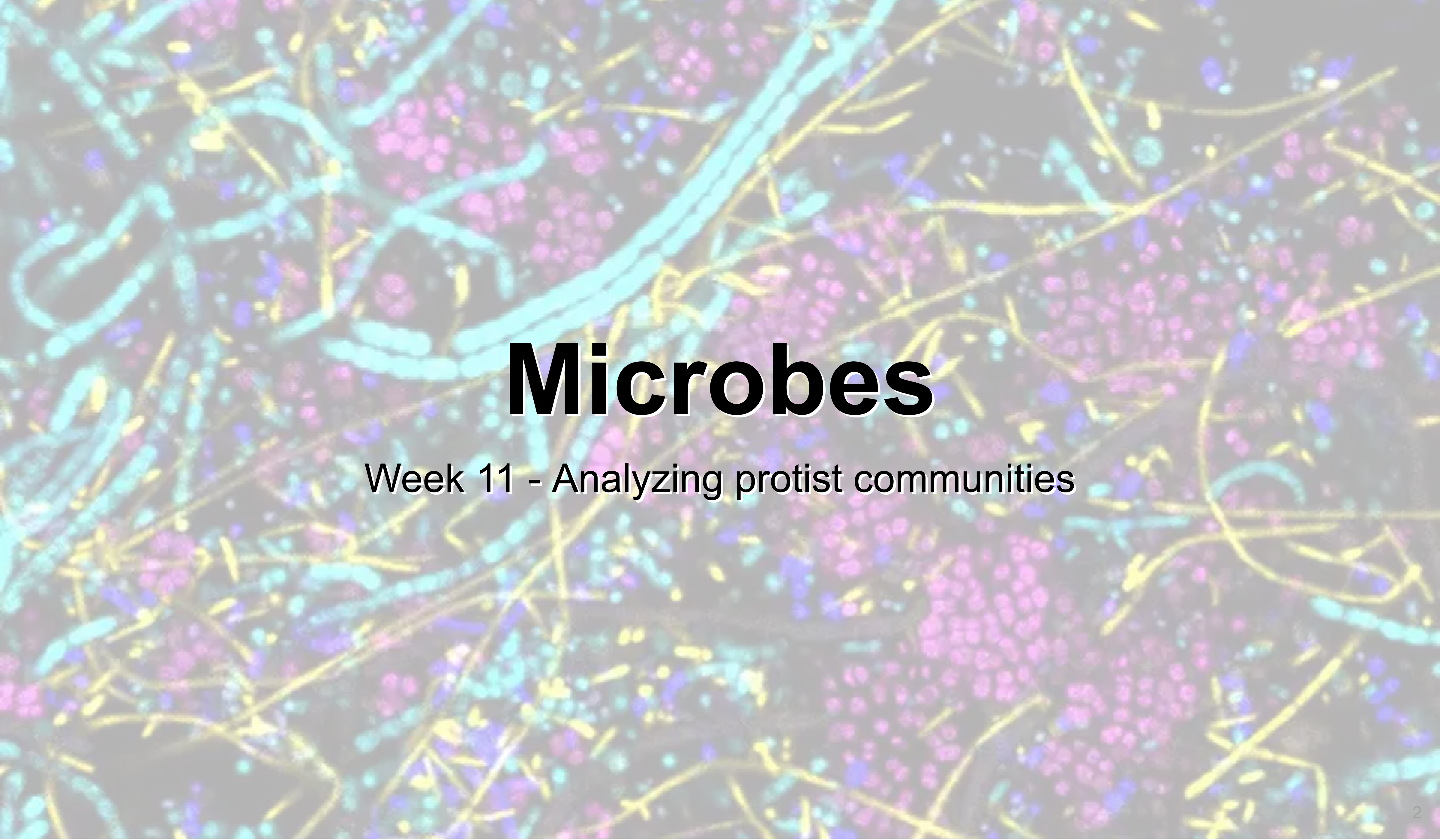
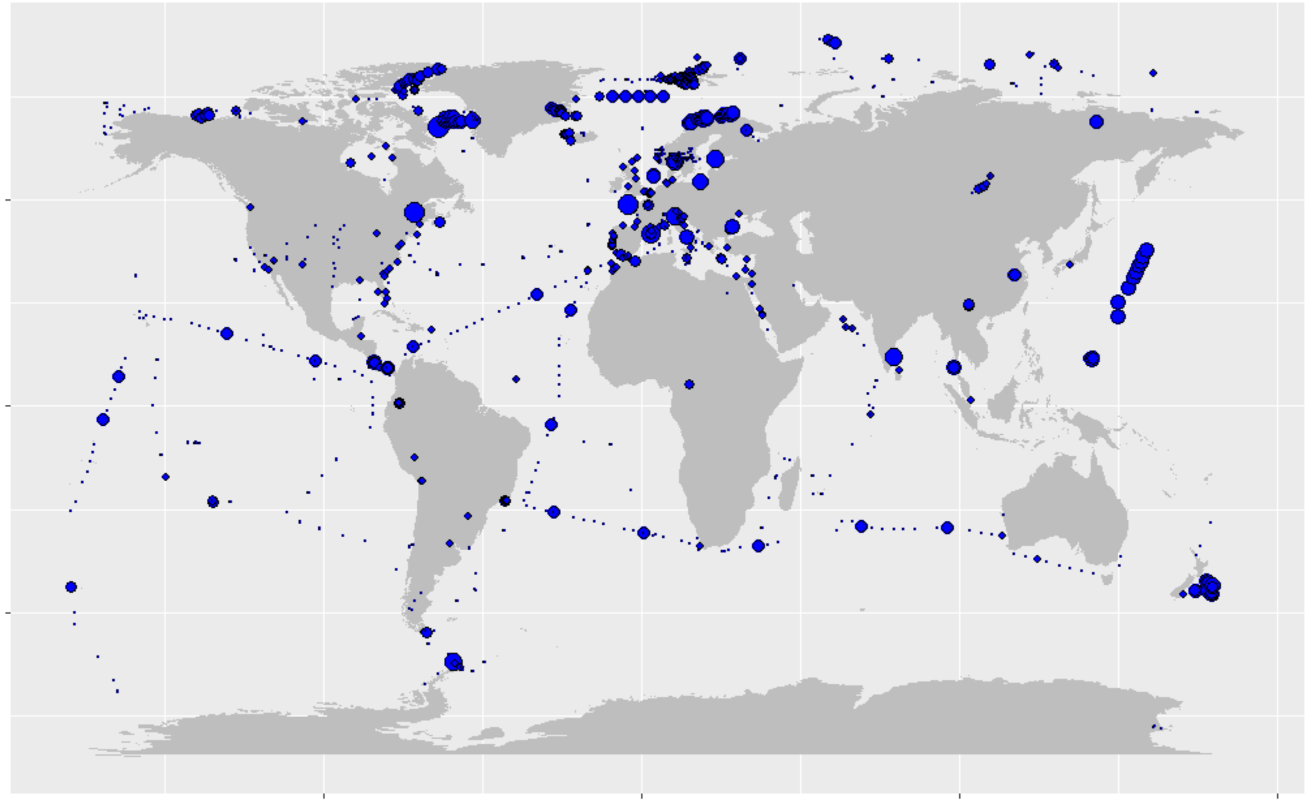


Microbes

Week 11 - Analyzing protist communities

MetaPR2

A database of metabarcodes



Daniel Vaulot

2022-10-24

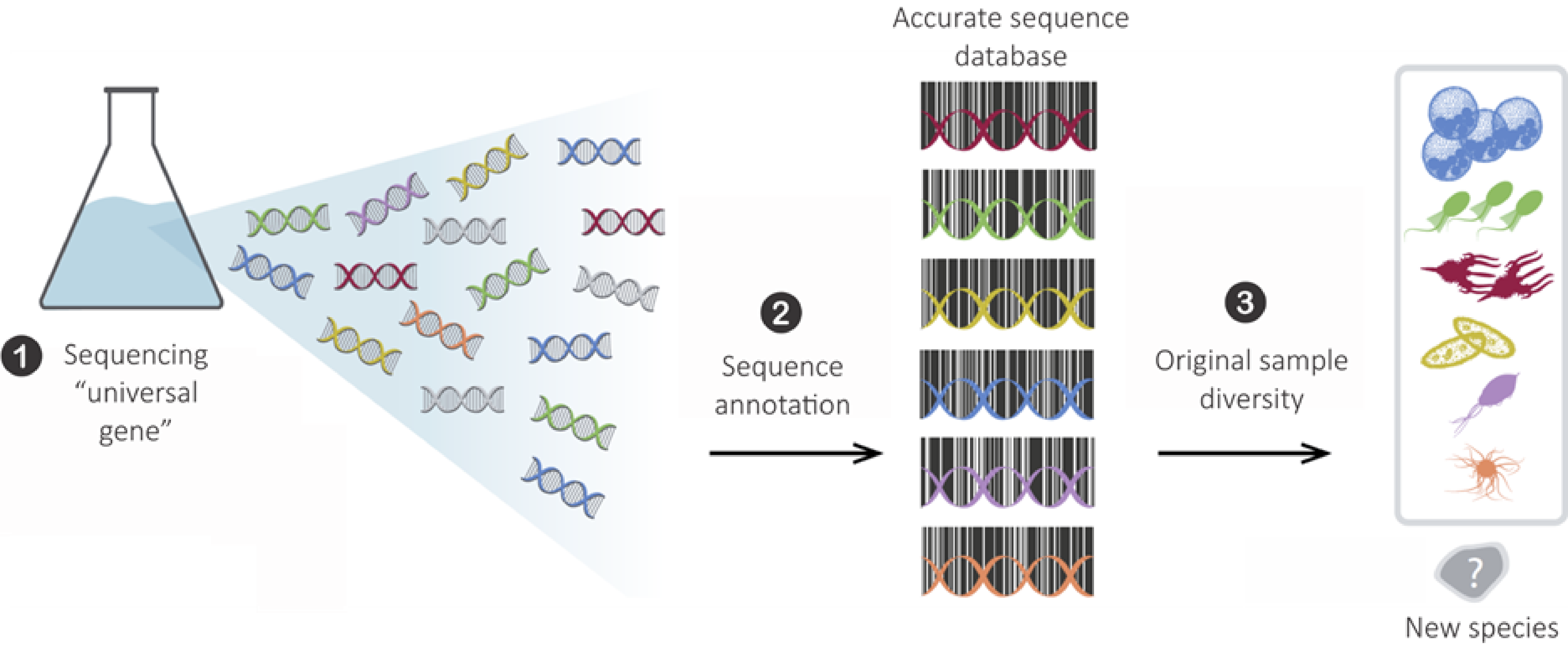
CNRS • SORBONNE UNIVERSITÉ
Station Biologique de Roscoff

NANYANG TECHNOLOGICAL UNIVERSITY
SINGAPORE

Outline

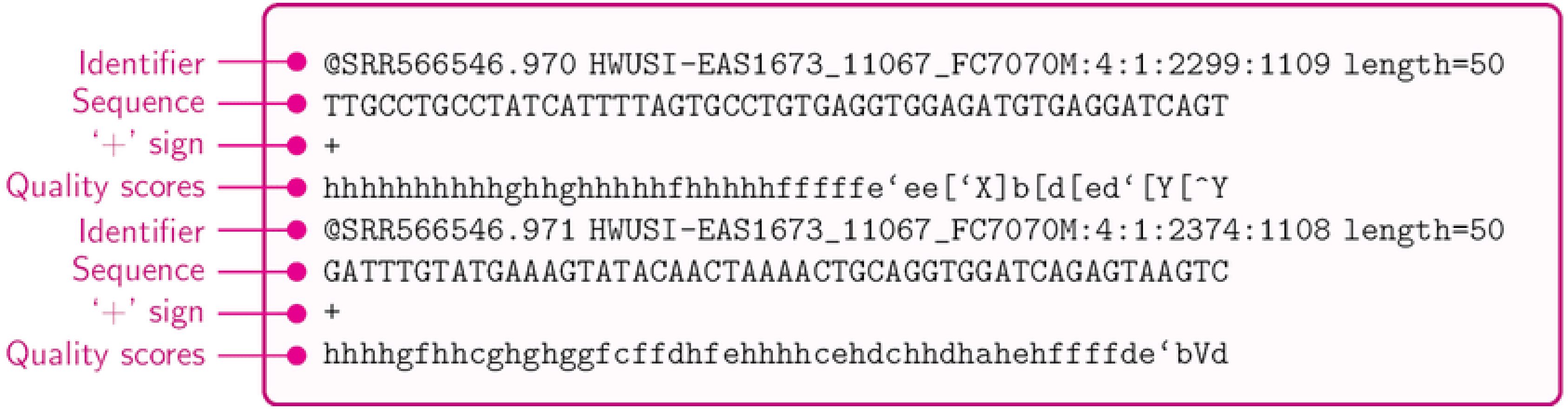
- Metabarcoding data
- Factors affecting protist communities
- Diversity
- Visualization/Analysis
- MetaPR2 in practice
- Final presentation

Metabarcoding



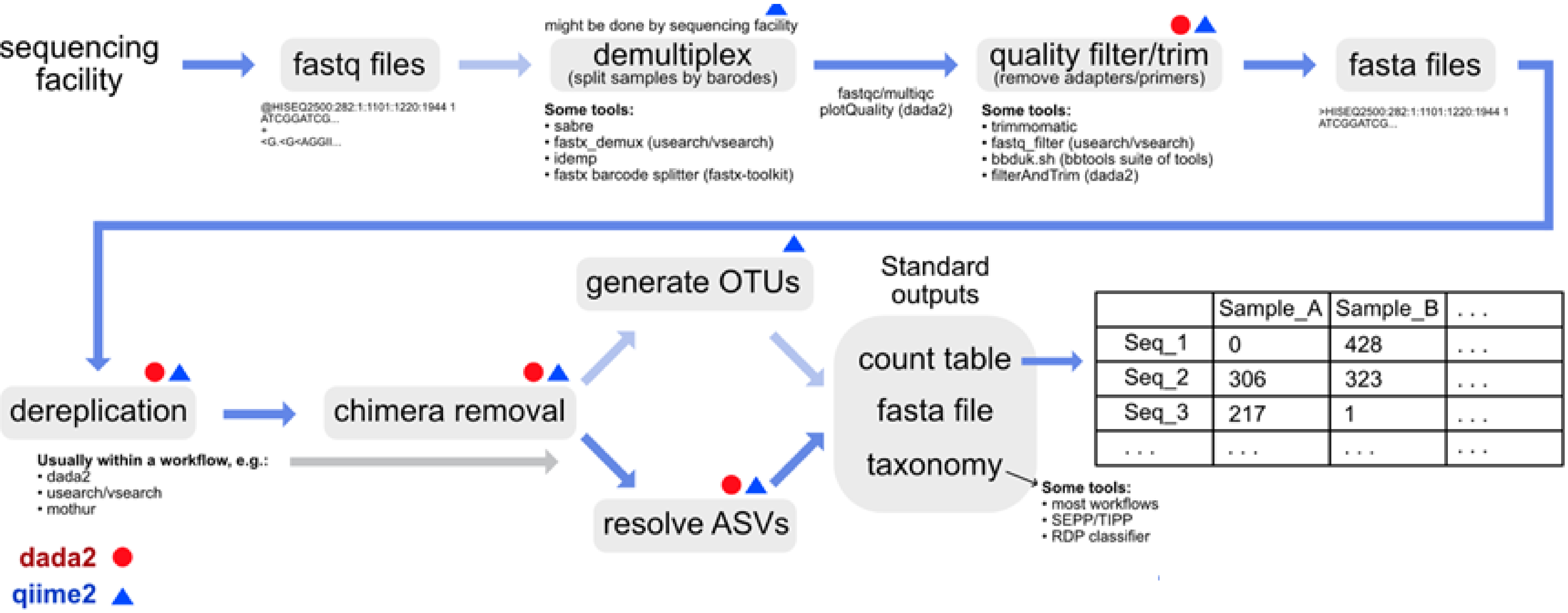
Metabarcoding

Fastq files



```
@SRR566546.970 HWUSI-EAS1673_11067_FC7070M:4:1:2299:1109 length=50
TTGCCTGCCTATCATTTTAGTGCCTGTGAGGTGGAGATGTGAGGATCAGT
+
hhhhhhhhhhghhghhhhhfhhhhhfffffe`ee[`X]b[d[ed`[Y[^Y
@SRR566546.971 HWUSI-EAS1673_11067_FC7070M:4:1:2374:1108 length=50
GATTTGTATGAAAGTATACAACTAAAACTGCAGGTGGATCAGAGTAAGTC
+
hhhhgfhhcghghggfcffdhfehhhhcehdchhdhahehffffde`bVd
```

Metabarcoding



sequencing facility

fastq files

```
@HISEQ2500:282:1:1101:1220:1944 1
ATCGGATCG...
+
<G.<G<AGGII...
```

might be done by sequencing facility ▲

demultiplex
(split samples by barcodes)

Some tools:
- sabre
- fastx_demux (usearch/vsearch)
- idemp
- fastx barcode splitter (fastx-toolkit)

fastqc/multiqc
plotQuality (dada2)

● ▲ quality filter/trim
(remove adapters/primers)

Some tools:
- trimmomatic
- fastq_filter (usearch/vsearch)
- bbduk.sh (bbtools suite of tools)
- filterAndTrim (dada2)

fasta files

```
>HISEQ2500:282:1:1101:1220:1944 1
ATCGGATCG...
```

dereplication ● ▲

Usually within a workflow, e.g.:
- dada2
- usearch/vsearch
- mothur

chimera removal ● ▲

generate OTUs ▲

resolve ASVs ● ▲

Standard outputs

count table

fasta file

taxonomy

Some tools:
- most workflows
- SEPP/TIPP
- RDP classifier

	Sample_A	Sample_B	. . .
Seq_1	0	428	. . .
Seq_2	306	323	. . .
Seq_3	217	1	. . .
. . .	. . .	. . .	. . .

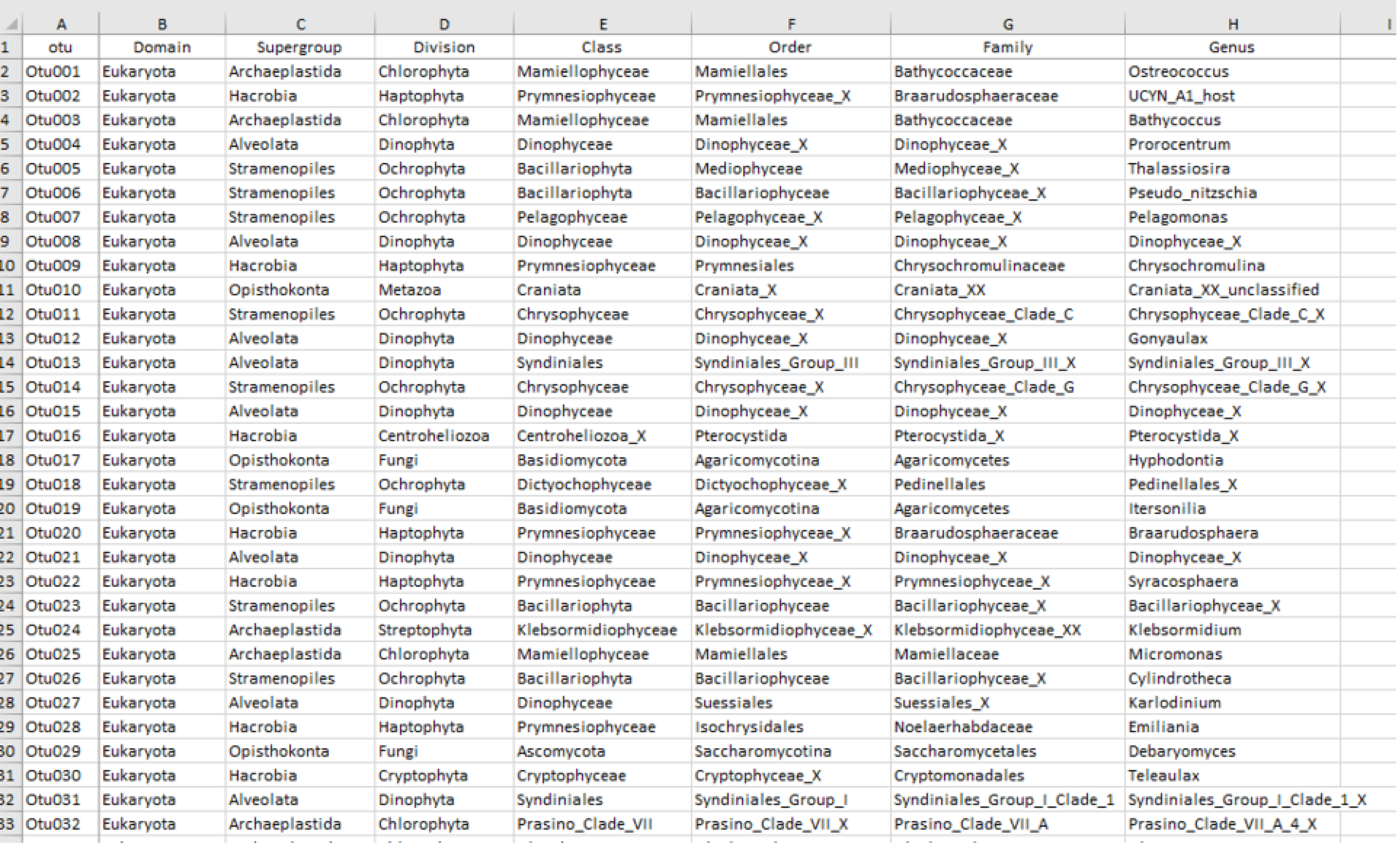
dada2 ●

qiime2 ▲

Metabarcoding

Data tables

ASVs Abundance Metadata Merged



otu	Domain	Supergroup	Division	Class	Order	Family	Genus
Otu001	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Ostreococcus
Otu002	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiophyceae_X	Braarudosphaeraceae	UCYN_A1_host
Otu003	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Bathycoccaceae	Bathycoccus
Otu004	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Prorocentrum
Otu005	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Mediophyceae	Mediophyceae_X	Thalassiosira
Otu006	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyceae	Bacillariophyceae_X	Pseudo_nitzschia
Otu007	Eukaryota	Stramenopiles	Ochrophyta	Pelagophyceae	Pelagophyceae_X	Pelagophyceae_X	Pelagomonas
Otu008	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Dinophyceae_X
Otu009	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiales	Chrysochromulinaceae	Chrysochromulina
Otu010	Eukaryota	Opisthokonta	Metazoa	Craniata	Craniata_X	Craniata_XX	Craniata_XX_unclassified
Otu011	Eukaryota	Stramenopiles	Ochrophyta	Chrysophyceae	Chrysophyceae_X	Chrysophyceae_Clade_C	Chrysophyceae_Clade_C_X
Otu012	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Gonyaulax
Otu013	Eukaryota	Alveolata	Dinophyta	Syndiniales	Syndiniales_Group_III	Syndiniales_Group_III_X	Syndiniales_Group_III_X
Otu014	Eukaryota	Stramenopiles	Ochrophyta	Chrysophyceae	Chrysophyceae_X	Chrysophyceae_Clade_G	Chrysophyceae_Clade_G_X
Otu015	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Dinophyceae_X
Otu016	Eukaryota	Hacrobia	Centroheliozoa	Centroheliozoa_X	Pterocystida	Pterocystida_X	Pterocystida_X
Otu017	Eukaryota	Opisthokonta	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Hyphodontia
Otu018	Eukaryota	Stramenopiles	Ochrophyta	Dictyochophyceae	Dictyochophyceae_X	Pedinellales	Pedinellales_X
Otu019	Eukaryota	Opisthokonta	Fungi	Basidiomycota	Agaricomycotina	Agaricomycetes	Itersonilia
Otu020	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiophyceae_X	Braarudosphaeraceae	Braarudosphaera
Otu021	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Dinophyceae_X	Dinophyceae_X	Dinophyceae_X
Otu022	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Prymnesiophyceae_X	Prymnesiophyceae_X	Syracosphaera
Otu023	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyceae	Bacillariophyceae_X	Bacillariophyceae_X
Otu024	Eukaryota	Archaeplastida	Streptophyta	Klebsormidiophyceae	Klebsormidiophyceae_X	Klebsormidiophyceae_XX	Klebsormidium
Otu025	Eukaryota	Archaeplastida	Chlorophyta	Mamiellophyceae	Mamiellales	Mamiellaceae	Micromonas
Otu026	Eukaryota	Stramenopiles	Ochrophyta	Bacillariophyta	Bacillariophyceae	Bacillariophyceae_X	Cylindrotheca
Otu027	Eukaryota	Alveolata	Dinophyta	Dinophyceae	Suessiales	Suessiales_X	Karlodinium
Otu028	Eukaryota	Hacrobia	Haptophyta	Prymnesiophyceae	Isochrysidales	Noelaerhabdaceae	Emiliania
Otu029	Eukaryota	Opisthokonta	Fungi	Ascomycota	Saccharomycotina	Saccharomycetales	Debaryomyces
Otu030	Eukaryota	Hacrobia	Cryptophyta	Cryptophyceae	Cryptophyceae_X	Cryptomonadales	Teleaulax
Otu031	Eukaryota	Alveolata	Dinophyta	Syndiniales	Syndiniales_Group_I	Syndiniales_Group_I_Clade_1	Syndiniales_Group_I_Clade_1_X
Otu032	Eukaryota	Archaeplastida	Chlorophyta	Prasino_Clade_VII	Prasino_Clade_VII_X	Prasino_Clade_VII_A	Prasino_Clade_VII_A_4_X

Factors affecting protist communities

Substrate

- Water
- Ice
- Sediment
- Soil
- Microbiome

Ecosystem

- Oceanic
- Coastal
- Rivers
- Lakes
- Terrestrial

Size fraction

- Total (0.2 µm -> 100 µm)
- Pico (0.2 µm -> 2-3 µm)
- Nano (2-3 µm -> 20 µm)
- Micro (20 µm -> 100-200 µm)
- Meso (100 µm -> 1000 µm)

Factors affecting protist communities

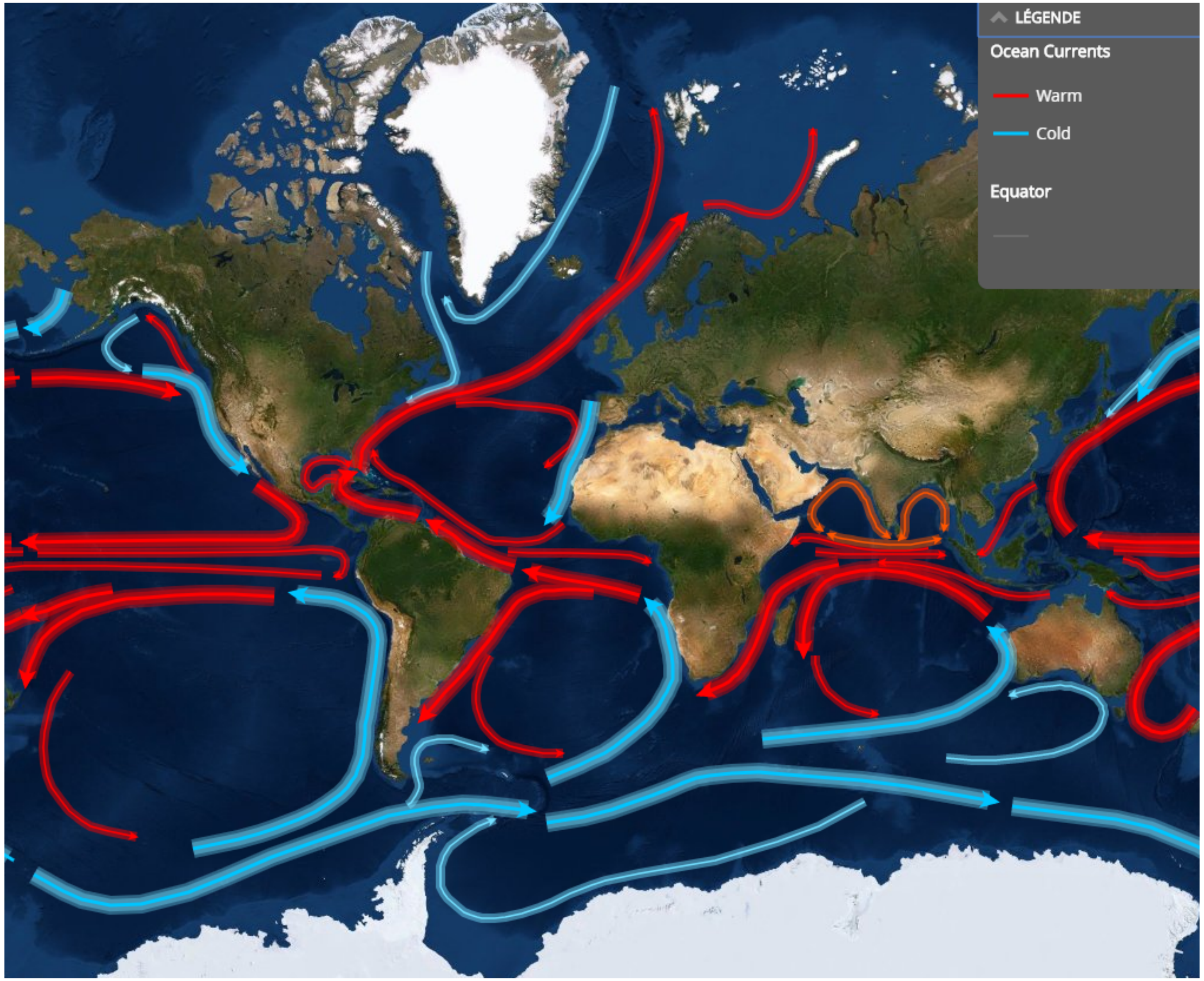
Environmental conditions

In oceanic waters:

- temperature
- salinity
- light
- nutrients

… which depend on:

- substrate (water vs.ice)
- latitude
- time of the year
- depth
- oceanic currents
- proximity of coast

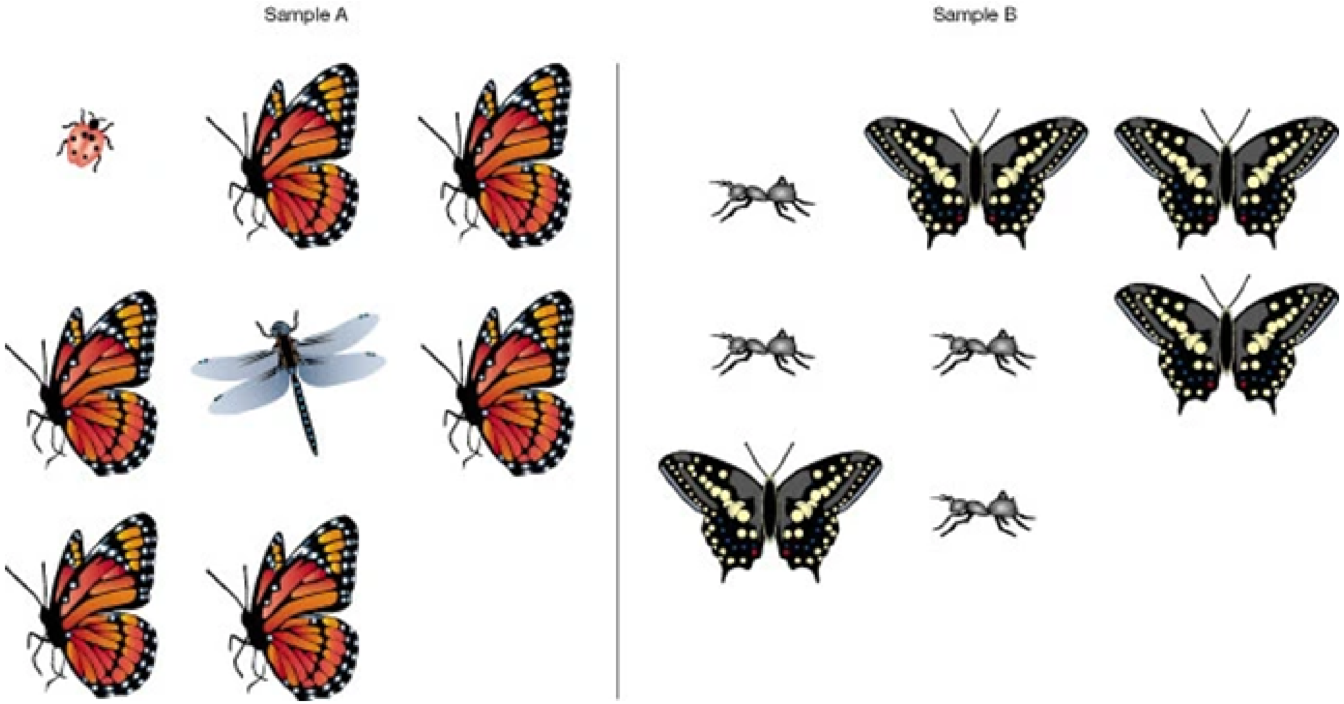


Diversity

Microbial species in a sample

- **species richness**: total number of species
- **species abundance**: proportion of each species

Richness vs. Evenness



Diversity

Alpha diversity - Diversity within a given sample

- **Chao 1** is a non-parametric estimator of the number of species in a community.
- **Shannon index**[1]

$$H = -\sum_{i=1}^{S} p_i \cdot \log p_i$$

Where:

p_i = fraction of the entire population made up of species i (proportion of a species i relative to total number of species present)

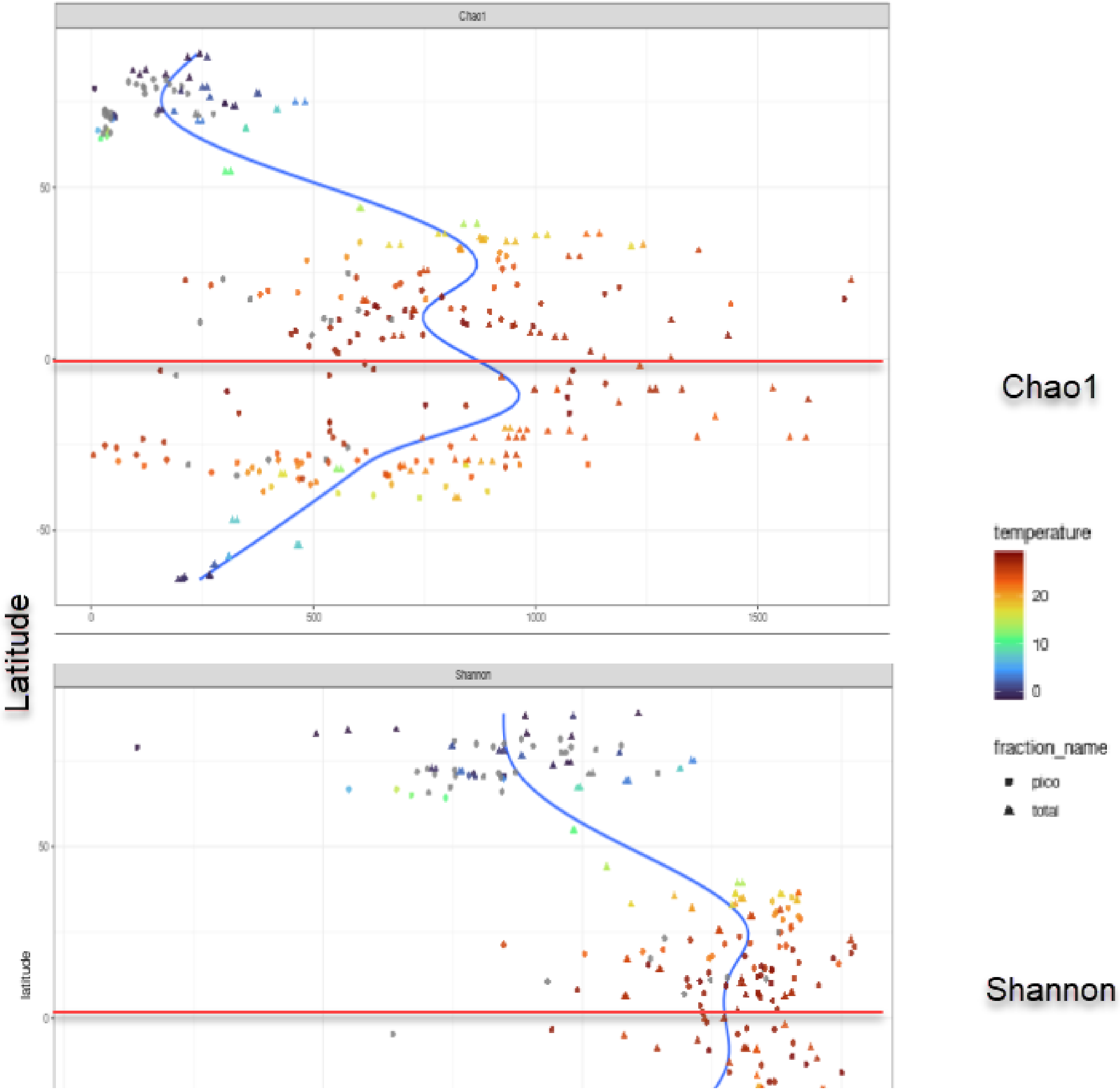
S = numbers of species encountered

A high value of H would be a representative of a diverse and equally distributed community and lower values represent less diverse community. A value of 0 would represent a community with just one species.

[1] Shannon's diversity index quantifies the uncertainty in predicting the species identity of an individual that is taken at random from the dataset.

Diversity

Alpha diversity - Effect of latitude



Diversity

Beta diversity - Compare diversity between samples

- Compute **distance** between samples:
  - **Bray-Curtis** dissimilarity: use abundance information
    - Varies between 0 and 1:
    - 0 means the two samples have the same composition
    - 1 means the two samples do not share any species

$$BC_{jk} = 1 - \frac{2\sum_{i=1}^{p} min(N_{ij},N_{ik})}{\sum_{i=1}^{p}(N_{ij}+N_{ik})}$$

where N_{ij} is the abundance of species i in sample j and p the total number of species

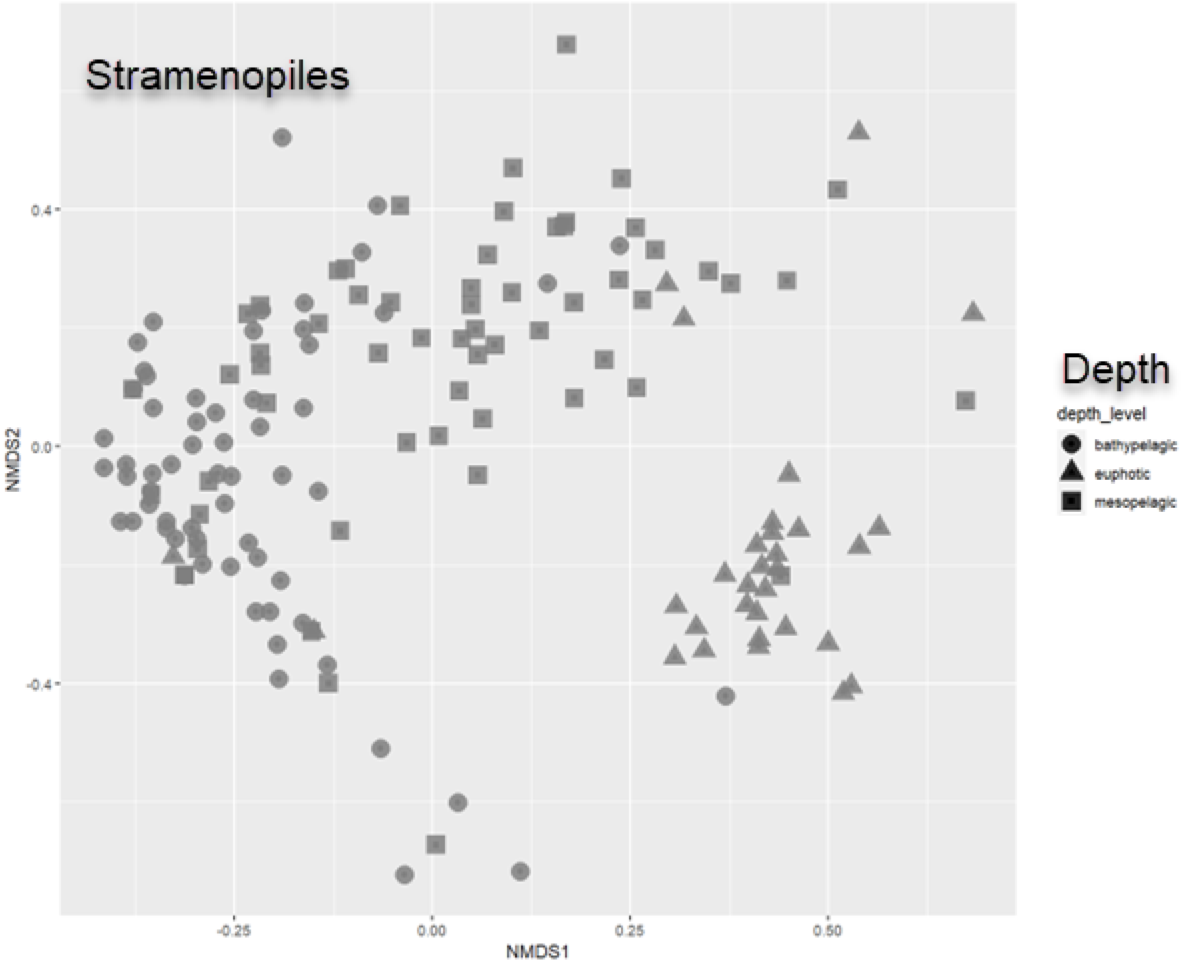
  - **Jaccard similarity** index
    - Number of common species between samples divided by total number of species in the two samples

$$J(A,B) = \frac{|A \cap B|}{|A \cup B|}$$

- **Ordinate** the samples
  - **NMDS**: Non-Metric Multidimensional Scaling

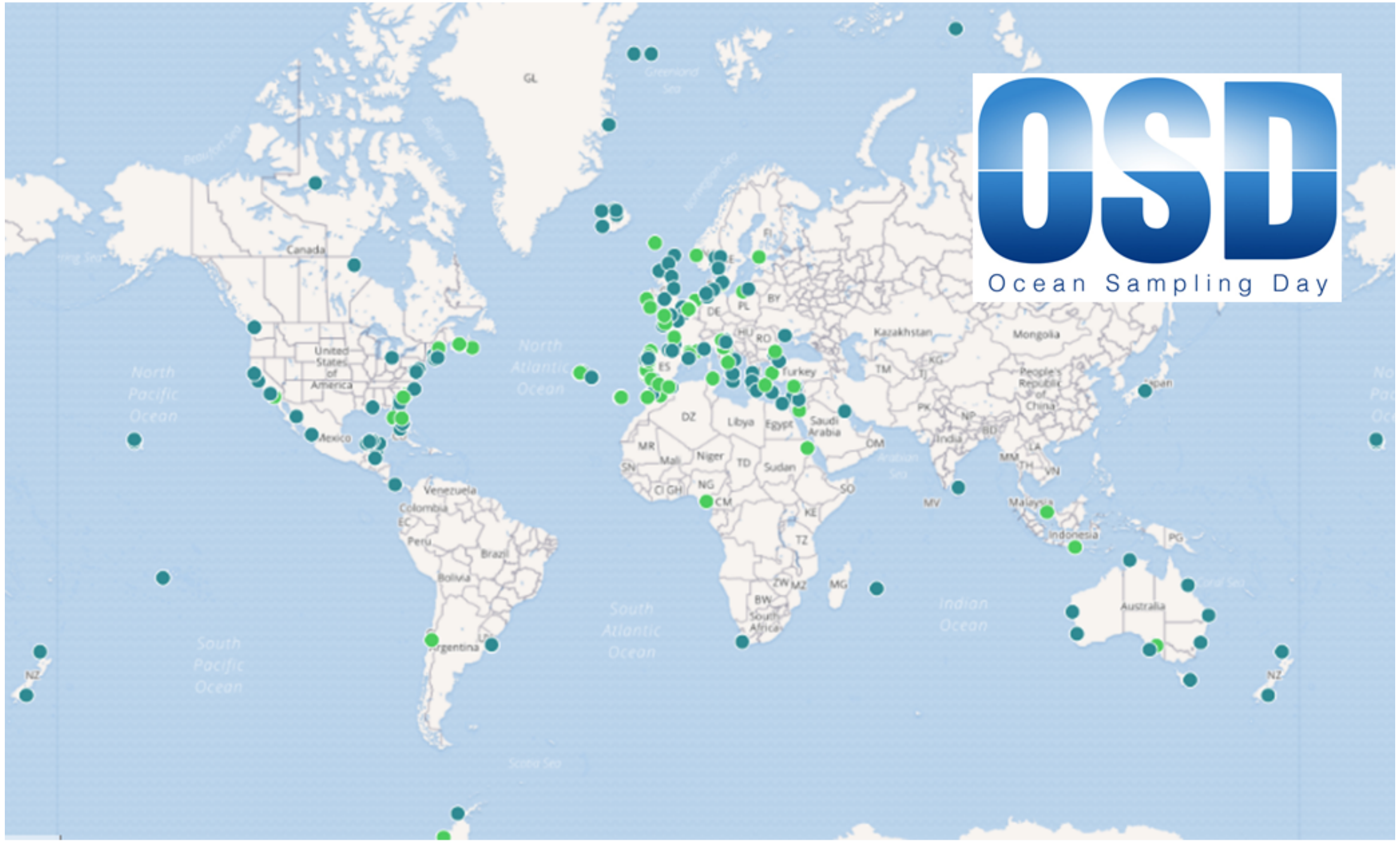
Diversity

Beta diversity - Effect of depth on Stramenopiles communities



MetaPR2 - Datasets

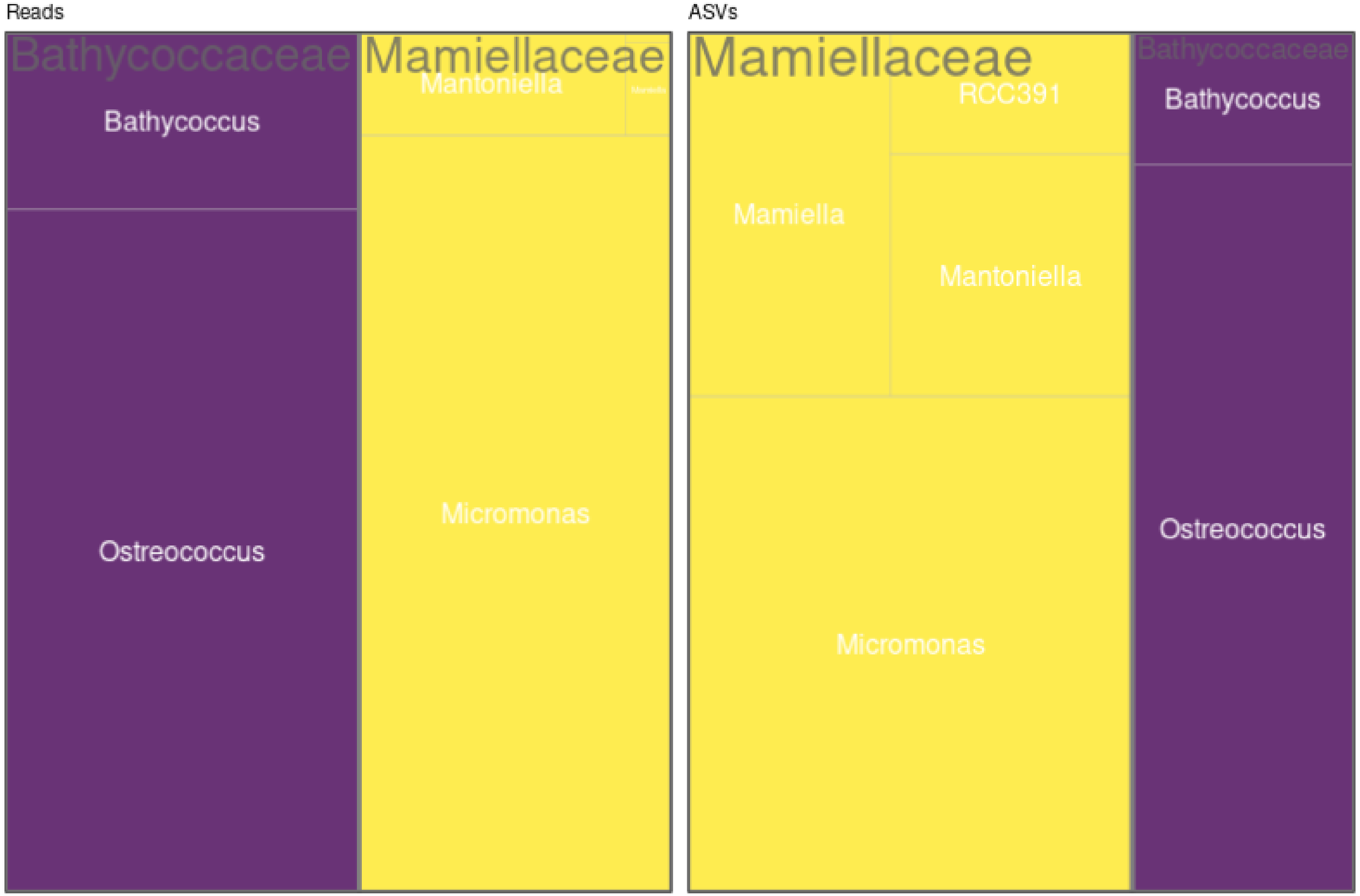
OSD Tara Oceans Malaspina Others (41 total)



MetaPR2 - Taxonomy

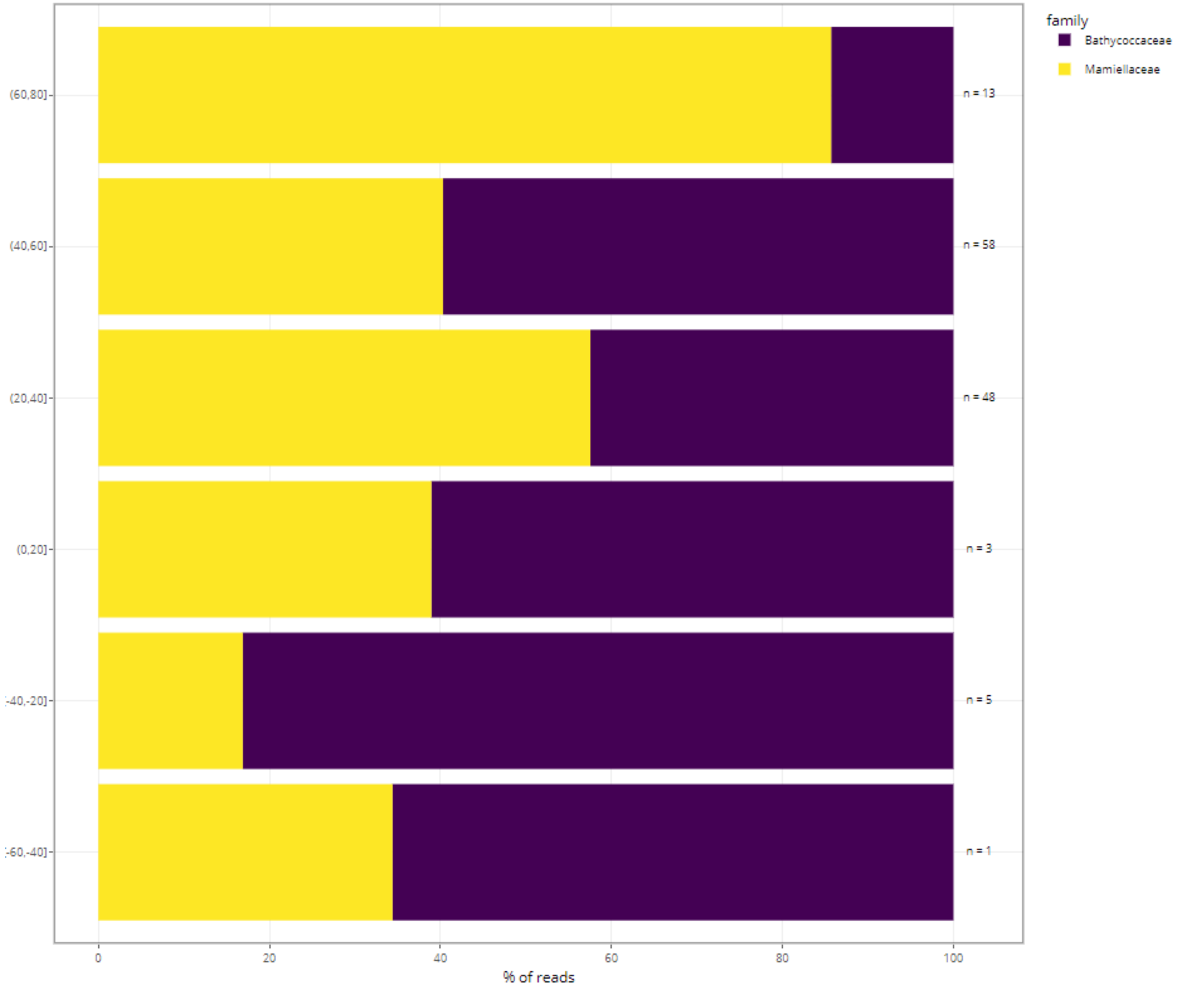
Eight levels:

- Kingdom: Eukaryota
- Supergroup: Archaeplastida
- Division: Chlorophyta
- Class: Mamiellophyceae
- Order: Mamielliales
- Family: Bathycoccaceae
- Genus: *Bathycococcus*
- Species: *B. prasinos*



MetaPR2 - Visualization

Barplots | Maps - Dominant | Maps - Pie charts | Diversity



MetaPR2 - In practice

Help

- Read in detail

Sample table

- dataset_name
- paper (can be useful to read)
- number of samples
- number of ASVs
- number of reads per sample (coverage)

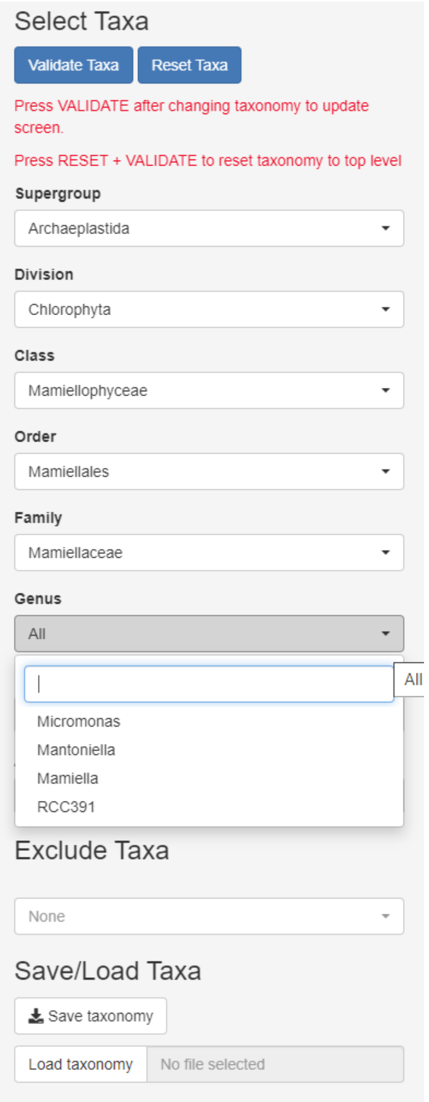
Sample selection

- Major datasets: OSD, Tara, Malaspina
- By habitat: oceanic, coastal etc…
    - Start by “marine global V4”
    - Extend to other habitats/datasets
- V4 vs V9
- DNA vs. RNA
- Ecosystems
- Sustrate: water, ice, soil…
- Size fractions: total, pico…
- Depth level: surface, euphotic…
- Minimum ASV: will filter out rare ASVs (e.g. 1000)
- Selection can be saved (yaml file)

MetaPR2 - In practice

Taxonomy

- Can select several taxa within one level
- Press validate every time you need to refresh
- Can exclude taxa to remove fungi, metazoa…
- Can save taxonomy and reload taxonomy (yaml file)



Press VALIDATE after changing taxonomy to update screen.

Press RESET + VALIDATE to reset taxonomy to top level

Supergroup: Archaeplastida

Division: Chlorophyta

Class: Mamiellophyceae

Order: Mamiellales

Family: Mamiellaceae

Genus: All

Micromonas
Mantoniella
Mamiella
RCC391

Exclude Taxa: None

No file selected

MetaPR2 - In practice

Treemaps

- Left panel: abundance (number of reads)
  - Reads are “normalized” to 100
- Right panel: diversity (number of ASVs)

Maps

- Read information at top
  - Taxo level
  - Number of samples with/without taxa
- Crosses where taxa absent
- Map types
  - Dominant
  - Pie chart
- Circle scale
  - Moving right increases size

Barplots

- taxonomy vs. function
- variables to use (but this depends on samples selected !)
  - fraction name
  - ecosystem
  - substrate
  - depth level
  - DNA_RNA
  - latitude
  - temperature
  - salinity
  - year, month, day for time series

MetaPR2 - In practice

Diversity

- Hit “Compute…” after refreshing taxonomy
- Time proportional to N samples and taxa
- Information about
  - Number of samples
  - Number of taxa (ASVs)

Alpha diversity

- X: Chao1, Shannon, Simpson (compare)
- Discretize continuous Y
- Change Y (see barplots)
- Change shape
- Change color

Beta diversity

- Ordination method (difference ?)
- Ordination distance (Bray, Jaccard…)
- Change color and shape

MetaPR2 - In practice

Download

- Download
    - datasets
    - samples
    - asv list with taxonomy
    - asv sequences

Only for those with extensive experience with data processing.

Final presentation

Taxonomic groups

Green algae

- *Prasinoderma*

- *Ostreococcus*

Ochrophyta (Stramenopiles)

- *Pelagomonas*, *Aureococcus*

- *Florenciella*

- Pinguiophyceae

Final presentation

Taxonomic groups

Diatoms

- *Pseudo-nitzschia*

- *Fragiliaropsis*

- *Minidiscus*

- *Rhizosolenia*

Dinoflagellates

- *Dinophysis*

- *Ceratium, Tripos*

Final presentation

Key points

- Look for key papers on this group
- What are the dominant species?
- What is the microdiversity [diversity within dominant species (ASVs)]?
- What is distribution ?
    - Substrate (water, ice…)
    - Ecosystems (marine, freshwater, terrestrial)
    - Size fraction
    - Depth layers (euphotic zone vs. meso and bathypelagic)
    - Latitudinal bands (polar, temperate, tropical)
    - Coastal vs Pelagic
- Alpha diversity
- Beta diversity

Final presentation

In practice

- Each group will have **max of 15’** to present their results. Your time will be cut after 15’.
- **Don’t overload** your presentation and run when talking. This will decrease the clarity of your presentation.
- **Share** equally time between group members.
- Introduce very briefly the main biological characteristics and ecological importance of your taxonomic group.
- Explain which **hypotheses/questions** your group were interested in.
- Explain the results you have observed. Focus on main points.
- Each group will have **5’ to answer questions**.

Final presentation

Evaluation

- Profs, TAs and PhD’s students will be judging your presentation (Only Profs will grade!):
  - **Grade scale**: 0 = unacceptable; 1 = poor; 2 = fair; 3 = good; 4 = outstanding

Criteria

- **Quality of presentation**
  - Slides (font size, amount on slide, legible and clear, references, no errors, etc).
  - Organization of presentation (outline, logical sequence, good transitions, easy to follow, etc).
  - Quality of oral presentation (well paced, projected voice, face audience, eye contact, confident, etc).
  - Did the group keep the audience interested? (show enthusiasm, command attention, did you learn something new?)
  - Was the presentation within the 15 minutes in length?
- **Content of presentation**
  - Was the presentation well structured ?
  - Did the group show an overall understanding of the topic? (background, objectives and significance thoroughly explained?).
  - Did the presentation cited the relevant material from the litterature?
  - Did the group answered questions accurately? Did the group possess good understanding of topic based on answers?