Ecological Characterization of Marine Chloroflexi through Metagenomic Big Data

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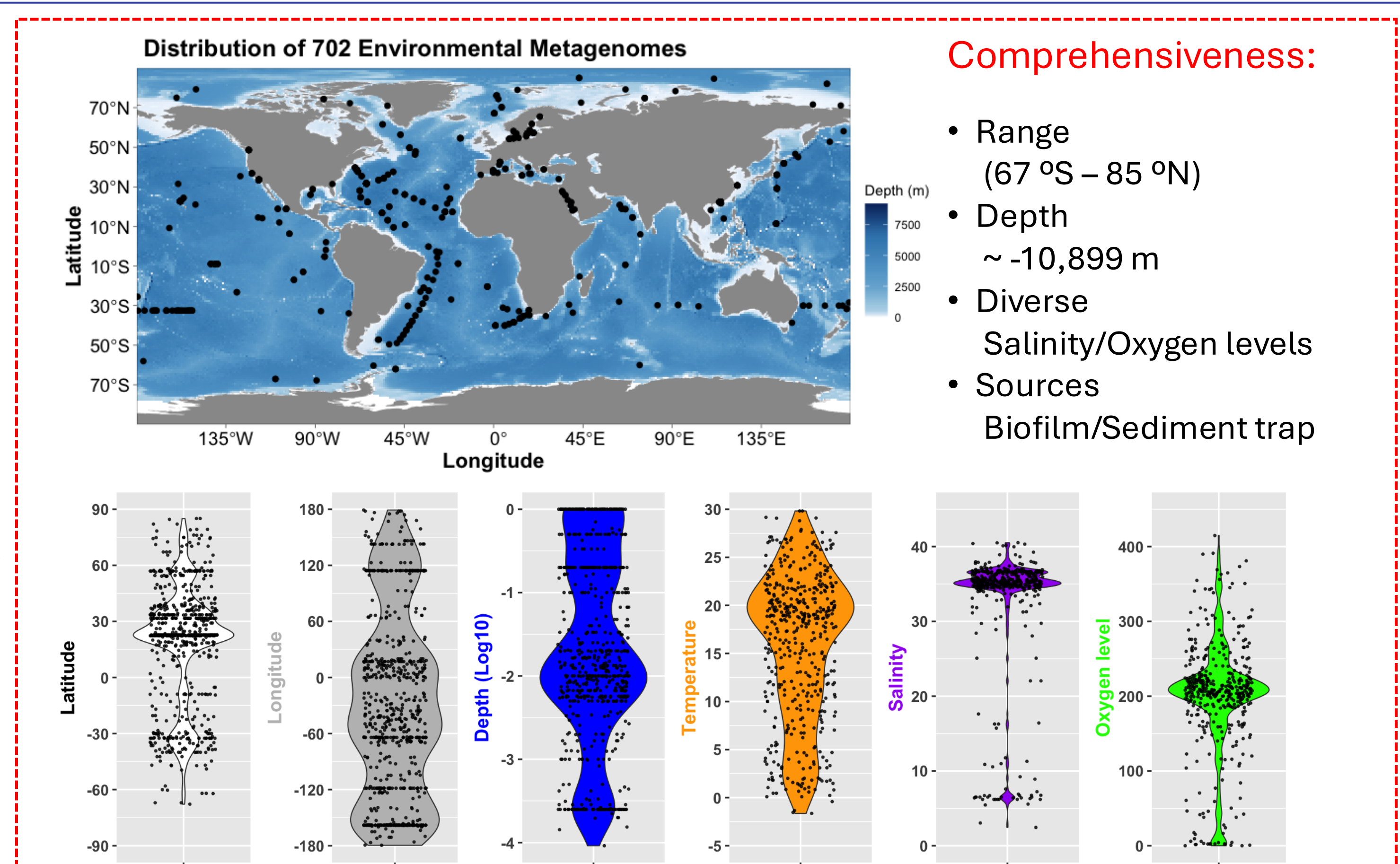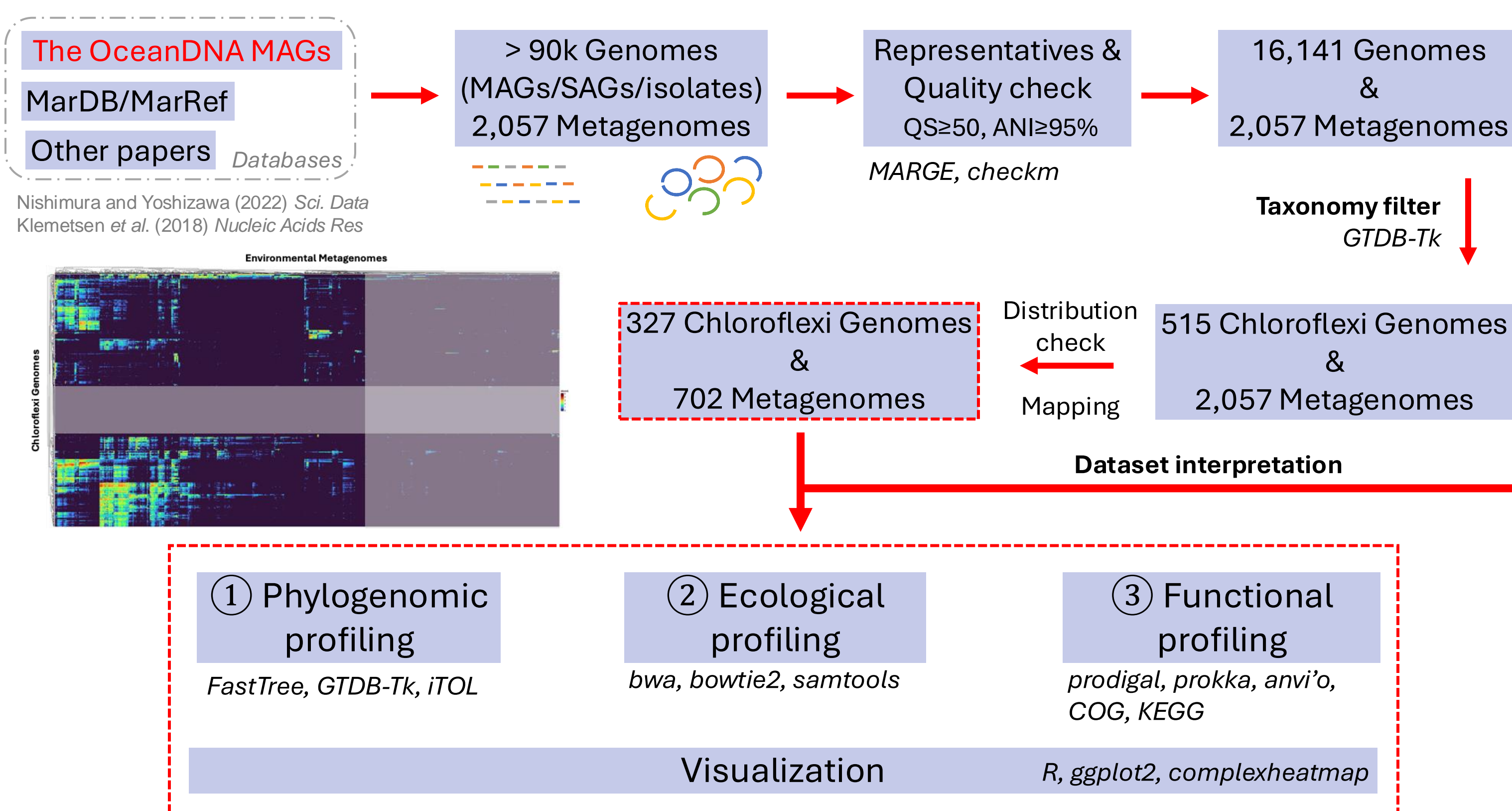
Posters

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Introduction

Bacteria of the phylum *Chloroflexota* (also known as Chloroflexi), which have great phylogenetic and functional diversity, are widely and abundantly distributed in global marine environments, notably in subseafloor ecosystems. However, our comprehension of these Chloroflexi and their ecophysiological adaptations has thus far remained limited due to the scarcity of sufficient data and pure culture isolates.

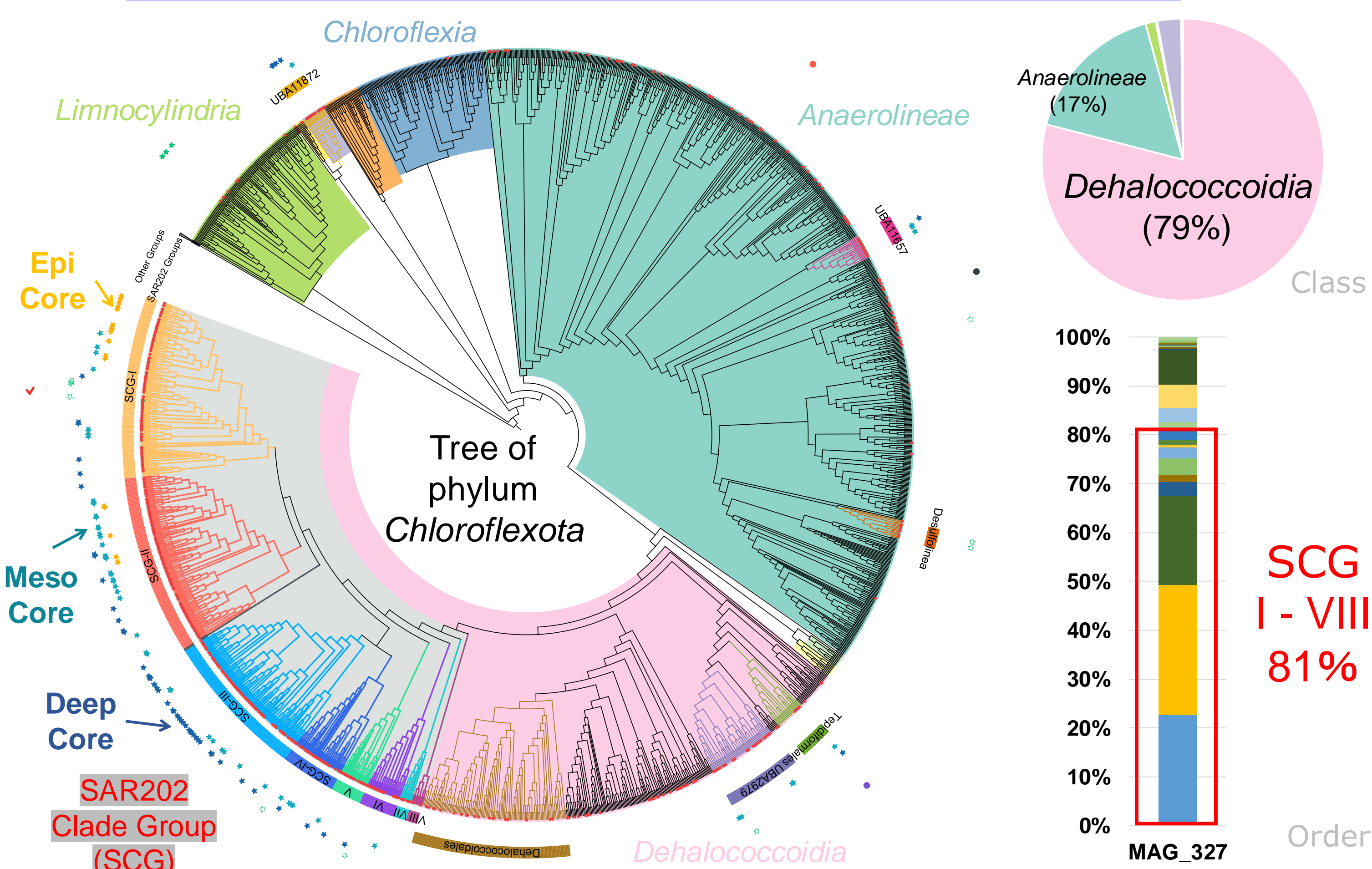Mehrshad *et al.* (2018) *Microbiome*, Lim *et al.* (2023) *Nat Commun.*, He *et al.* (2024) *Commun Biol.*

Purpose:

To unravel the ecological niches and possible adaptive processes of these mystic marine Chloroflexi using global metagenomic big data (①What? ②Where? ③How?)
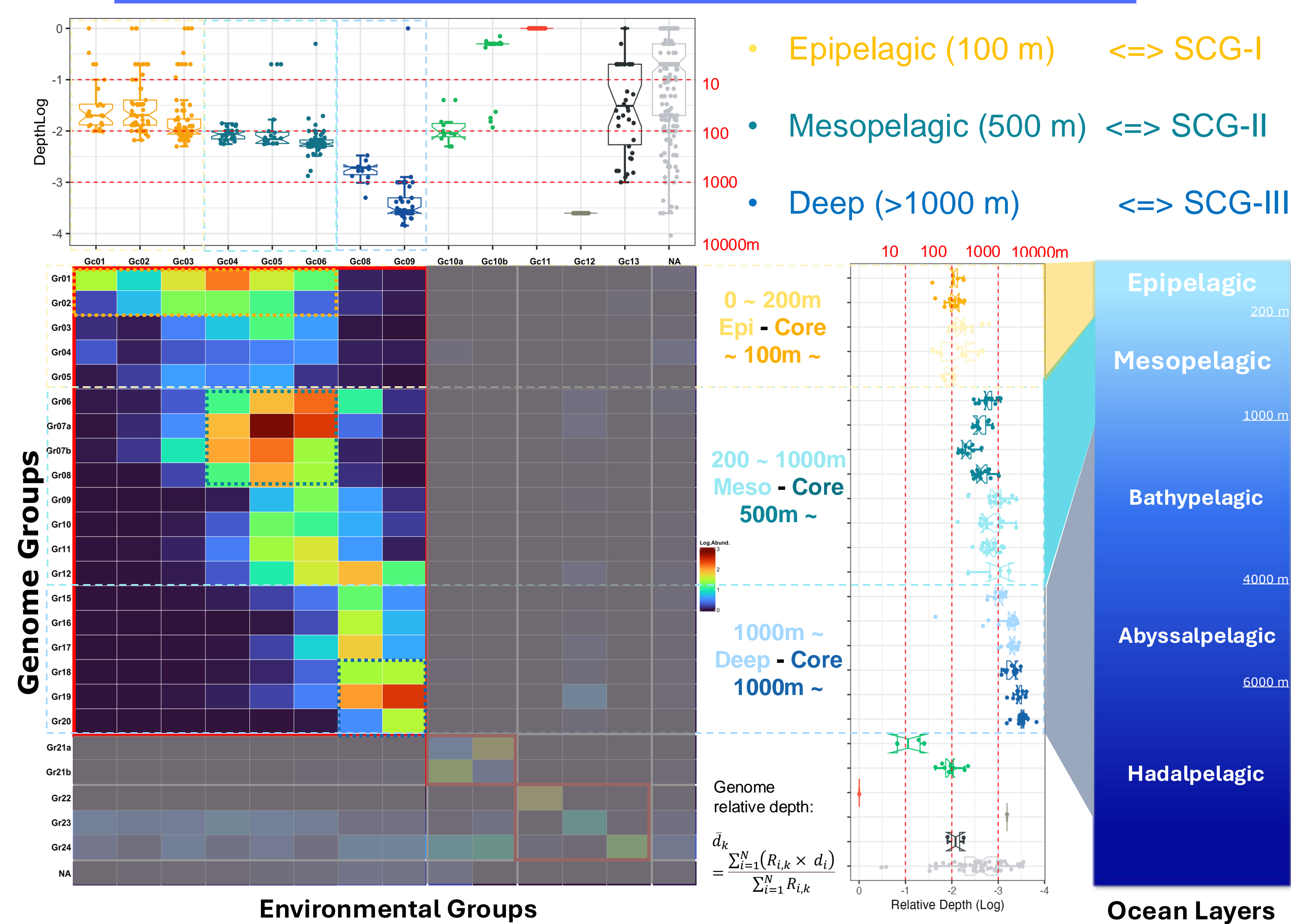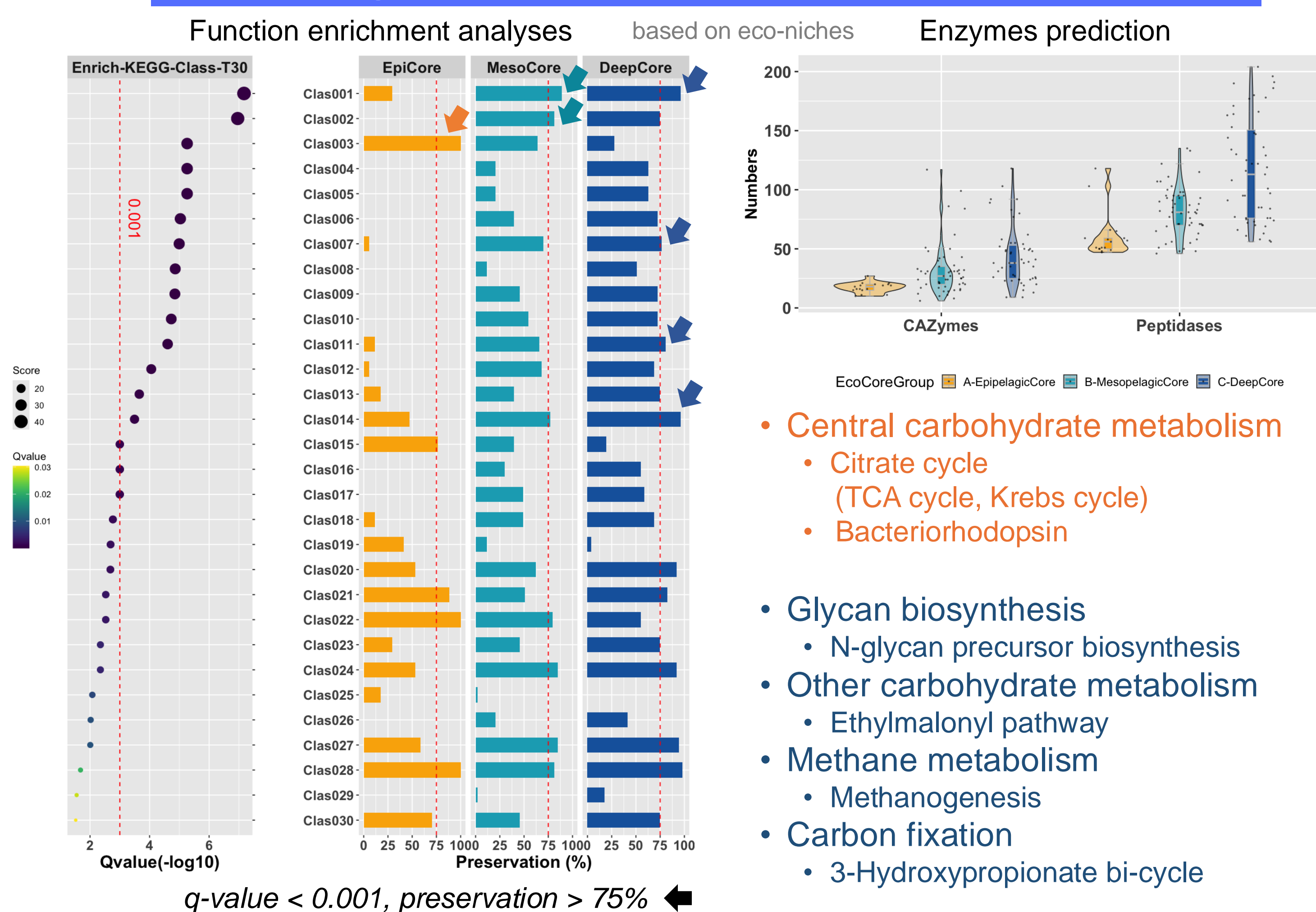
Methods

Databases:
- The OceanDNA MAGs
- MarDB/MarRef
- Other papers

Nishimura and Yoshizawa (2022) *Sci. Data*
Klemetsen *et al.* (2018) *Nucleic Acids Res*

Environmental Metagenomes

> 90k Genomes (MAGs/SAGs/isolates) 2,057 Metagenomes
→ Representatives & Quality check (QS≥50, ANI≥95%) *MARGE, checkm*
→ 16,141 Genomes & 2,057 Metagenomes
→ Taxonomy filter *GTDB-Tk*
→ 515 Chloroflexi Genomes & 2,057 Metagenomes
→ Distribution check / Mapping → 327 Chloroflexi Genomes & 702 Metagenomes
→ Dataset interpretation

① Phylogenomic profiling — *FastTree, GTDB-Tk, iTOL*
② Ecological profiling — *bwa, bowtie2, samtools*
③ Functional profiling — *prodigal, prokka, anvi'o, COG, KEGG*

Visualization — *R, ggplot2, complexheatmap*

Distribution of 702 Environmental Metagenomes

Comprehensiveness:
- Range (67 ºS – 85 ºN)
- Depth ~ -10,899 m
- Diverse Salinity/Oxygen levels
- Sources Biofilm/Sediment trap

Results

① Dominant in class *Dehalococcoidia* (SAR202 clade)

Tree of phylum *Chloroflexota*

- *Chloroflexia*
- *Limnocylindria*
- *Anaerolineae*
- Epi Core
- Meso Core
- Deep Core
- SAR202 Clade Group (SCG)
- *Dehalococcoidia*

Class:
- *Dehalococcoidia* (79%)
- *Anaerolineae* (17%)

SCG I - VIII 81%

MAG_327 (Order)

② Vertical distribution pattern in marine environment

Epipelagic (100 m) <=> SCG-I
Mesopelagic (500 m) <=> SCG-II
Deep (>1000 m) <=> SCG-III

Genome Groups / Environmental Groups

Ocean Layers:
- Epipelagic
- Mesopelagic
- Bathypelagic
- Abyssalpelagic
- Hadalpelagic

0 ~ 200m Epi - Core ~ 100m ~
200 ~ 1000m Meso - Core 500m ~
1000m ~ Deep - Core 1000m ~

Genome relative depth:
$$d_k = \frac{\sum_{i=1}^{N}(R_{i,k} \times d_i)}{\sum_{i=1}^{N} R_{i,k}}$$

Relative Depth (Log)

③ Ecological niche specific functions

Function enrichment analyses (based on eco-niches)

Enzymes prediction

EcoCoreGroup: A-EpipelagicCore, B-MesopelagicCore, C-DeepCore

CAZymes / Peptidases

- Central carbohydrate metabolism
  - Citrate cycle (TCA cycle, Krebs cycle)
  - Bacteriorhodopsin
- Glycan biosynthesis
  - N-glycan precursor biosynthesis
- Other carbohydrate metabolism
  - Ethylmalonyl pathway
- Methane metabolism
  - Methanogenesis
- Carbon fixation
  - 3-Hydroxypropionate bi-cycle

q-value < 0.001, preservation > 75%

Conclusion

What?
- Dominant taxa in the marine Chloroflexi is SAR202 clade members, and eight groups (I-VIII) are classified.

Where?
- The vertical/depth distribution pattern of marine Chloroflexi is mainly characterized by three ecological niches (Epi-Meso-Deep).

How? Keep digging …
- Niches are associated with phylogenetic taxa and specific enriched functions are identified, such as bacteriorhodopsins in the Epipelagic group, and diverse carbohydrate metabolisms in the Deep group.

Abbreviations: MAG Metagenome-assembled genomes, **SAG** Single-amplified genomes, **UTokyo・AORI** Atmosphere and Ocean Research Institute, The University of Tokyo, Kashiwa, Chiba, Japan, **JAMSTEC・CeBN** Research Center for Bioscience and Nanoscience (CeBN), Japan Agency for Marine-Earth Science and Technology (JAMSTEC), Yokosuka, Kanagawa, Japan, **UTokyo・GSFS** Department of Natural Environmental Studies, Graduate School of Frontier Sciences, The University of Tokyo, Kashiwa, Japan.