystone functions observed were carbon cycling functions, specifically methanotrophy



Conclusions

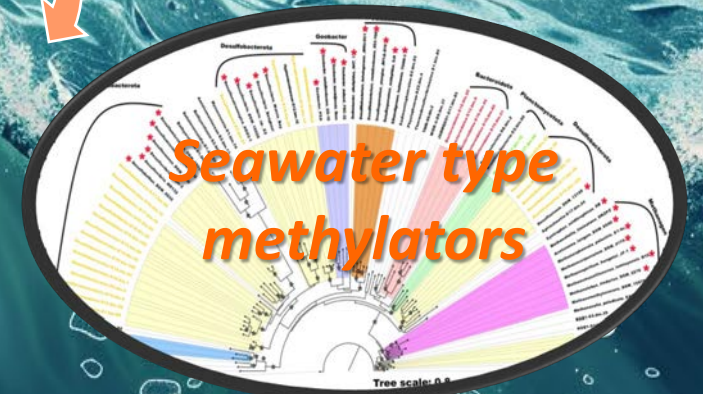


Desulfobacterota and Bacteroidota



Freshwater type methylators

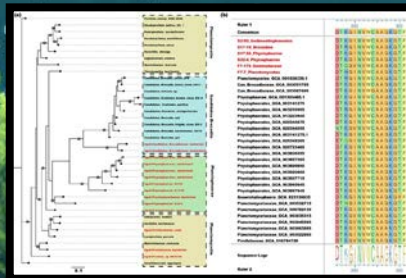
Hg methylators were more diverse
82 MAGs from the 17 Phyla
47.62%



Seawater type methylators

61 MAGs from the 12 Phyla
54.1%

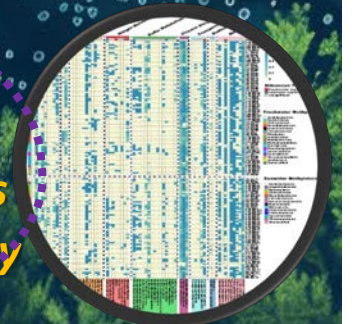
Horizontal gene transfer



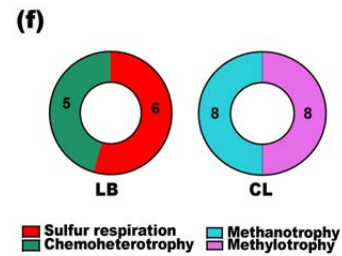
Hg methylators from Planctomycetota
Potential Anammox methylators

Genome composition and adaptation strategies

- Multiple terminal oxidases*
- Carbon cycling pathway*
- B-type vitamins biosynthesis*
- Biofilm biosynthesis pathway*
- Element cycle*



Co-occurrence network analysis between putative methylators and functions from non-mercury methylators



Co-occurrence network analysis between putative methylators and functions from non-mercury methylators

