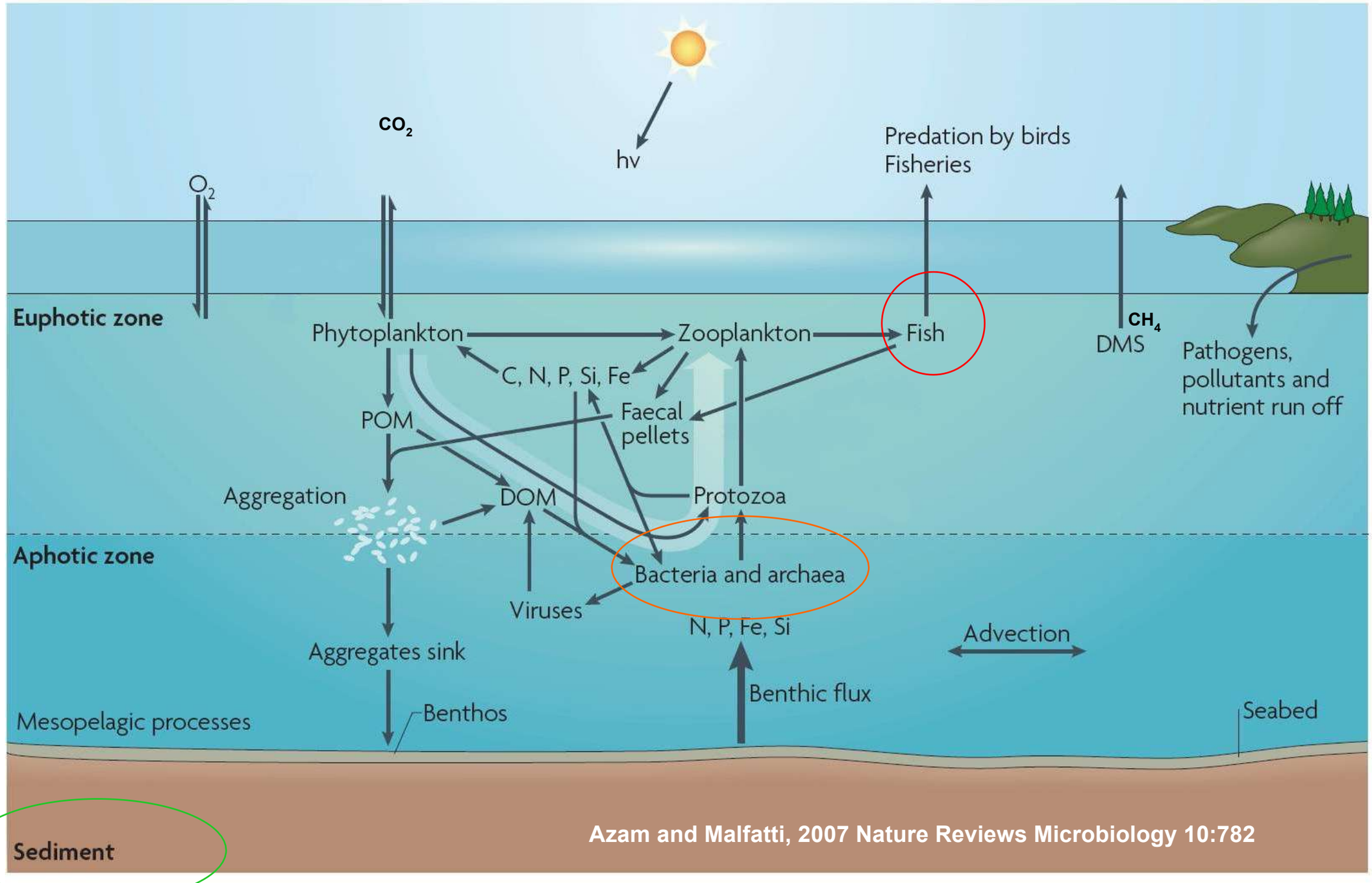
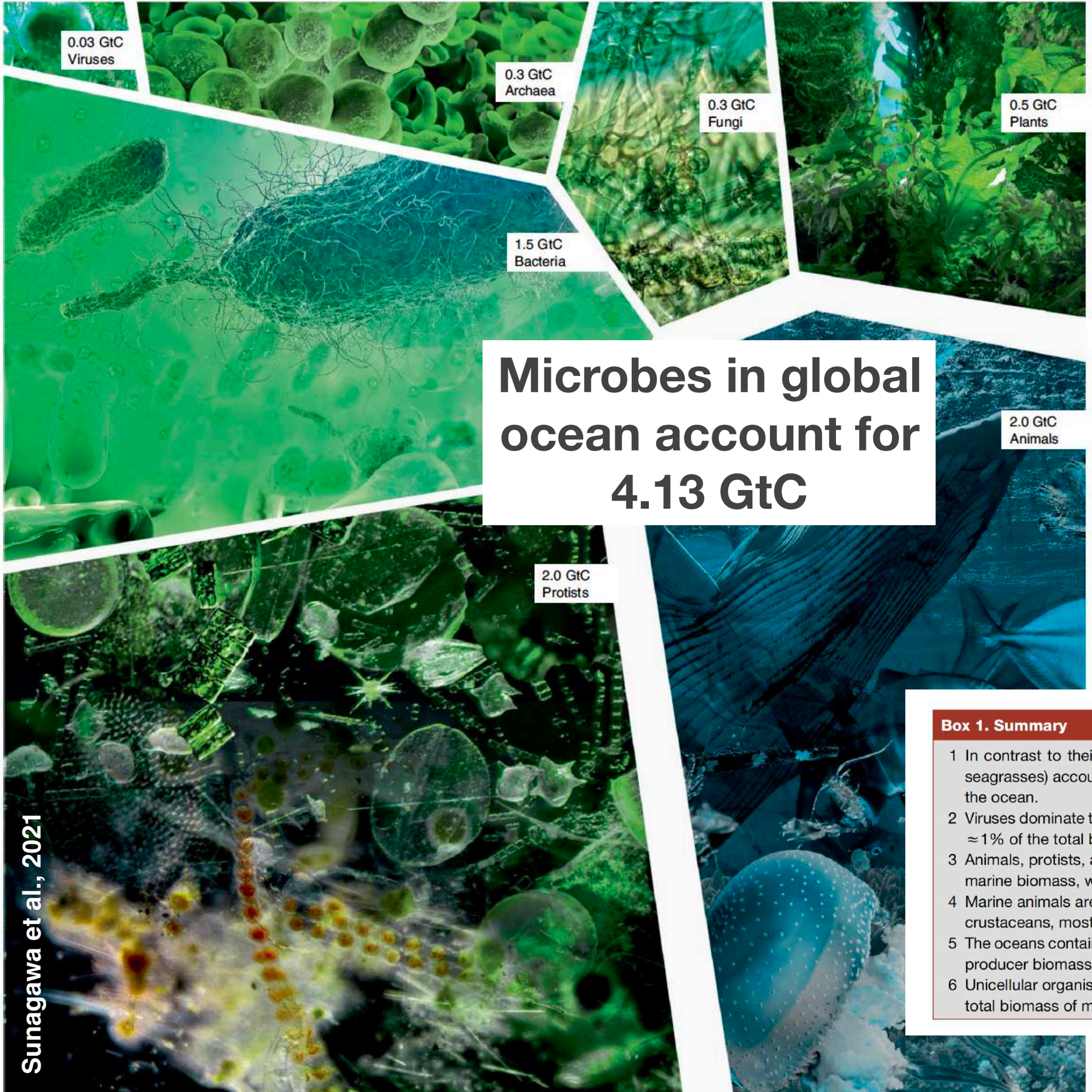
L03a: Diversity of Marine Bacteria, Archaea, Eukarya and Viruses

Marine microbes are highly diverse



Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782

Gigatons of carbon

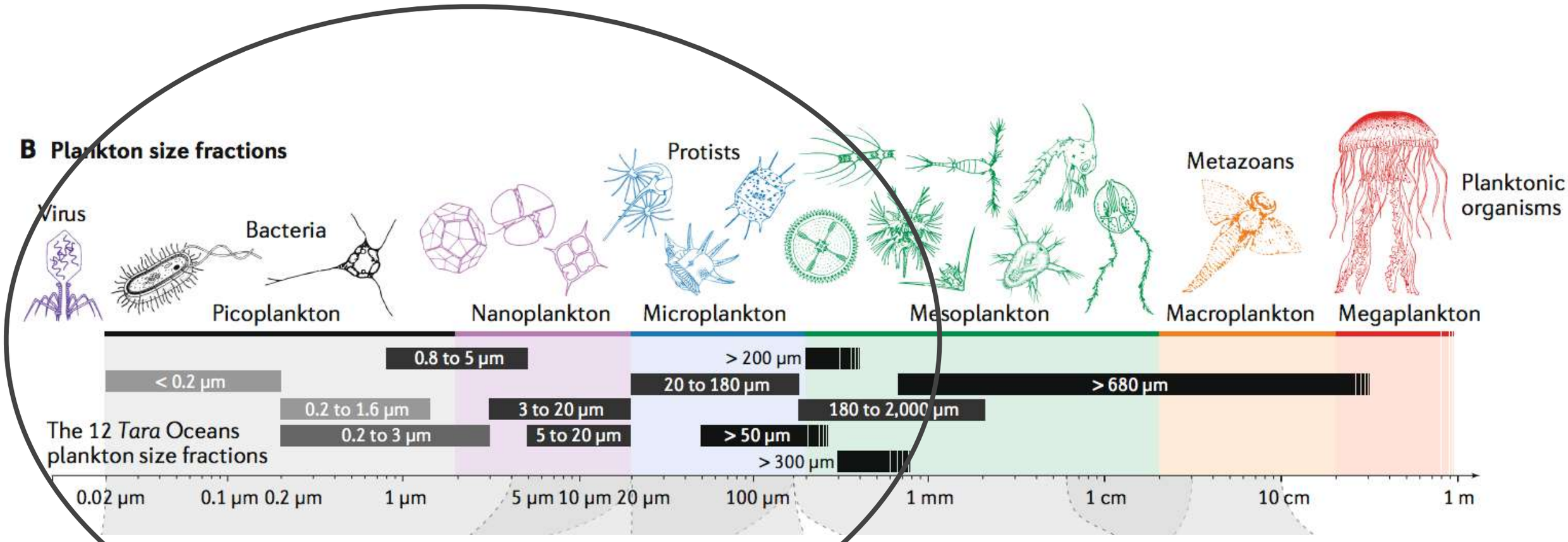


Bar-On & Milo, 2019

Box 1. Summary

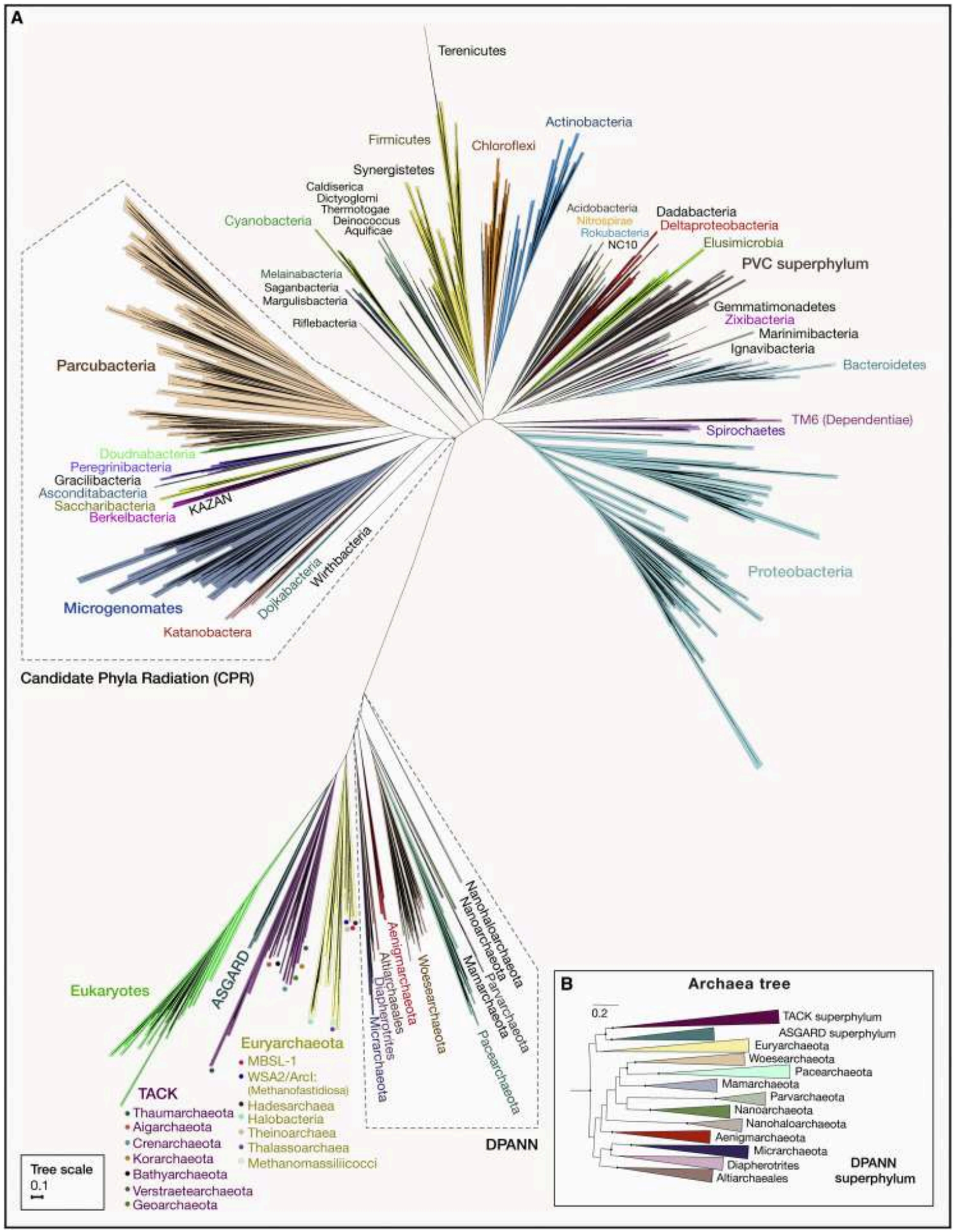
1 In contrast to their domination on land, plants (green algae and seagrasses) account for less than 10% of the total biomass in the ocean.
2 Viruses dominate the ocean in terms of number but constitute only ≈1% of the total biomass.
3 Animals, protists, and bacteria together account for ≈80% of the marine biomass, whereas on land they comprise only ≈2%.
4 Marine animals are dominated by small mesopelagic fish and crustaceans, mostly copepods, shrimp, and krill.
5 The oceans contain much more consumer biomass (≈5 Gt C) than producer biomass (≈1 Gt C).
6 Unicellular organisms contribute approximately two-thirds of the total biomass of marine organisms.

Size-range of marine microbes



Sunagawa et al., 2020

Diversity of Marine Bacteria


A
Terenicutes
Actinobacteria
Firmicutes
Chloroflexi
Synergistetes
Caldiserica
Dictyoglomi
Thermotogae
Deinococcus
Aquificae
Cyanobacteria
Acidobacteria
Nitrospirae
Rokubacteria
NC10
Dadabacteria
Deltaproteobacteria
Elusimicrobia
PVC superphylum
Melainabacteria
Saganbacteria
Margulisbacteria
Riflebacteria
Gemmatimonadetes
Zixibacteria
Marinimibacteria
Ignavibacteria
Bacteroidetes
Parcubacteria
TM6 (Dependentiae)
Spirochaetes
Doudnabacteria
Peregrinibacteria
Gracilibacteria
Asconditabacteria
Saccharibacteria
Berkelbacteria
KAZAN
Proteobacteria
Microgenomates
Dojkabacteria
Wirthbacteria
Katanobacteria
Candidate Phyla Radiation (CPR)
Nanohaloarchaeota
Nanoarchaeota
Parvarchaeota
Aenigmarchaeota
Altiarchaeales
Diapherotrites
Micrarchaeota
Woesearchaeota
Mamarchaeota
Pacearchaeota
ASGARD
Eukaryotes
Euryarchaeota
MBSL-1
WSA2/ArcI:
(Methanofastidiosa)
Hadesarchaea
Halobacteria
Theinoarchaea
Thalassoarchaea
Methanomassiliicocci
TACK
Thaumarchaeota
Aigarchaeota
Crenarchaeota
Korarchaeota
Bathyarchaeota
Verstraetearchaeota
Geoarchaeota
DPANN
Tree scale
0.1
B
Archaea tree
0.2
TACK superphylum
ASGARD superphylum
Euryarchaeota
Woesearchaeota
Pacearchaeota
Mamarchaeota
Parvarchaeota
Nanoarchaeota
Nanohaloarchaeota
Aenigmarchaeota
Micrarchaeota
Diapherotrites
Altiarchaeales
DPANN
superphylum

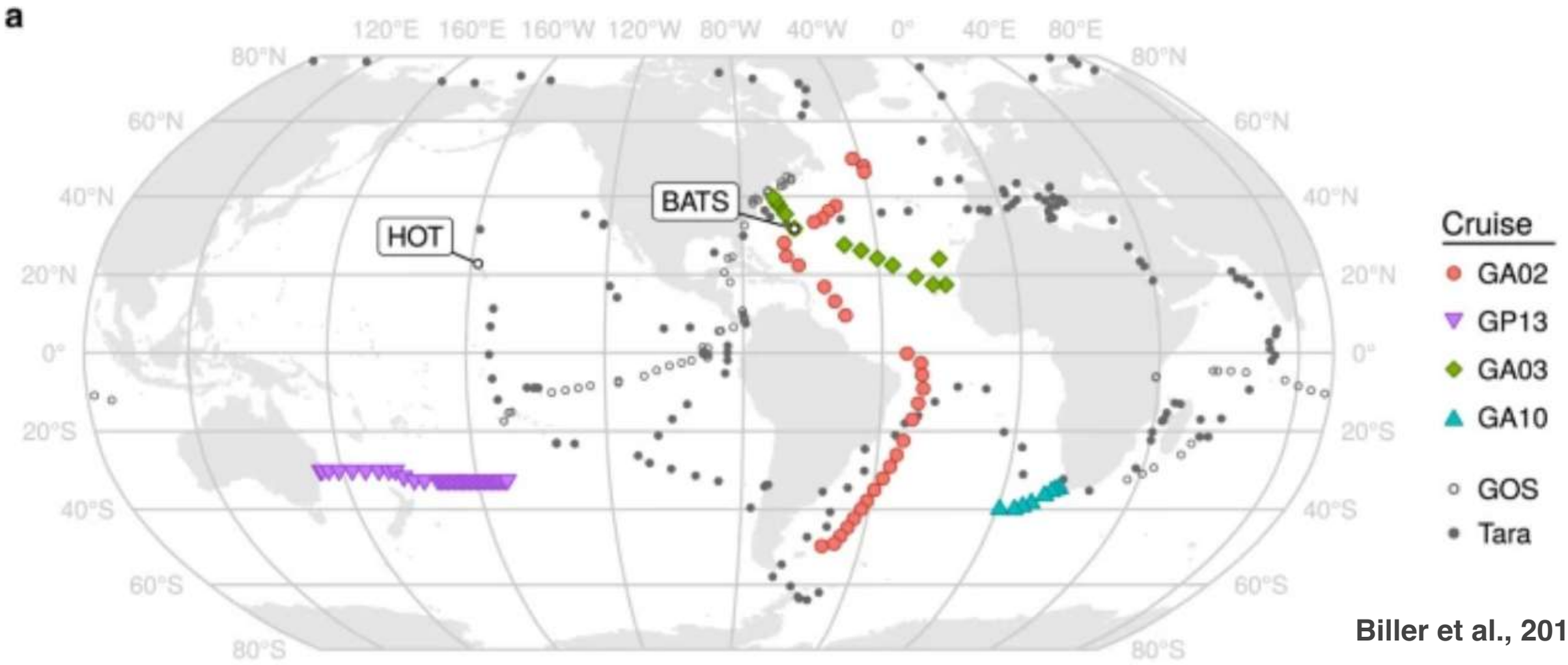
GLOBAL OCEAN SURVEY, GOS





GEOTRACES and microbial exploration

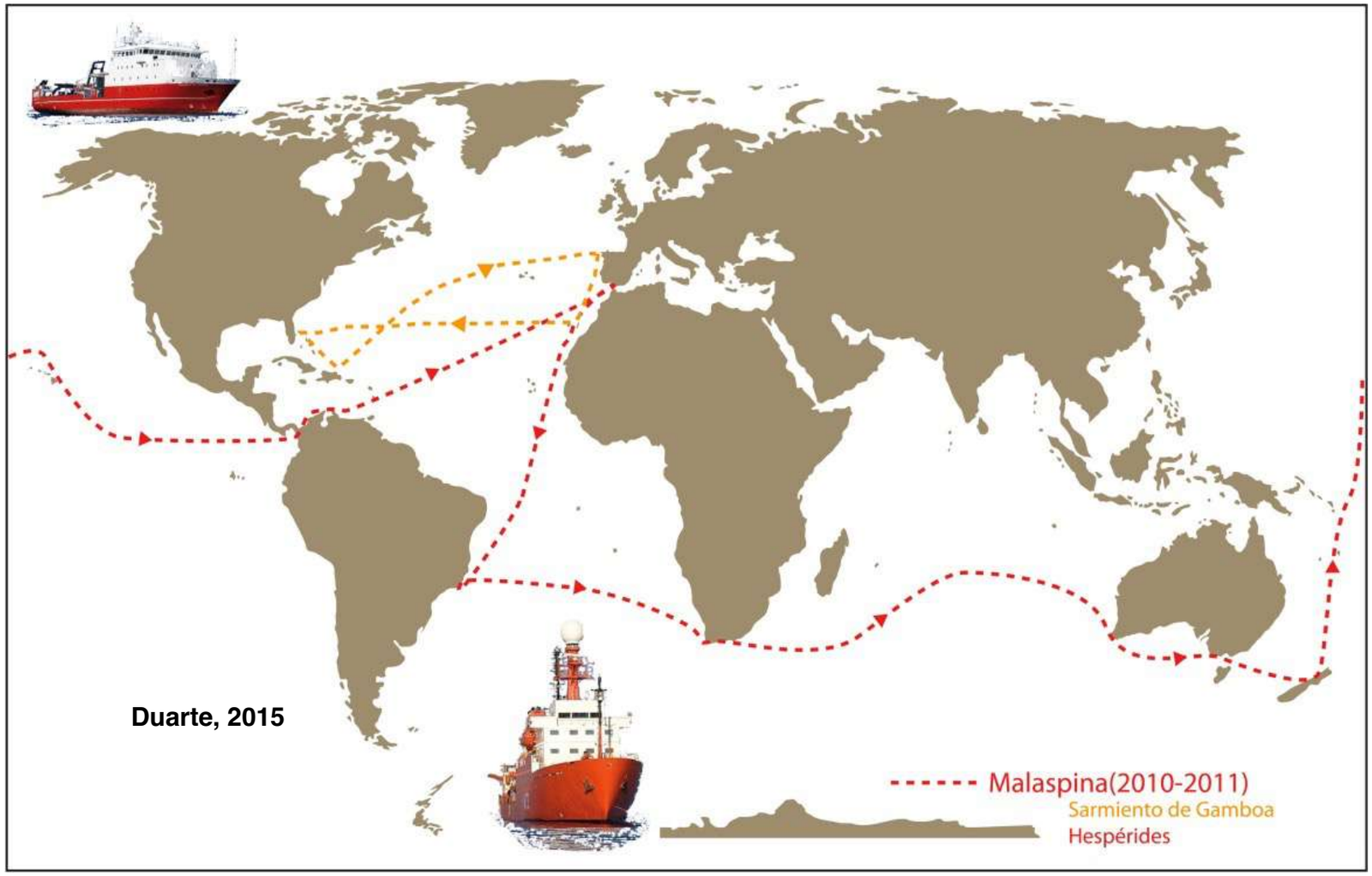
Figure 1: GEOTRACES metagenomic sampling locations.

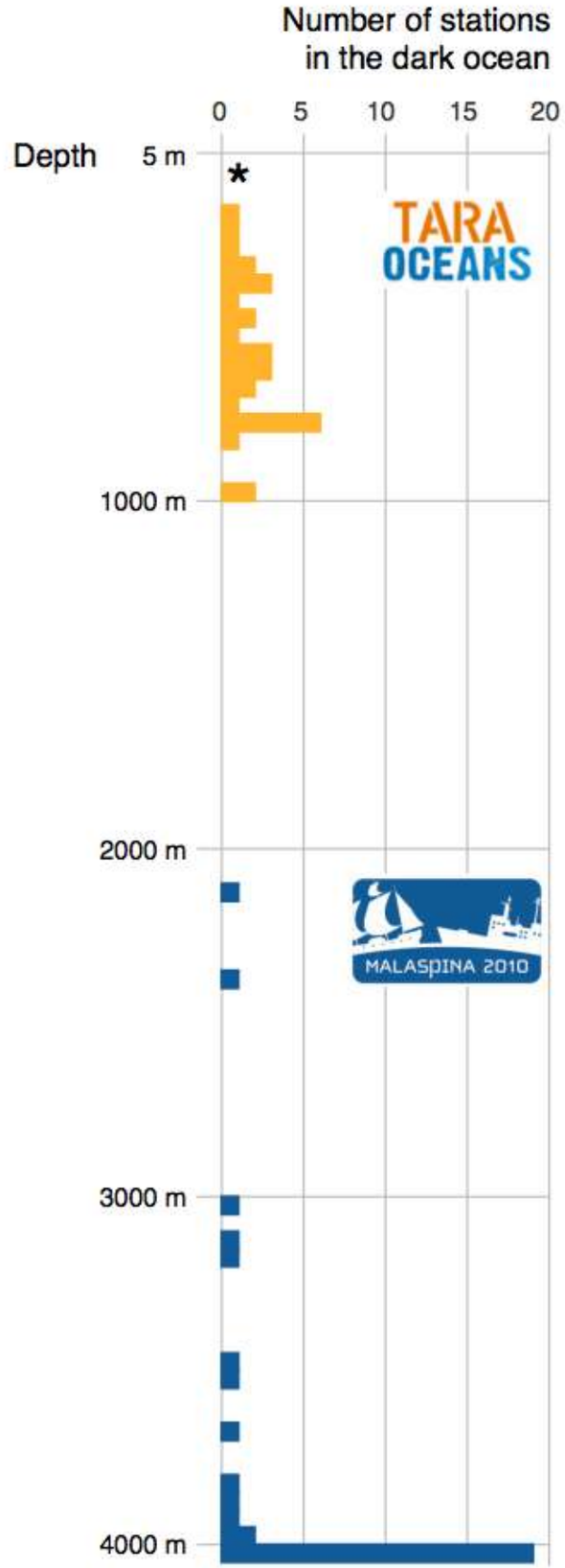


Biller et al., 2018

GEOTRACES is an international study of the marine biogeochemical cycles of trace elements and their isotopes, to accelerate research under a global program (since 2008, Anderson et al., 2014)

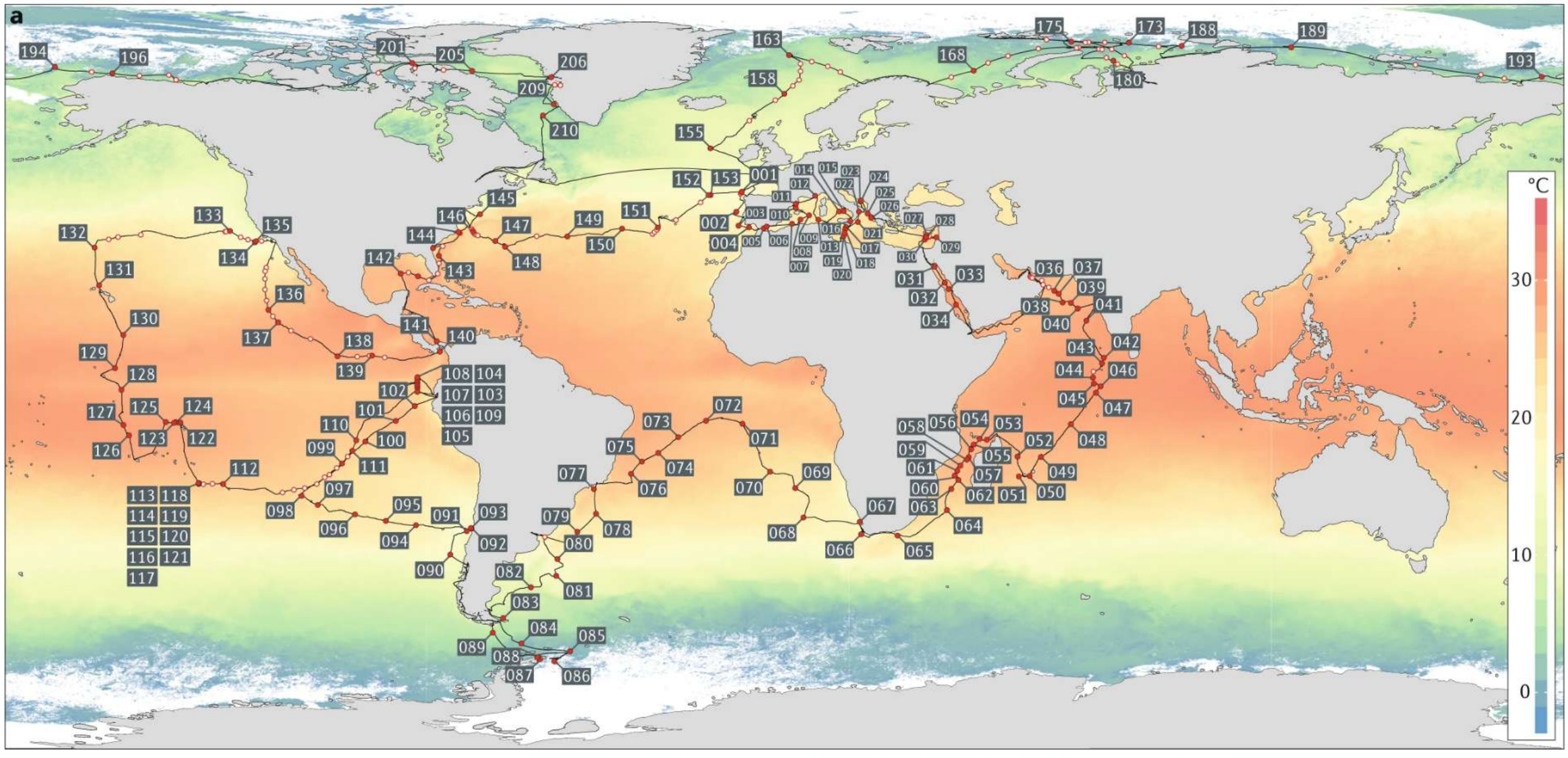
MALASPINA





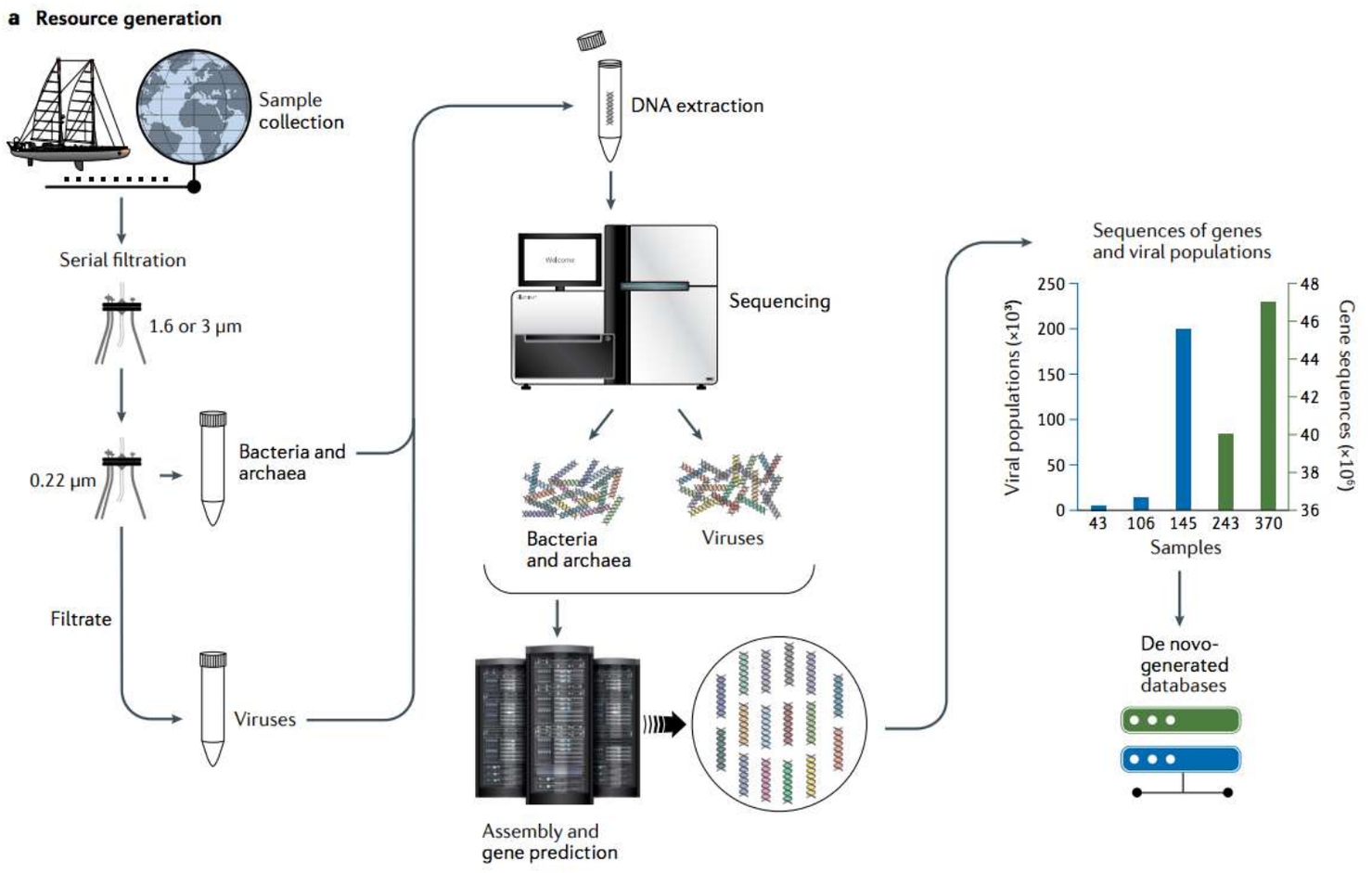
Acinas et al., 2019

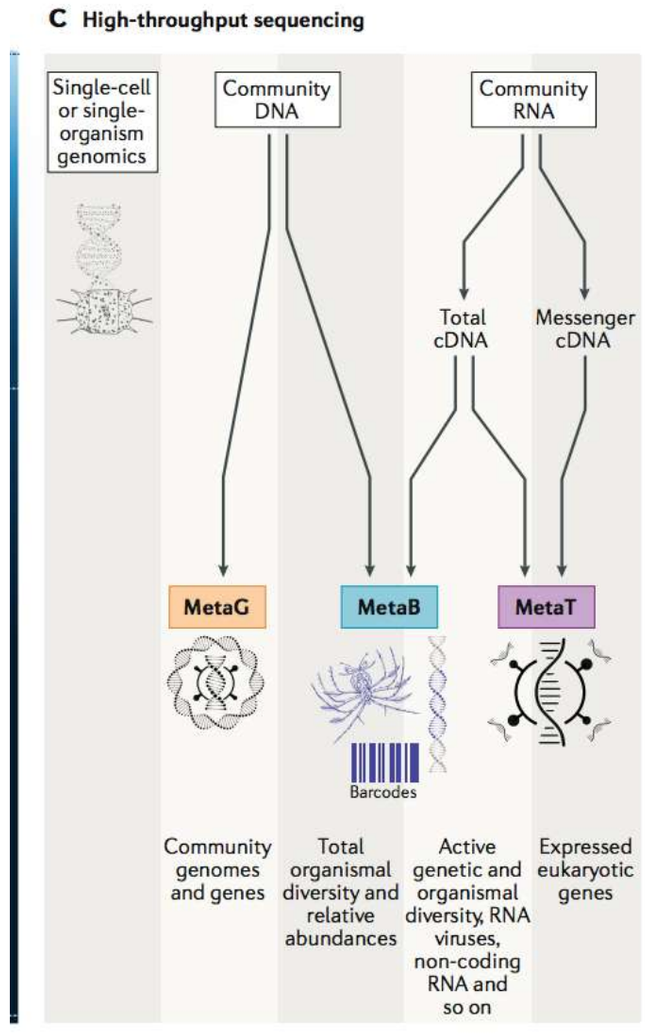
TARA OCEANS



Sunagawa et al., 2020

DNA technology as a tool to characterize microbial ecosystems





Sunagawa et al., 2020

Sequencing effort dictates gene discovery in marine microbial metagenomes

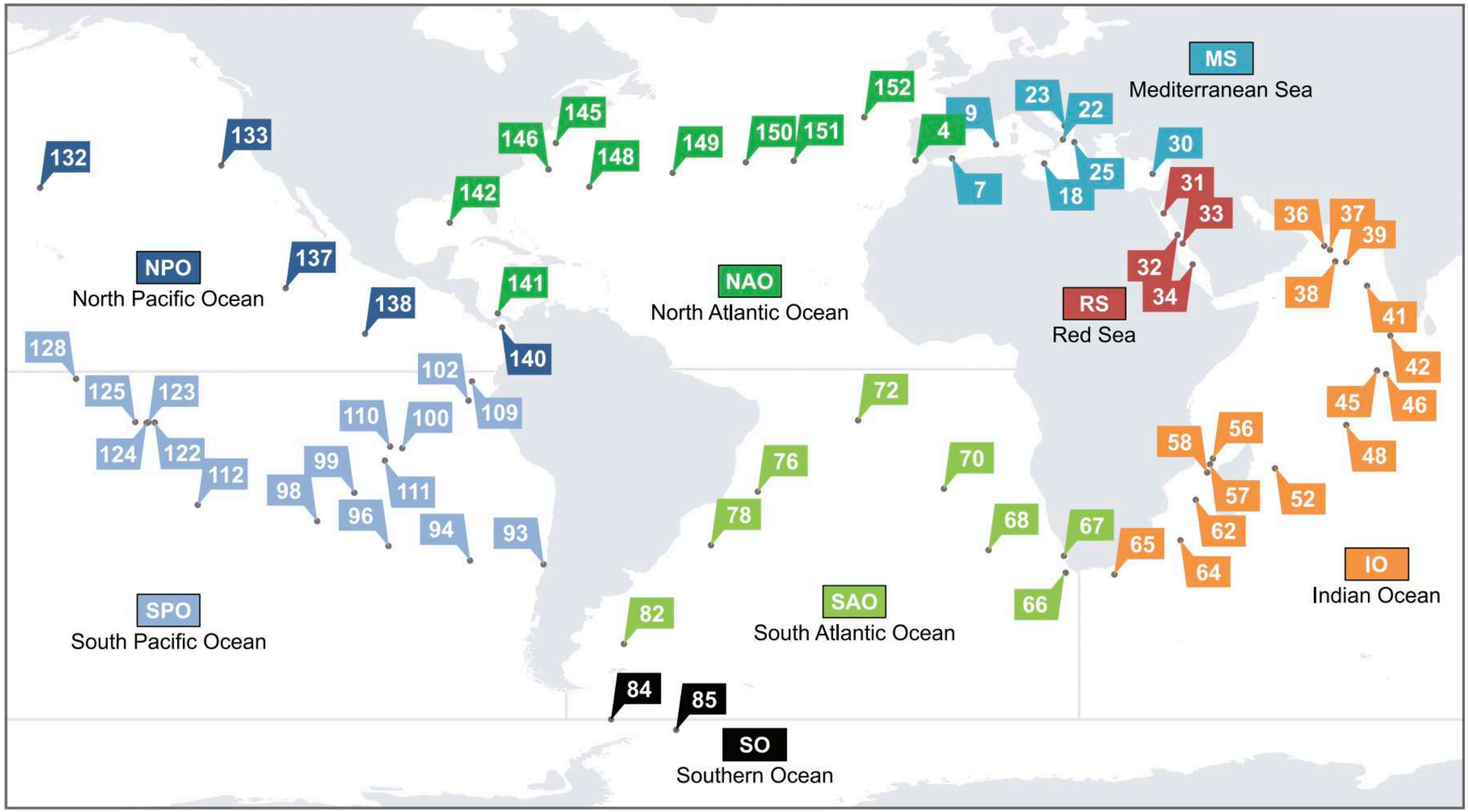
Table 1. The number of non-redundant gene sequence clusters predicted in various metagenome projects exploring marine pelagic microbial communities and mammalian enteric microbiomes and corresponding yield relative to the sequencing depth applied.

Project (gene catalogue)	Analytical procedures[b]	Samples	Sequenced depth (Tbp)[c]	Gene/protein sequences ($\times 10^6$)[a]			Yield (10^6 per Tbp)		Original data source[f]
				Redundant	Non-redundant				
					Genes[d]	Proteins[e]	Genes	Proteins	
Marine									
Global Ocean Sampling (GOS)	GP + GC	44	0.00625	13.6	4.5	3.9	720.0	624.0	Rusch *et al*. (2007)
Baltic Sea reference metagenomes	GP + GC	81	0.586	8.7	8.6	8.3	14.7	14.2	Hugerth *et al*. (2015)
Tara Ocean (OM-RGC)	GP + GC	243	5.821	61.3	33.3	27.7	5.7	4.8	Sunagawa *et al*. (2015)
RSCK2011[g,h]	AM + GP + GC	45	0.0483	2.0	1.3	1.2	26.9	24.8	Thompson *et al*. (2017)
Station ALOHA (HOTGC)[g,i]	RAM + GP + GC	103	0.638	47.3	29.6	26.1	46.4	40.9	Mende *et al*. (2017)
GEOTRACES program[g,j]	GP + GC	610	5.024	72.9	29.1	24.1	5.8	4.8	Biller *et al*. (2018)
MALASPINA-Deep (MDSGC)[g]	RAM + GP + GC	60	0.121	11.8	6.7	6.3	55.4	52.1	Acinas *et al*. (2019)
MALASPINA-profiles (MRGC)[g]	AM + GP + GC	116	1.714	61.6	32.7	29.0	19.1	16.9	P. Sanchez *et al*. in prep.
MESOCOSM[g]	AM + GP + GC	65	0.163	5.1	2.8	2.6	17.2	16.0	This study
Non-marine									
Human gut microbiome I[g]	GP + GC	124	0.577	9.7	4.1	3.8	7.1	6.6	Qin *et al*. (2010)
Human gut microbiome II	GP + GC	1267	6.298	121.3	18.5	16.0	2.9	2.5	Li *et al*. (2014)
Mouse gut microbiome[k]		184	0.781	22.2	2.6	n.d.	3.3	n.d.	Xiao *et al*. (2015)
Rat gut microbiome	GP + GC	98	0.222	26.8	7.6	6.9	34.2	31.0	Pan *et al*. (2018)
Pig gut microbiome[k]		287	1.761	62.9	7.7	n.d.	4.4	n.d.	Xaio *et al*. (2016)

Abbreviations: OM-RGC, Ocean Microbial Reference Gene Catalogue; MDGC, Malaspina Deep-Sea Gene Collection; MPRGC, Malaspina Reference Gene Catalogue; Red Sea Centre Cruise 2011; HOTGC, Hawaii Ocean Time-series Gene Catalogue, AM, assembled; RAM, re-assembled; GP, gene prediction; GC, gene cluster with mmseq2; n.d. not determined.

TARA OCEANS (0-1000 m)

A *Tara* Oceans sampling stations



Sunagawa et al., 2015

Proteobacteria

- **Most abundant sequences in ocean environment**
- **Metabolic functions: autotrophy, heterotrophy, an oxygenic phototrophy, methylotrophy, sulfate reduction, nitrogen fixation**
- **O_2 requirement**
- **CO_2 fixation**
- **Alpha-**
- **Beta-**
- **Gamma-**
- **Delta-**
- **Epsilon**
- **Zeta-**

Alphaproteobacteria

- From oligotrophic genome to copiotrophic lifestyle including intracellular symbiont
- From 20-50 % of bacterial abundance via 16SrRNA gene (amplicon sequencing)
- Caulobacterales: cellular differentiation with stalk, extrusion—> epigenetic control —> important for biofilm formation and larval settlement (e.g. corals, sponges, oysters..)
- Rhodospirillales and Magnetococcales: magnetotaxis —> have magnetic particles —> oxic/anoxic interface —> 50MYA speciation (in salt marshes and sediment)
- SAR11, most abundant bacterium

Betaproteobacteria

- Copiotrophic heterotrophic lifestyle associated with plants and animals
- Chemolithoautotrophic: sulfur oxidizing bacteria
- From ~5-10 % of bacterial abundance via 16SrRNA gene (amplicon sequencing)
- Include also rare biosphere

Gammaproteobacteria

- Present everywhere with the exception of hot or alkaline environment
- Alteromonadales, Oceanospirillales, Vibrionales —> almost entirely marine
- From 25-30% of bacterial abundance via 16SrRNA gene (amplicon sequencing)
- From oligotrophic genome to copiotrophic lifestyle
- Many are uncultivated of sulfide-oxydizing bacteria as intracellular symbiont of invertebrate (e.g. tube worm) and mat and sediment
- Vibrionaceae: pathogens and symbiont, large genome, 2 chromosomes
- Oceanospirillales: break down complex organic compounds —> with Osedax worm and sediment by whale falls, hydrocarbon spill (i.e. Alcanivorax); degradation of DMSP (=dimethylpropionosulfonate)
- Thiotrichaceae: filamentous forms, chemotaxis, sulfur-oxidizing chemolithotrophs (using nitrate in anoxic conditions)

Deltaproteobacteria

- **Anaerobic sulfate bacteria and sulfur-reducing bacteria (SRB)**
- **Sediments, anoxic mud, rich of organic matter, particle associated**
- **With methanogenic archaea —> organic matter remineralisation in coastal and shelf-sediment**
- **SRB: organic compounds and H_2—> dissimilative sulfate reduction —>H_2S is toxic but chemotrophic and phototrophic bacteria can use it**
- **Cable bacteria (multi cells): coupling O_2 reduction to water to sulfide oxidation to sulfate via long distance *e*- transport**

Epsilonproteobacteria

- Anaerobic respiration with organic or inorganic electron donor and acceptors
- Hydrothermal vents —> important primary producers chemolithoautotrophy
- Important in colonizing new vents
- Mycobacteria: large genome, social behaviours, gliding motility, make spores, prey on bacteria
- Bdellovibrionales: prey on Gram-Negative bacteria, top-down population control —> new probiotic

Zetaproteobacteria

- Hydrothermal vents, brine, Fe-rich waters
- Steel-corrosion
- Microaerophilic Fe(II)-oxidizing chemolitoautothrops

Cyanobacteria

- **Evolution 2.5 MYA**
- **Oxygenic photosynthesis, membranes to anchor photosystems**
- **Chl a, accessory pigments: phycobilins and phycoerythrin**
- **Surface ocean, sea ice, shallow sediments, microbial mats, on algae and animal surfaces**
- **Some anaerobically growth using H_2, H_2S, or organic compounds as electron donor**
- **Single cells to filaments, to endosymbionts**
- **Nitrogen fixation evolved about 3 BYA in anoxic conditions**
    - **UCYN-A in symbiosis with a microalge Prymnesiophyte, N_2 fixing**
    - ***Crocosphaera,* filamentous, N_2 fixing**
    - **Microbial mats: secondary products, toxin production**

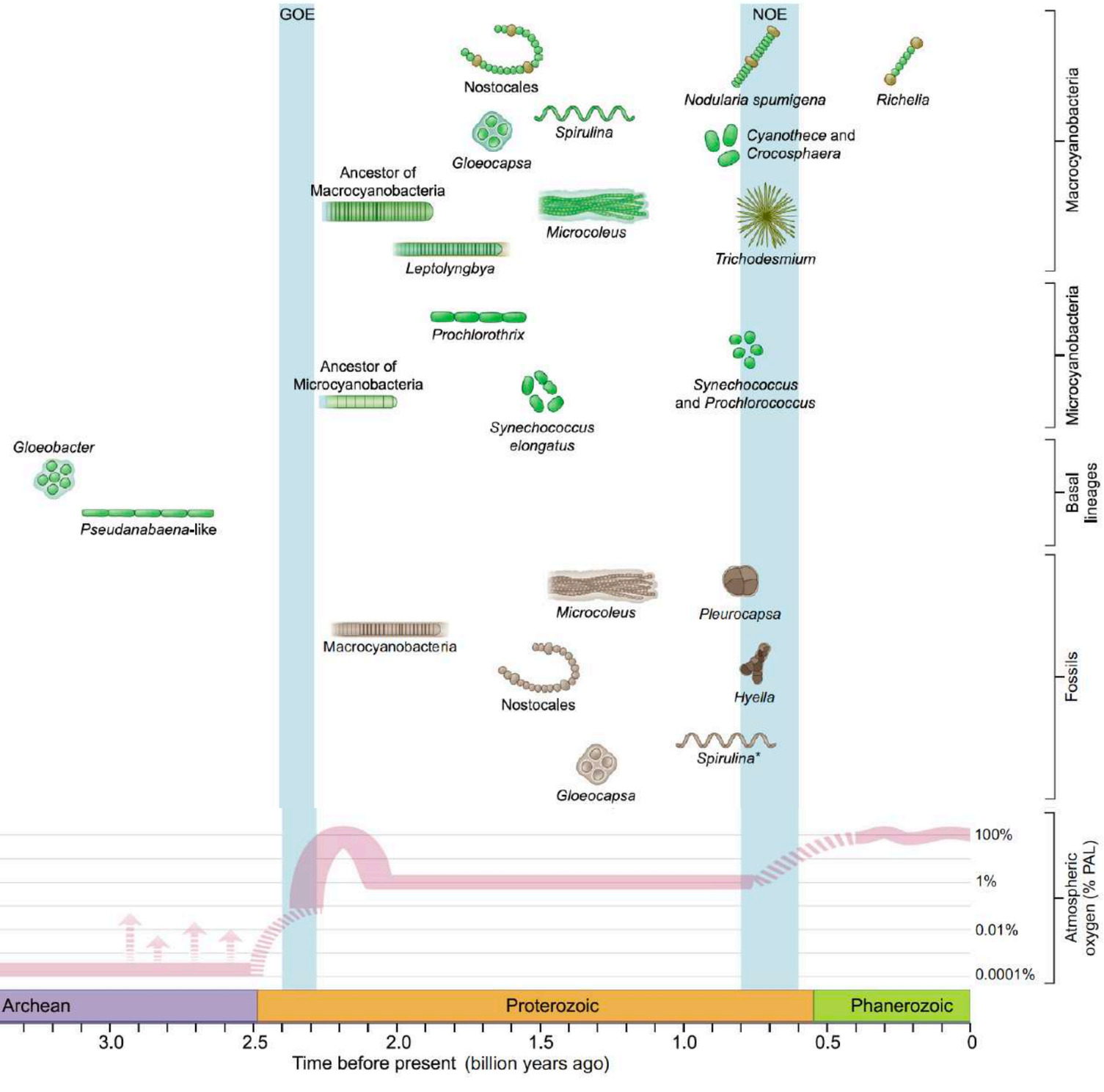
Cyanobacteria

- ***Procholorococcus*** **—> 20% O_2 production to the atmosphere**
  - **Streamlined genome**
  - **Different ecotype, according to light preferences**
  - **Assimilate to glucose and sugars**
  - **Produce outermsmbrane vesicles**
- ***Synechococcus*** **—>very abundant**
  - **Different ecotypes, open ocean *vs* coastal**
  - **Circadian clock regulation**
- ***Trichodesmium*** **—> colony-former**
  - **Nitrogen fixation**
  - **Tropical waters**

Timeline of oxygen concentration in the atmosphere, the antiquity of cyanobacterial lineages based on molecular clock estimates and fossil occurrences

Cyanobacteria derived from early branches live in **nonmarine habitats** or as **benthos** within the shallow, sunlit oceans prior to the Neoproterozoic expansion of phytoplankton, the most widespread and productive cyanobacteria were (**mostly) filamentous mat-forming forms and other - microbial benthos**

https://www.nature.com/scitable/topicpage/the-molecular-clock-and-estimating-species-divergence-41971/



Sánchez-Baracaldo et al., 2021

Primordial Cyanobacteria community: microbial mats

Microbial mats are **highly productive ecosystems** in which feedbacks between cyanobacteria and sulfate-reducing bacteria take place within a few **densely packed vertical millimetres**

Geobiological studies point to the widespread importance of microbial mat ecosystems on the Precambrian Earth it is unknown the their quantitative contribution to global primary production

While estimates of **benthic** oxygenic primary productivity **exceed** those for the **pelagic** realm by 1000–10000-fold on a per-volume basis, sunlit benthic habitats (including the terrestrial, biological soil crusts) occupy a small area in contrast to the open ocean

The **global biogeochemical impact of oxygenic photosynthesis** may have increased markedly when **cyanobacteria started colonizing the open ocean**



An image of a vertically stratified microbial community, taken from a hypersaline mat at Guerrero Negro basin, Mexico. (Photo Credit: John Spear and Norman Pace)

https://kids.frontiersin.org/articles/10.3389/frym.2022.654148

Modern microbial mats



https://kids.frontiersin.org/articles/10.3389/frym.2022.654148

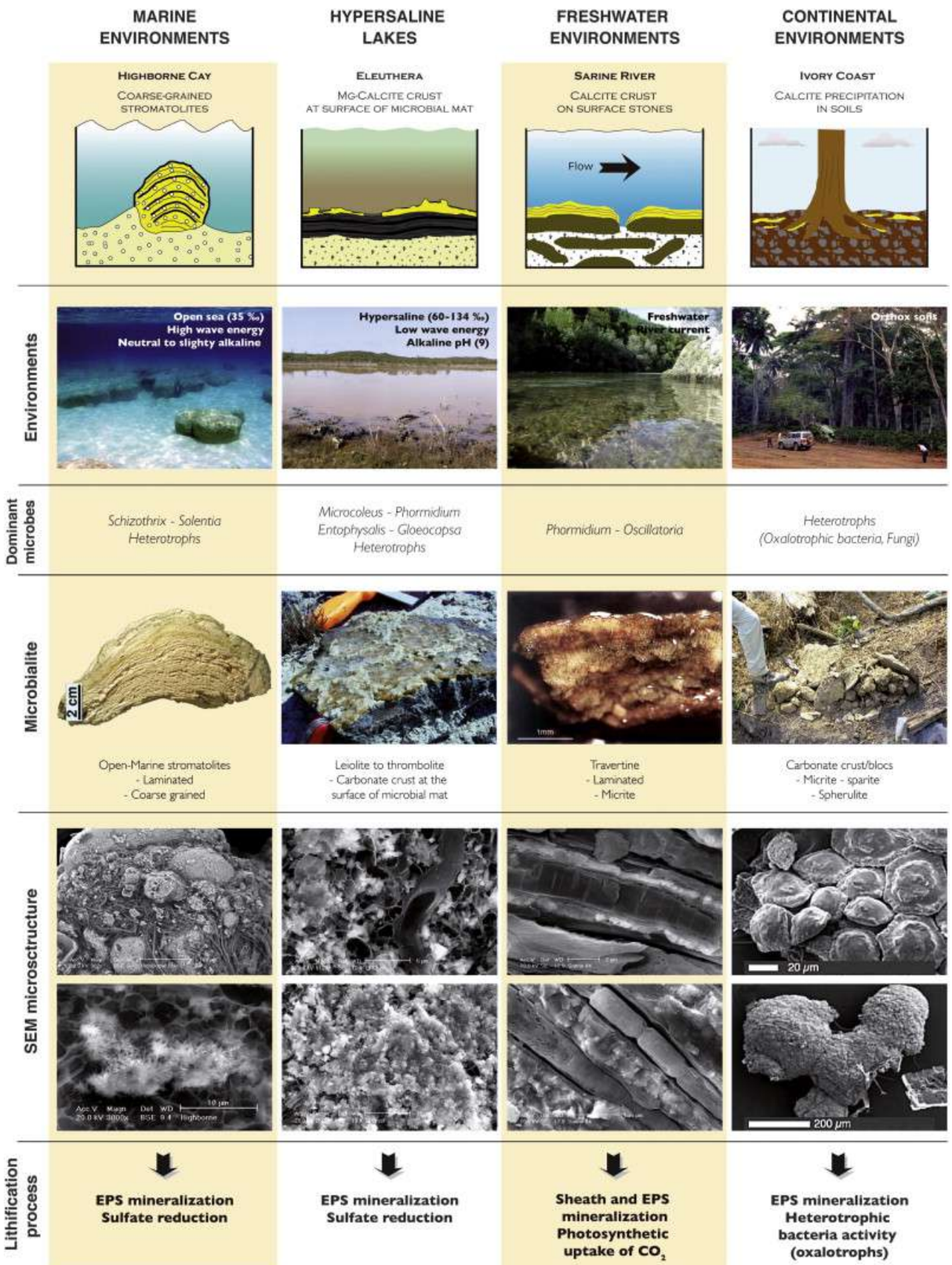
https://kids.frontiersin.org/articles/10.3389/frym.2022.654148



Examples of structures made by microorganisms using soil, water, rocks, and minerals.

(A) Oncolites from Casey Falls locality, Canning Basin, Western Australia (photograph credit: Heidi Allen). (B) Endoevaporite. (C) Microbial mat. (D,E,G,H) Microbial mats in the Yucatán Peninsula in Mexico. (F) Microbial mats from the Middle Island Sinkhole, Lake Huron in North America (photo credit: John Bright, NOAA Thunder Bay National Marine Sanctuary). (I) A cross-section of a microbial mat from Guerrero Negro, Mexico.

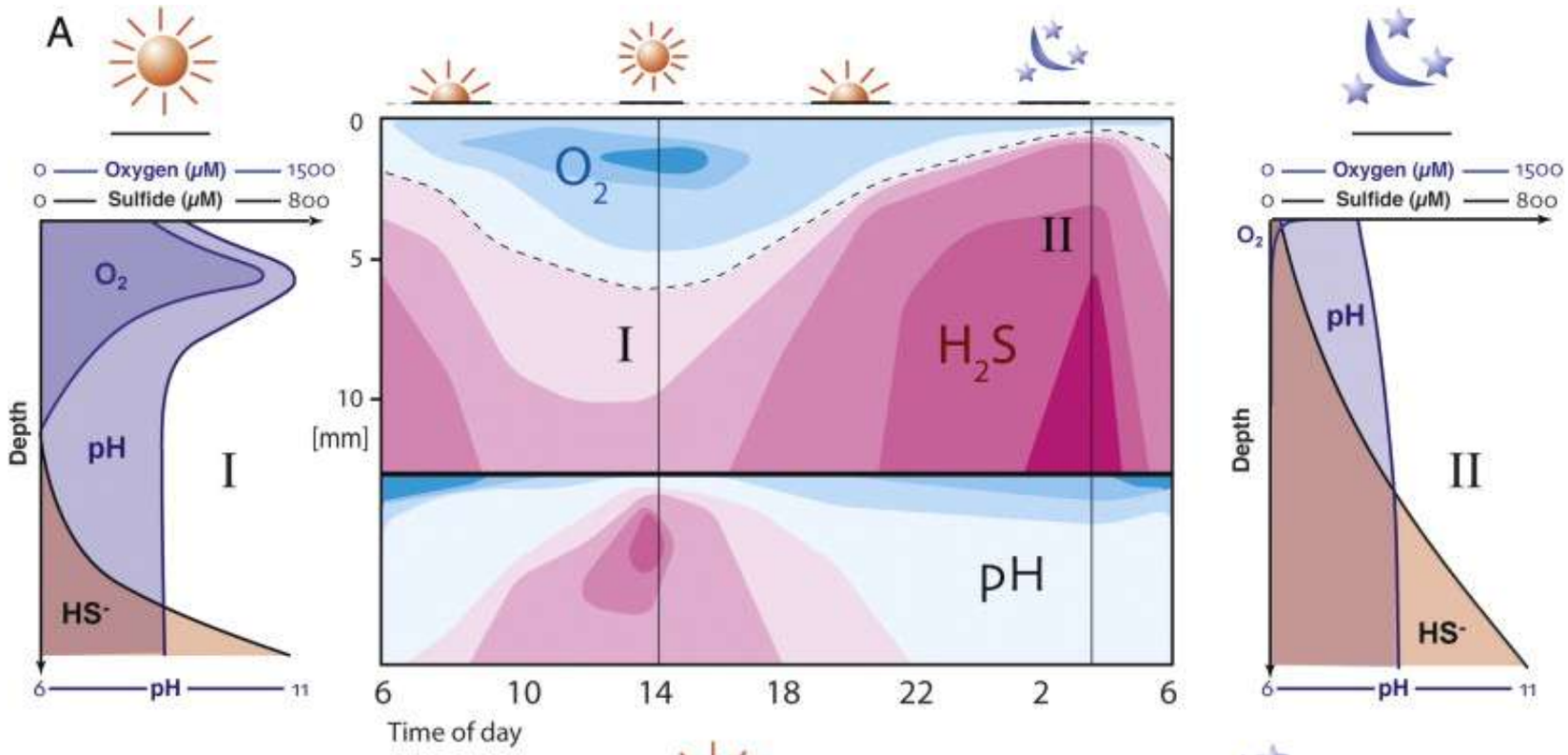
Examples of modern microbialites

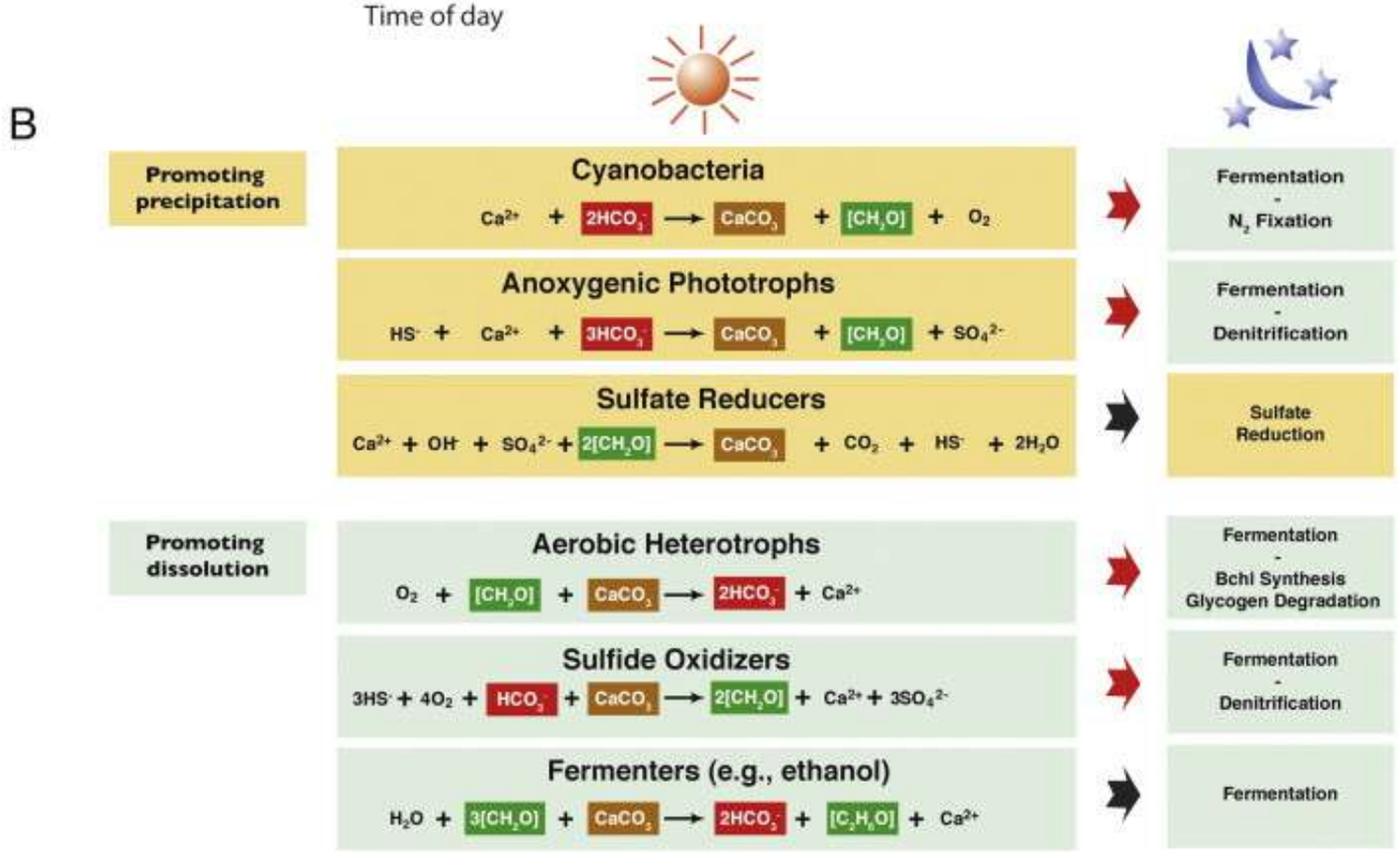


A. Open-marine stromatolite (Exumas, Bahamas). B. Hypersaline microbialites (Eleuthera, Bahamas). C. Freshwater travertine (Sarine River, Switzerland). D. Carbonate deposits in tropical soils (Ivory Coast, Africa)

Dupraz et al. 2009

Diel fluctuations of vertical geochemical gradients in a microbial mat and combined metabolic–geochemical reactions

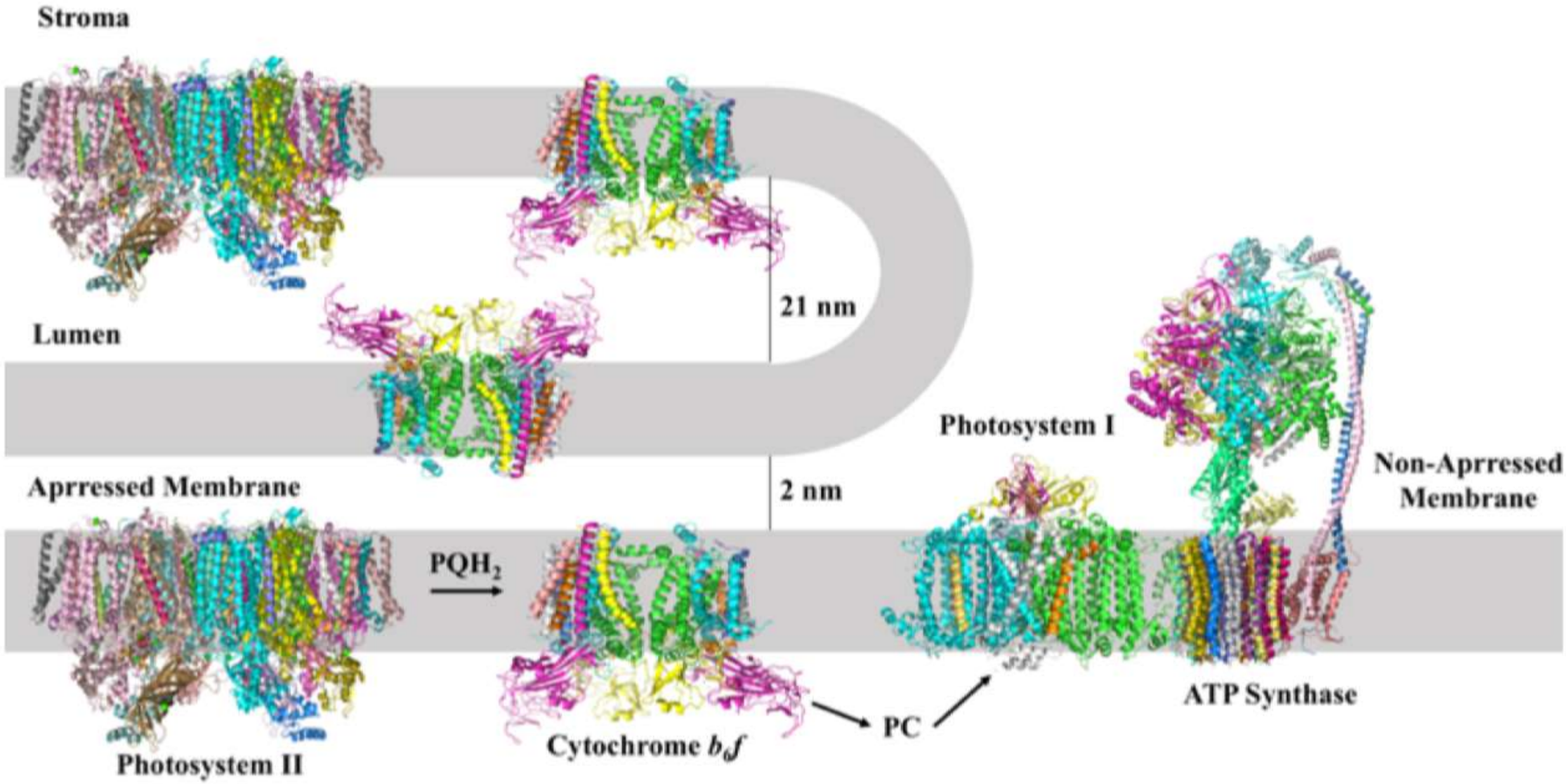




Dupraz et al. 2009

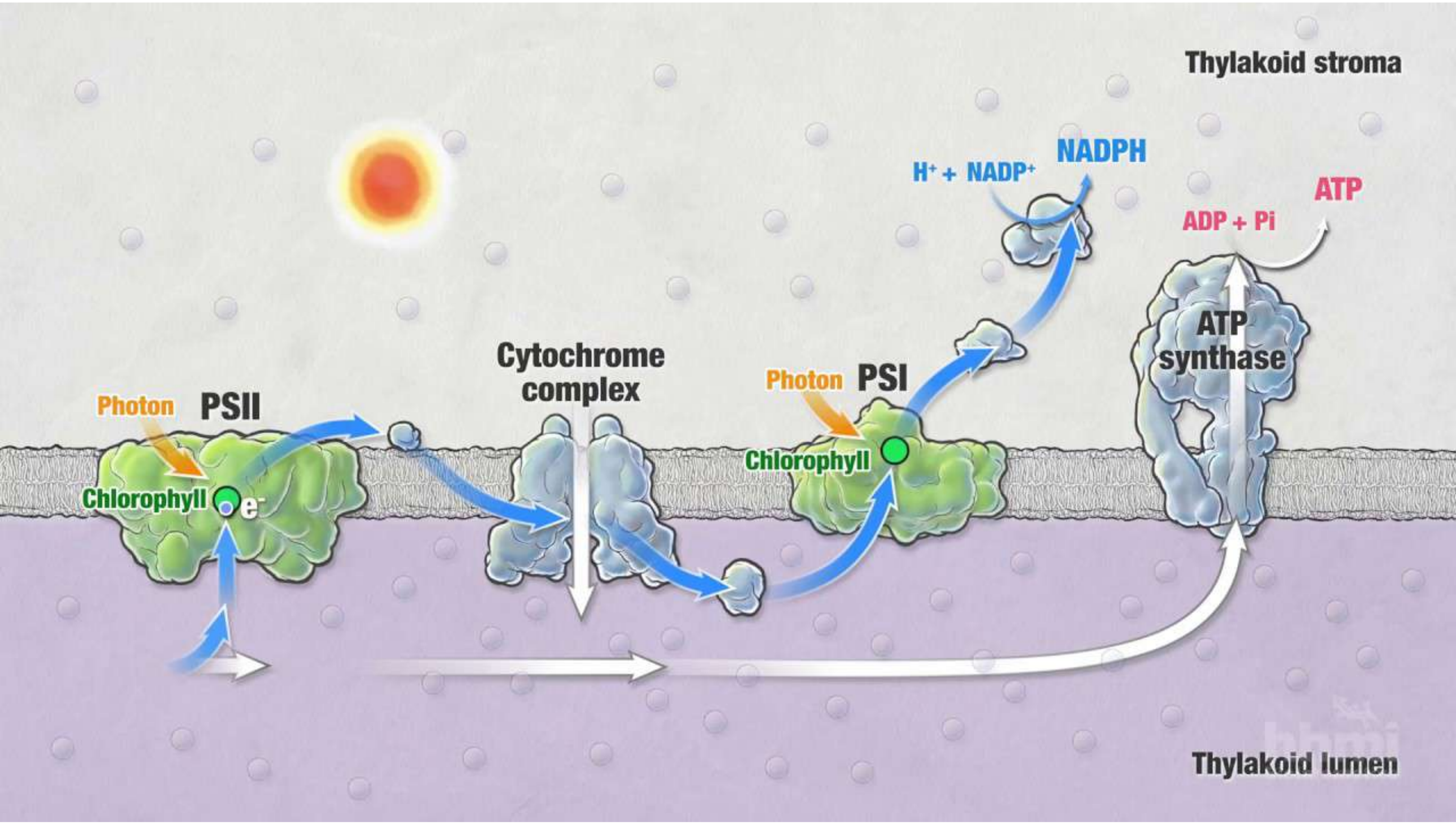
Photosynthetic reaction centres

Eight known extant bacterial phyla contain groups with photosynthetic reaction centres (RCs, **https://pdb101.rcsb.org/motm/22)**: Cyanobacteria, Proteobacteria, Chloroflexi, Acidobacteria, Chlorobi, Firmicutes, Gemmatimonadetes, and Candidatus Eremiobacterota

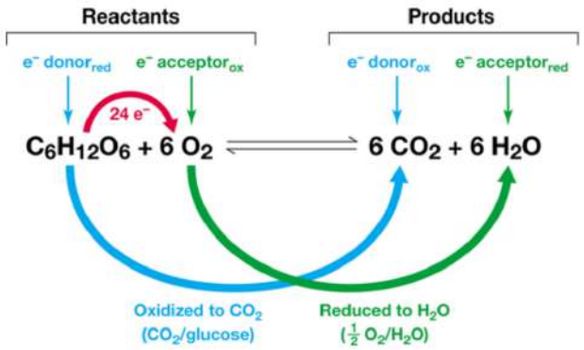


Bhaduri et al. 2021

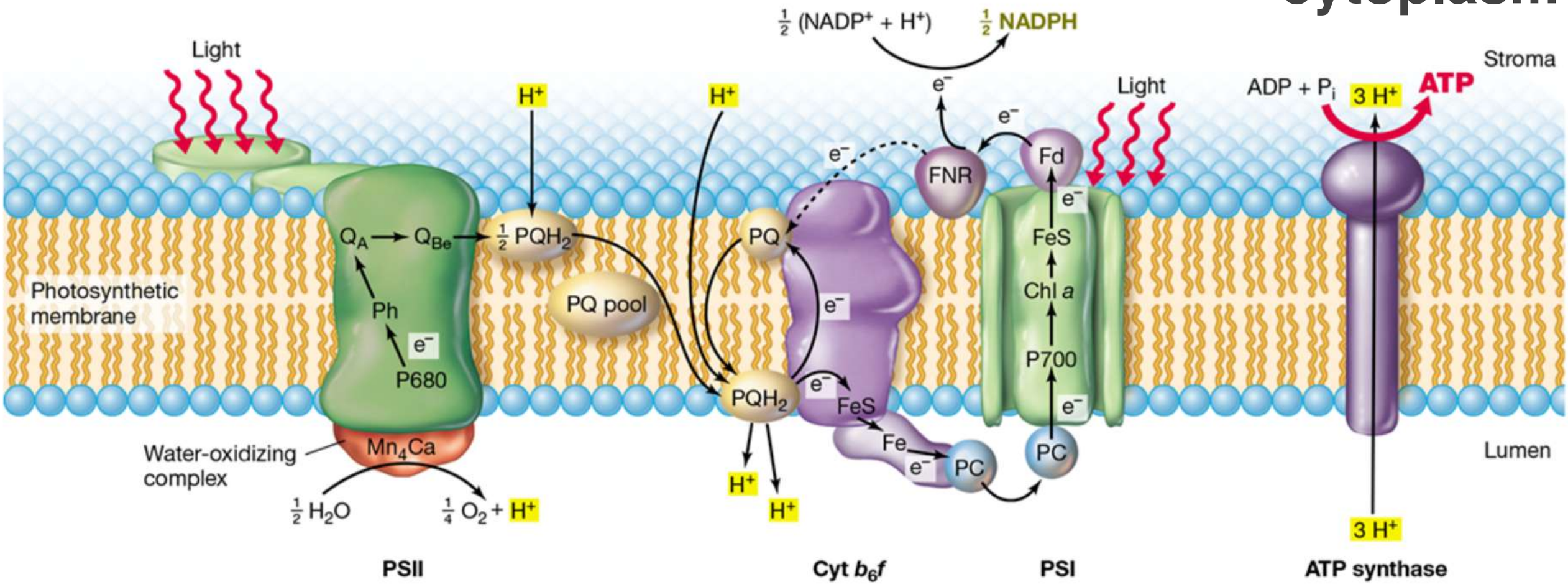
The availability of electron donors likely limited primary production by early anoxygenic photoautotrophs providing strong selection pressure for bacteria able to extract electrons from readily abundant water.


Thylakoid stroma
NADPH
H⁺ + NADP⁺
ATP
ADP + Pi
ATP synthase
Cytochrome complex
Photon
PSII
Photon
PSI
Chlorophyll
e⁻
Chlorophyll
Chlorophyll
Thylakoid lumen

Oxygenic Photosynthesis



cytoplasm

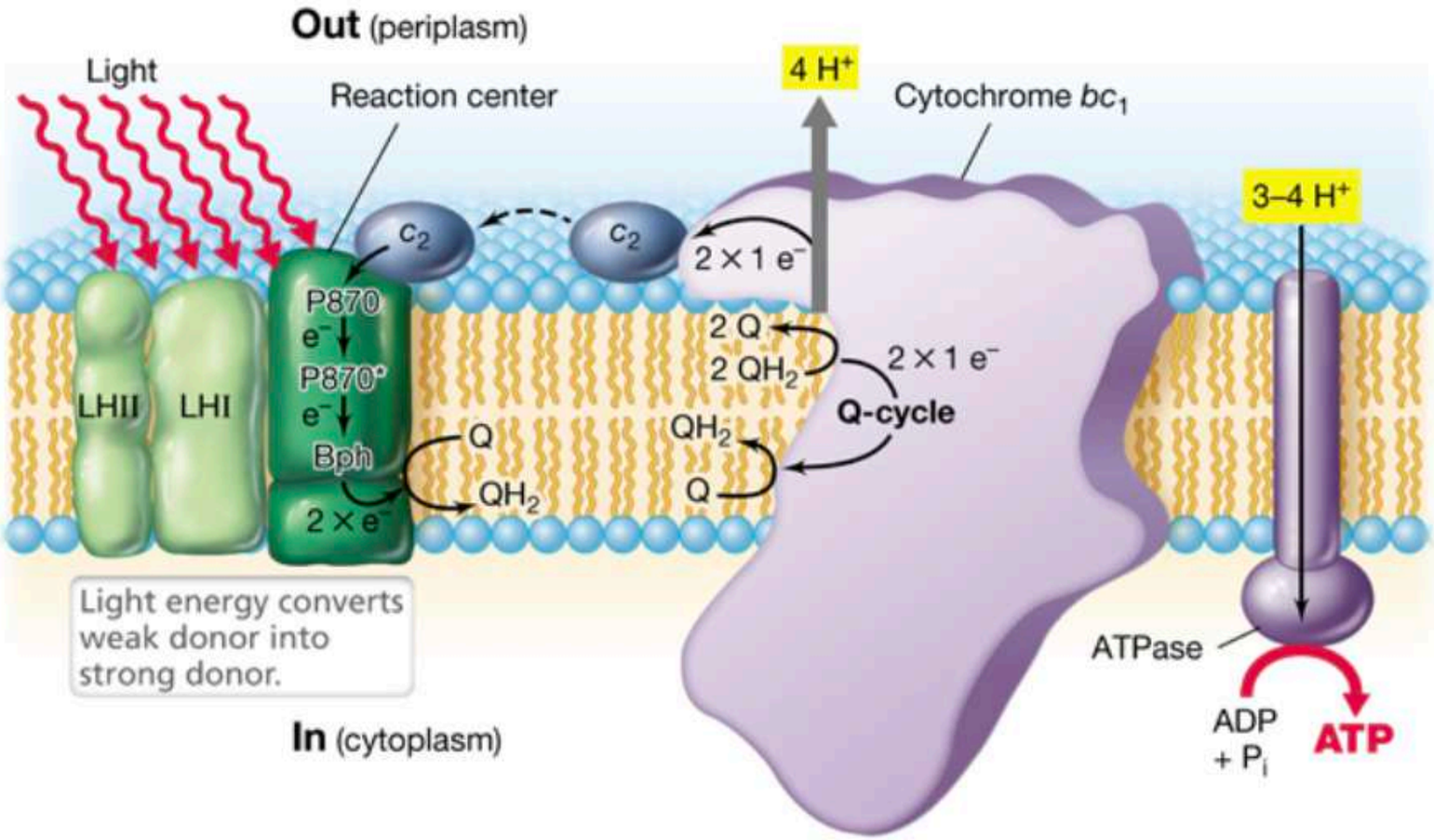


Madigan et al. 2020

- Splitting of H2O
- Generation H+ motive force
- Generation of NADPH —> **C fixation (from CO_2)** via Calvin–Benson–Bassham cycle
- ATP production

Anoxygenic Photosynthesis

Figure 3.25 Electron flow in anoxygenic photosynthesis in a purple bacterium.



(a) **Electron transport in purple bacteria**



(b) **Bloom of purple bacteria in a salt marsh**

Madigan et al. 2020

Circadian rhythms in gene transcription

Circadian clocks have **evolved** within the cyanobacteria (an extremely diverse group of oxygenic photosynthesizing bacteria) and many, if not all, eukaryotes

These clocks effectively tune gene expression patterns, and thus metabolic activity and behavior, to distinct daily frequencies

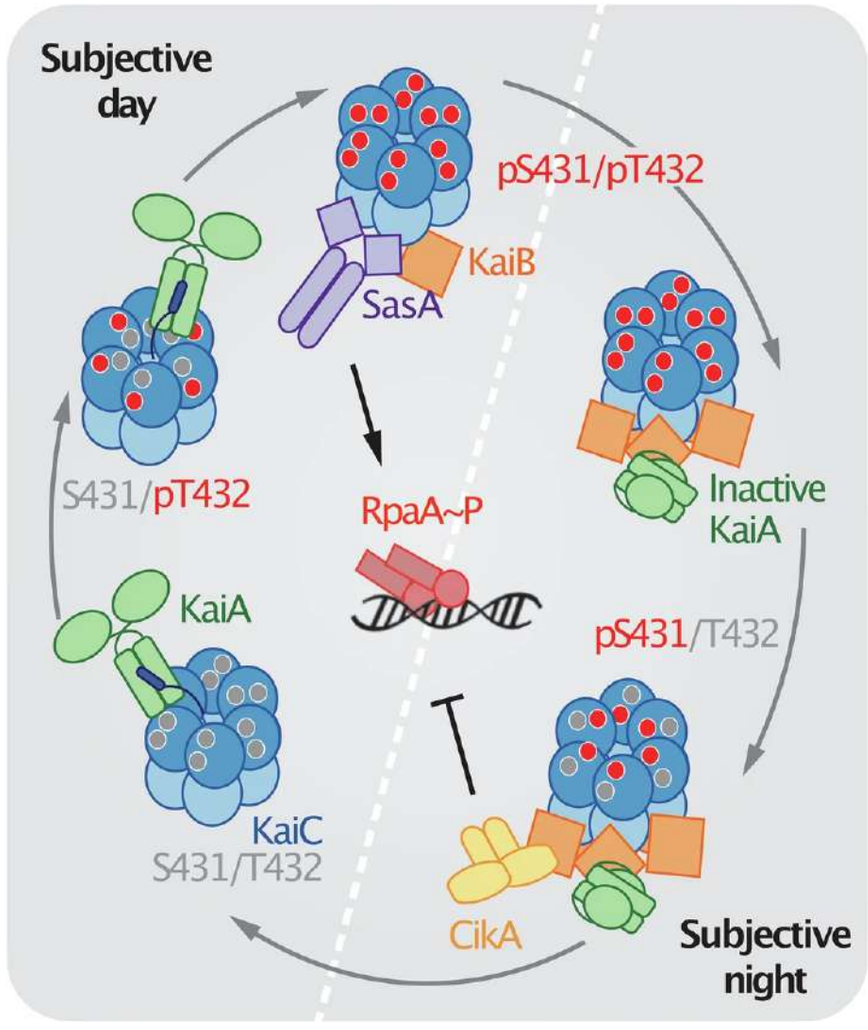
Suggesting a **strong evolutionary advantage for anticipating regular fluctuations in the environment**

Regulation timing of cell division and metabolism in order to have enough nutrients, competence (DNA uptake) and type IV

Two-component systems on a 24 hr cycle; The KaiABC system is the core oscillator, but the clock also needs to communicate its timing to the rest of the cell. Two important proteins are SasA and CikA. They help regulate the phosphorylation state of RpaA, a response regulator and transcriptional regulator

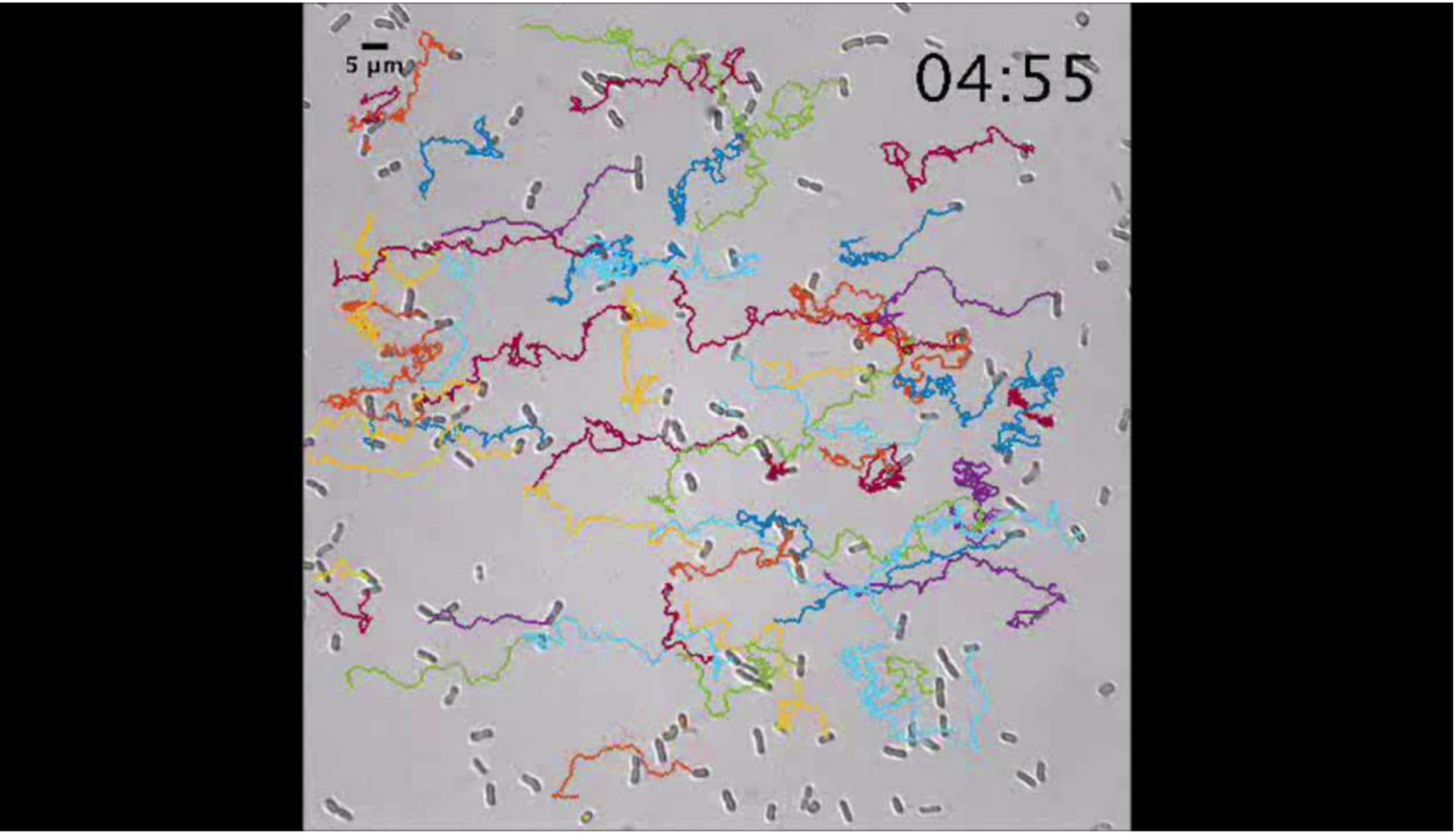
The three hallmarks of bona fide circadian clocks are that:

i) their phase can be entrained by environmental cues
ii) they continue to generate circadian rhythms under constant environmental conditions
iii) their periodicity is relatively insensitive to modest changes in ambient temperature due to a temperature-compensation mechanism

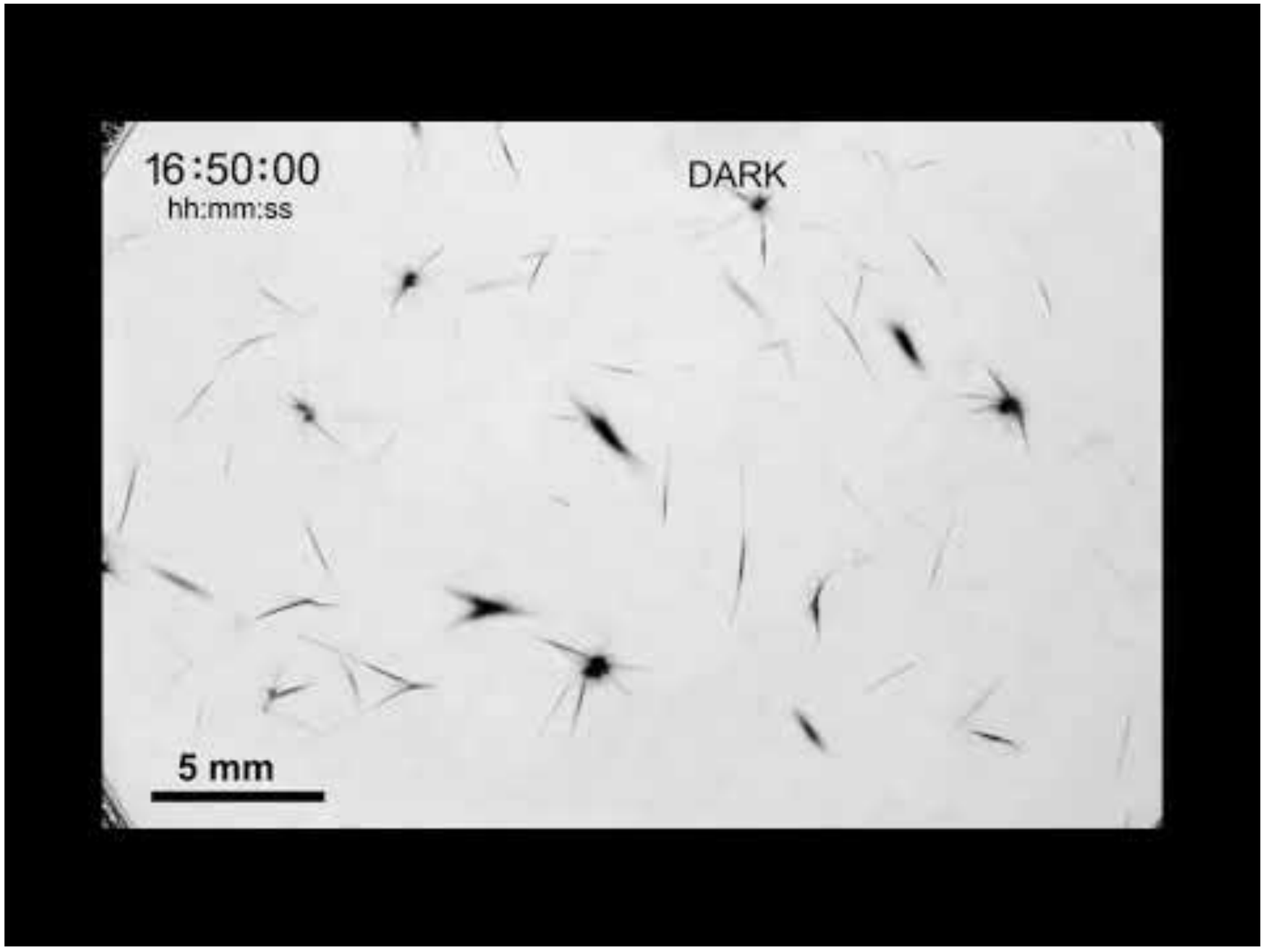


Chang and LiWang, 2005

Synechococcus phototaxis



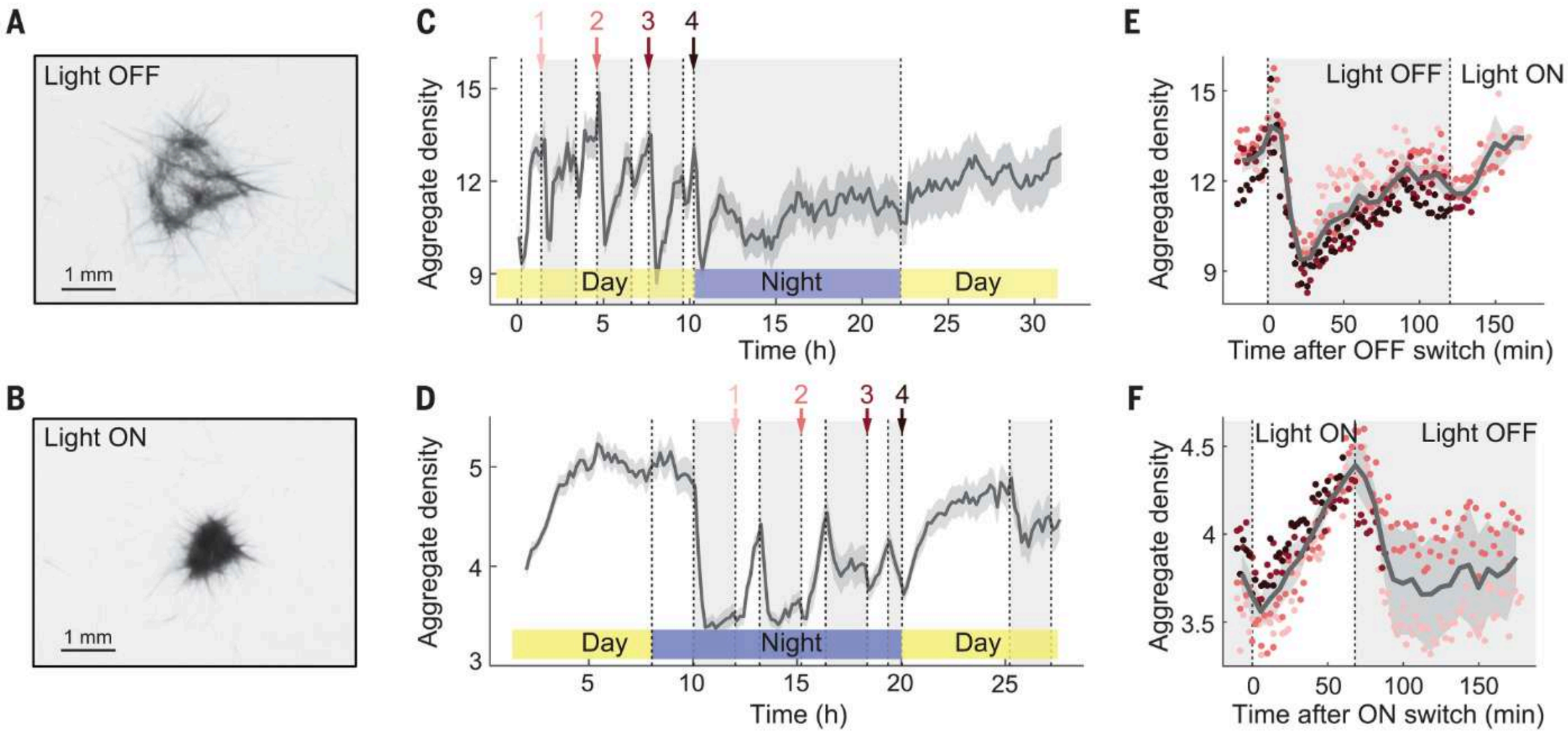
Trichodesmium gliding motility



Pfreundt et al. 2023

Credit: Science (2023). DOI: 10.1126/science.adf2753

Trichodesmium aggregate density changes rapidly upon switches in light intensity



Pfreundt et al. 2023

Trichodesmium dust capturing, as a source of Fe



Planctomycetes

- Floating fungi
- Marine sediments, surfaces and marine waters
- Expandable periplasmic space
- Belong to superphylum: Planctomycetes-Verrucomicrobia-Chlamydide, PVC
- Aerobic chemoheterotrophs
- From 6% of bacterial abundance via 16SrRNA gene (amplicon sequencing)and < 1% in open ocean
- Associated with marine snow and algae bloom
- Break down complex heteropolysaccharides
- Annamoxisome, anaerobic oxidation of ammonia

Bacteroidetes

- From 20% of bacterial abundance via 16SrRNA gene (amplicon sequencing) in coastal and open ocean
- Attached to phytoplankton particulate aggregates, sediment, hydrothermal vents, polare region and associated with animals
- Pigments, carotenoid
- Degrade agar and simple and complex carbohydrates (e.g. chitin)
- Present in human gut—> pollution indicator

Chloroflexi

- From 40% of bacterial abundance via 16SrRNA gene (amplicon sequencing) in deep ocean
- Wide habitat
- Diverse metabolisms: anoxygenic photosynthesis, aerobic or anaerobic heterotrophy
- Degradation of hydrocarbons
- DOM to refractory DOM
- Still unknown

Aquificae & Thermotogae

- **Primitive branching**
- **Aquificae:**
    - ✳ **Thermophilic, microaerophilic oxidizing H_2 —> using H_2, tiosulfate or sulfur as *e*- donor and O_2 as *e*- acceptor, fixing C via reductive TCA cycle**
    - ✳ **Primary production in hydrothermal vent system**
- **Thermotogae:**
    - ✳ **Anaerobic, thermophilic, as well as mesophilic**
    - ✳ **Unique outer membrane**
    - ✳ **Shallow and deep hydrothermal vents**
    - ✳ **Fermentative and anaerobic chemoorganotrophs —> carbohydrates**
    - ✳ **Fix N_2 and reduce sulfur to H_2S**

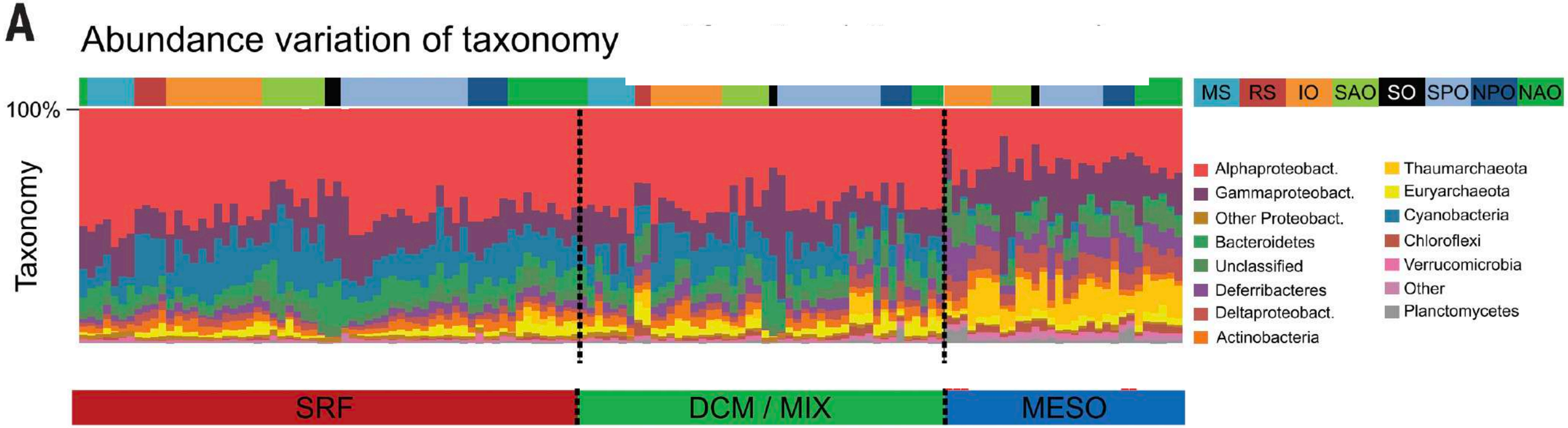
Firmicutes

- Gram-positive, thick peptidoglycan
- Low G-C content
- Free-living or filamentous
- Bacilli and Clostridia
- ~4 % in water column and ~ 8% in sediment bacterial abundance via 16SrRNA gene (amplicon sequencing)
- Produce spores
- Degrade complex polysaccharides —> gut associated

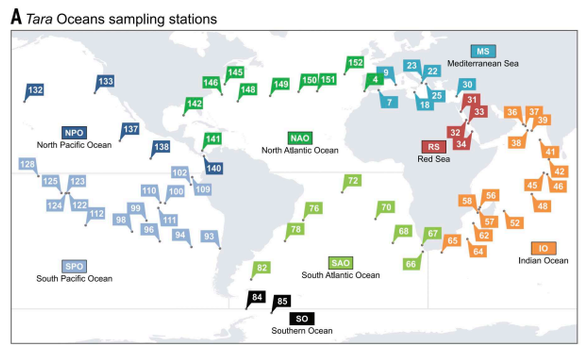
Actinobacteria

- Gram-positive
- High G-C content
- Free-living or filamentous
- Sediments, marine particles in water column
- Decompose organic matter
- Produce secondary metabolites and antibiotics

Tara: 0-1000 m water column, I

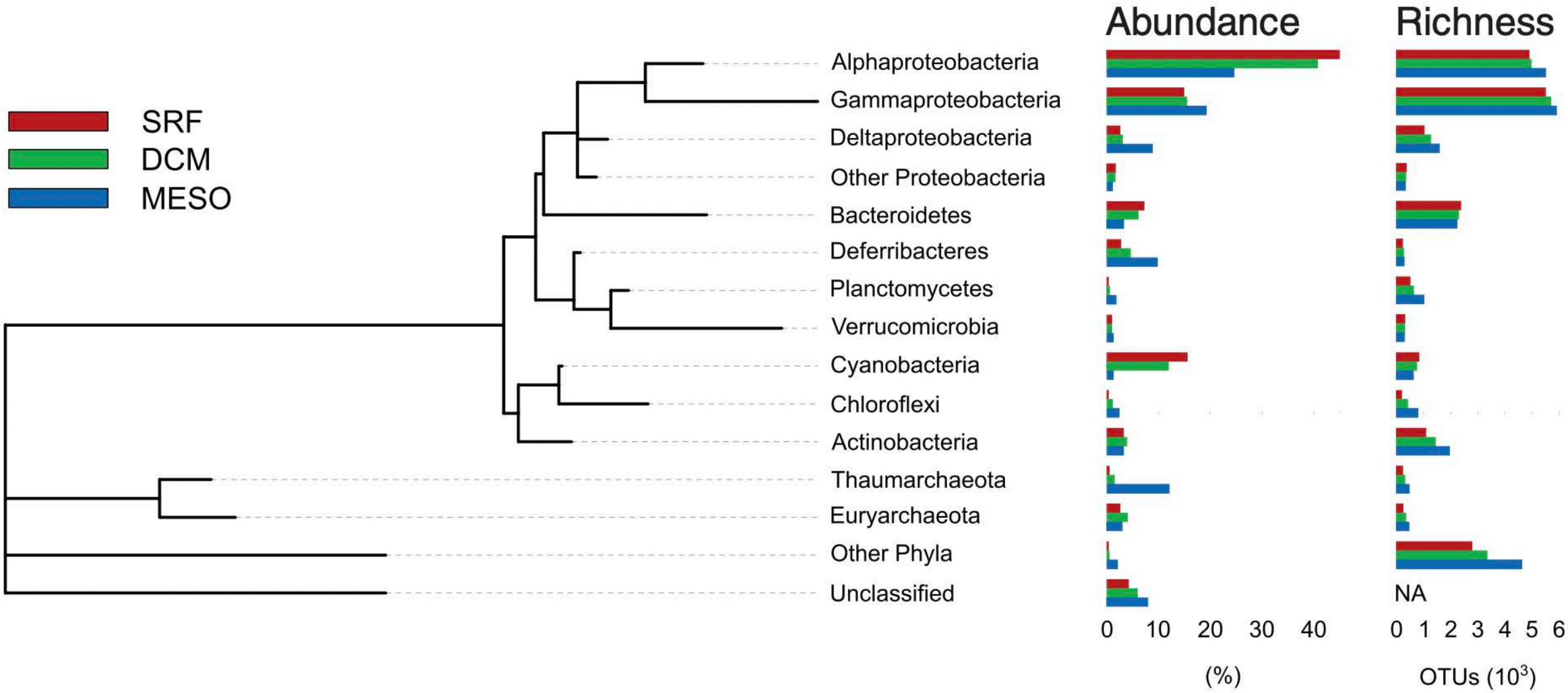


- Alphaproteobacteria the most abundant in surface and DCM layers
- Alpha- and Gammaproteobacteria and Bacteroideted most abundant in surface and DCM layers
- In Mesoplelagic, Deltaproteobacteria, Atinobacteria and Archaea are dominant with Alpha- and Gammaproteobacteira
- Cyanobacteria dominant in surface waters



Sunagawa et al., 2015

Tara: 0-1000 m water column, II

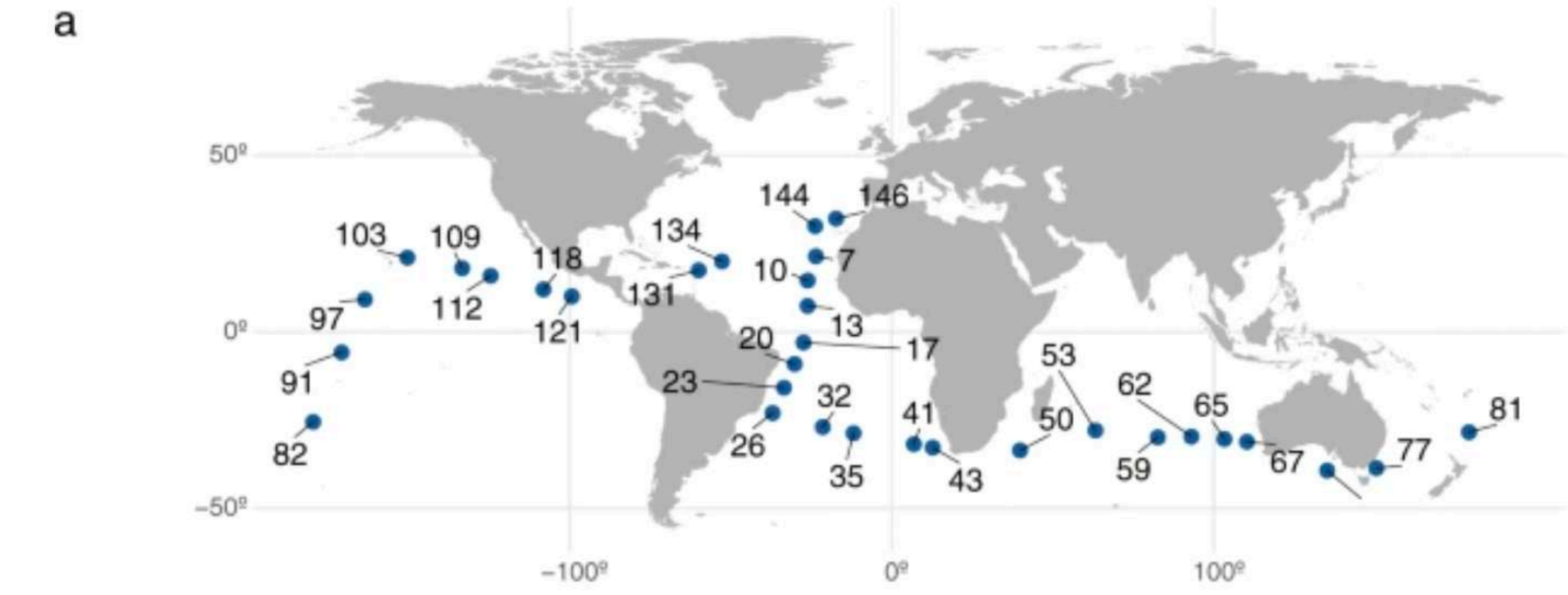


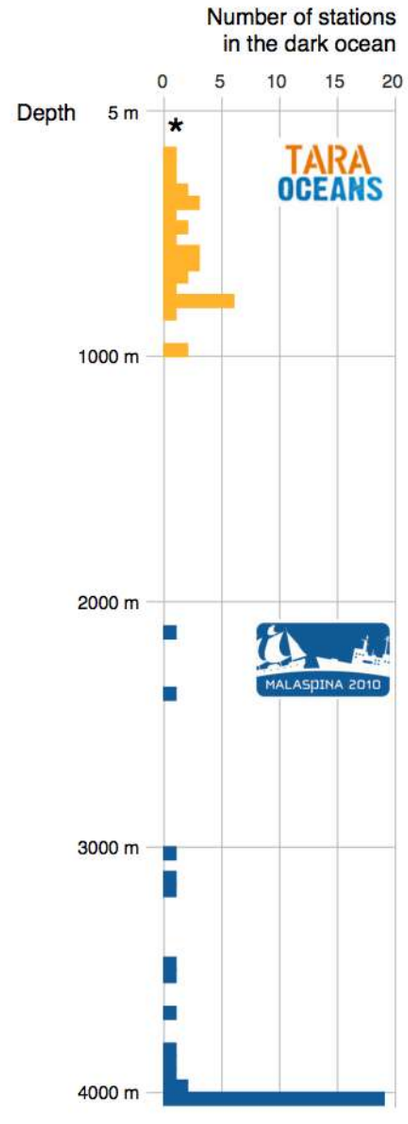
- **Highest abundance of Alphaproteobacteria, Gammaproteobaceira and Cyanobacteria**
- **Highest richness of Alpha-, Gammaproteobacteria and Bateroidetes**

Sunagawa et al., 2015

MALASPINA: 2000-4000 m water column I

Fig. 1: Malaspina Deep Ocean Genetic Resources.

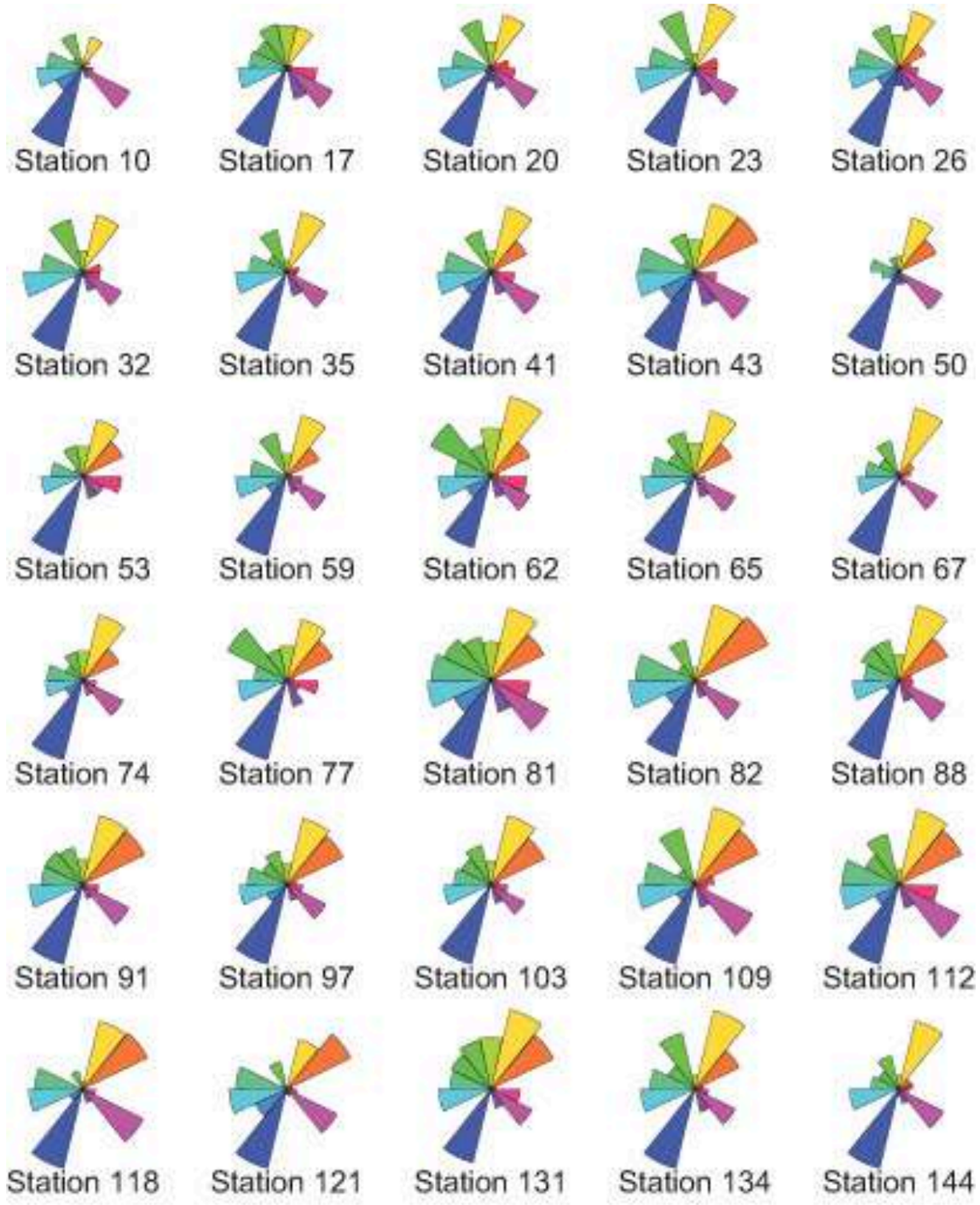




Acinas et al., 2019 and 2021

MALASPINA: 2000-4000 m water column II

- **Alpha- and Gammaproteobacteria the most abundant**
- **Thaumarcheota and Actinobacteria abundant**





Salazar et al., 2015