



# The Protist Ribosomal Reference database

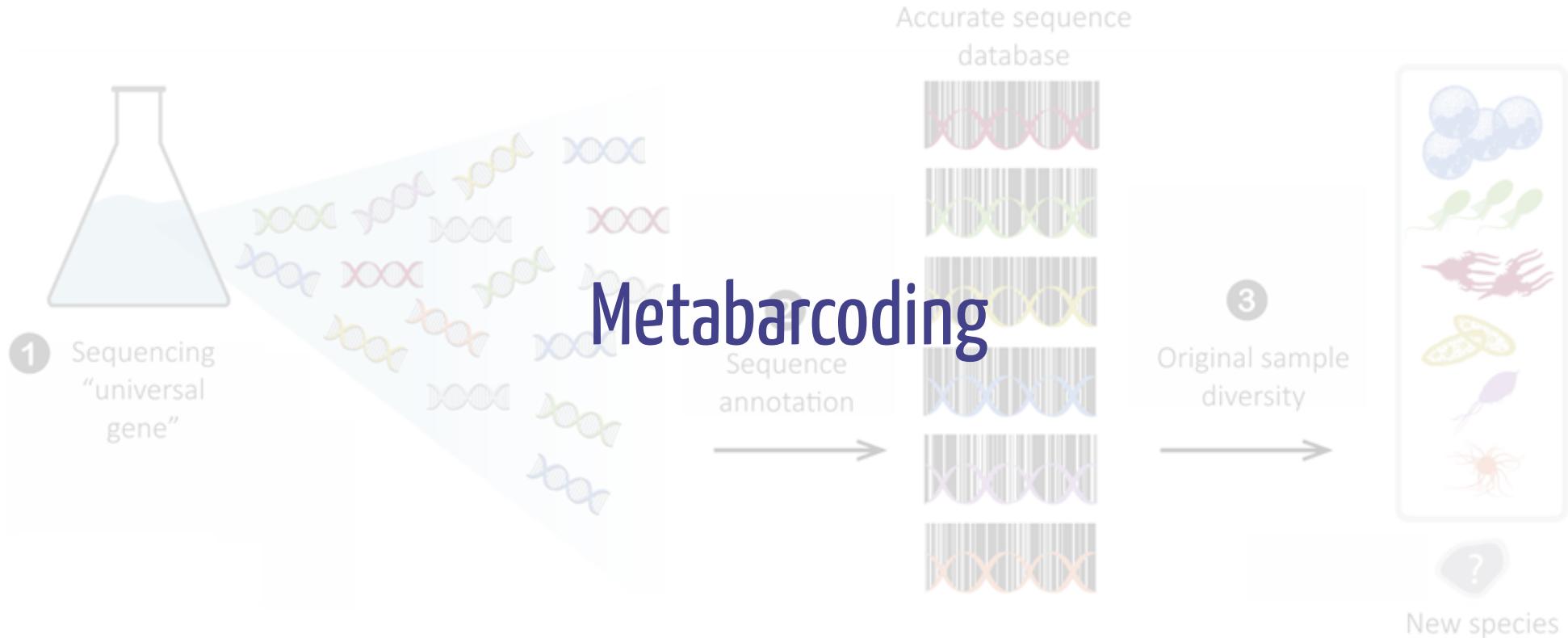
Daniel Vaulot and the PR<sup>2</sup> team

Protist-Online - 2020-06-24

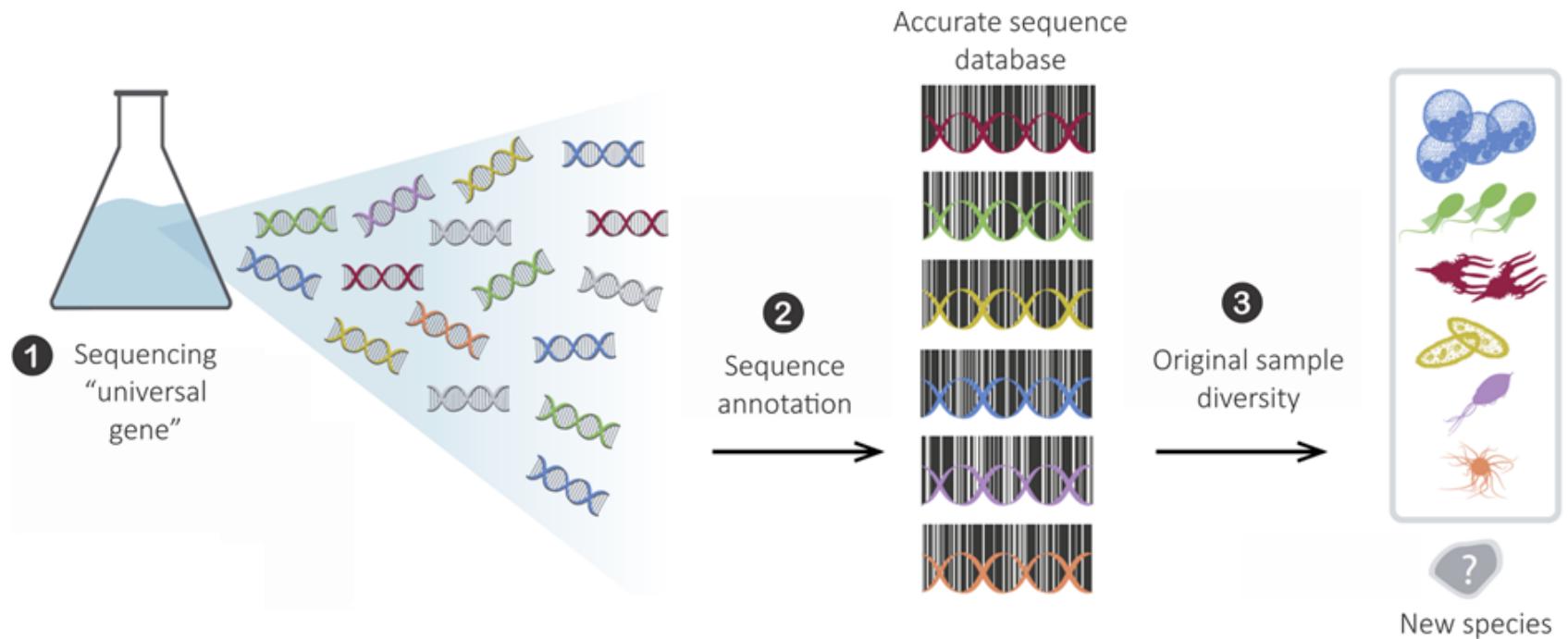


# Outline

- The explosion of metabarcoding
- PR<sup>2</sup>
- Major uses
- A database of metabarcodes: meta PR<sup>2</sup>
- What's next ?

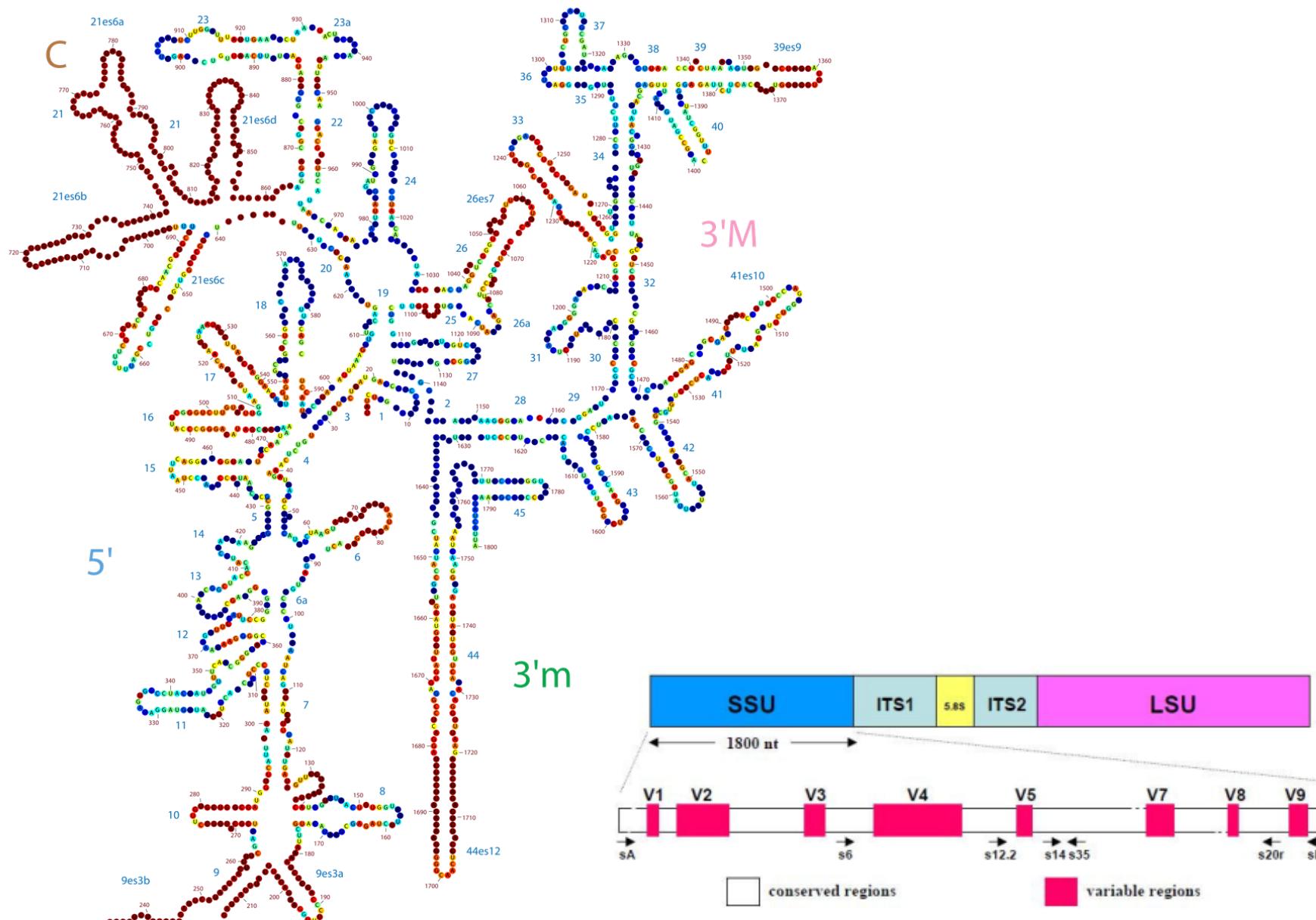


# Principle



# Target gene

- 18S rRNA
- ITS
- 16S plastid
- rbcL



*Saccharomyces cerevisiae*  
small subunit ribosomal RNA

# Assignment

## Reference database

- Genbank
  - Taxonomy very bad
- Silva
  - OK for prokaryotes
  - Eukaryotes bad

The screenshot shows the official website for the SILVA rRNA database project. At the top, there are three logos: elixir Core Data Resource, de.NBI (German Network for Bioinformatics Infrastructure), and the SILVA logo (high quality ribosomal RNA databases). Below the logos is a navigation bar with links: Home, SILVangs, Browser, Search, ACT, Download, Documentation, Projects, FISH & Probes, and Contact.

**SILVA**

Welcome to the SILVA rRNA database project  
A comprehensive on-line resource for quality checked and aligned ribosomal RNA sequence data.  
SILVA provides comprehensive, quality checked and regularly updated datasets of aligned small (16S/18S, SSU) and large subunit (23S/28S, LSU) ribosomal RNA (rRNA) sequences for all three domains of life (*Bacteria, Archaea and Eukarya*).  
SILVA are the official databases of the software package ARB.  
For more background information → [Click here](#)

**SILVAngs**

**silva**  
Check out our service for Next Generation Amplicon data

**SILVA Alignment, Classification and Tree (ACT) Service**  
The SILVA ACT service combines alignment, search and classify as well as reconstruction of trees in a single web application.  
SILVA ACT is available at: → [www.arb-silva.de/act](http://www.arb-silva.de/act)

**SILVA Tree Viewer**  
The SILVA Tree Viewer is a web application to browse and query the SILVA guide trees.  
A technical preview is available at → [www.arb-silva.de/treereader](http://www.arb-silva.de/treereader). The Tree Viewer for SILVA 132 LSU can be found → [here](#).

**News**

21.12.2019 **Merry Christmas & Happy New Year 2020**  
The SILVA Team wishes you a Merry Christmas & Happy New Year. Many thanks for all your feedback and support to improve SILVA and SILVangs. Looking forward to see you again in 2020.

16.12.2019 **SILVA 138 SSU released**  
Finally it is done... SILVA 138 SSU is released. LSU will follow in Spring 2020. Please note: The Treeviewer and SILVangs are still on SILVA 132. Updates are planned for January 2020. Many thanks for your patience, the next release will come faster (hopefully).

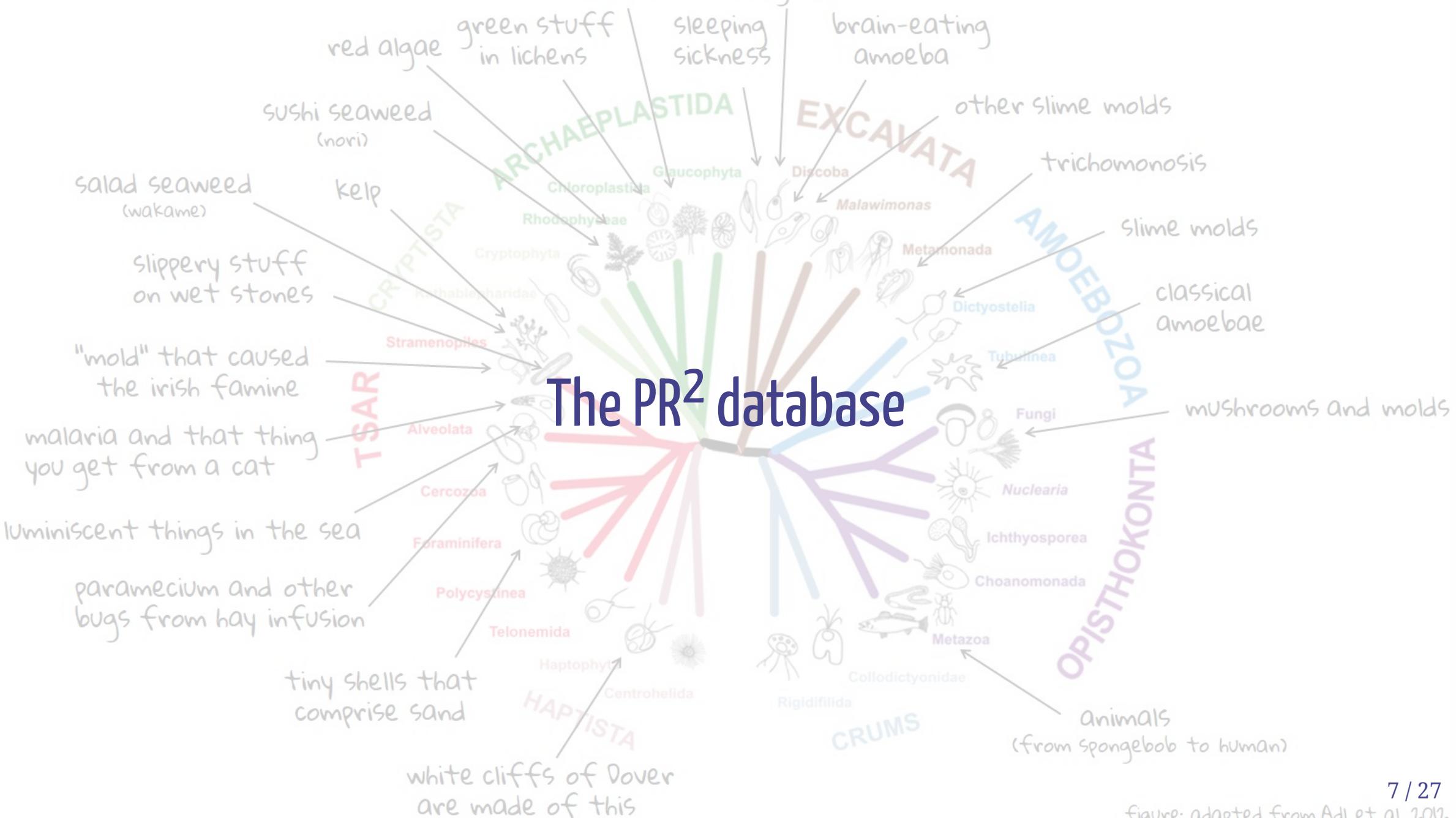
24.10.2019 **SILVA SSU Release 138 in November**  
We know you all are looking forward to the release of SILVA SSU 138. We are currently in the last steps of creating the release and plan to release SILVA SSU 138 in the week of November 11. We are sorry for the additional delay and the inconveniences it may cause.

03.09.2019 **Update on SILVA Release 138**  
We are sorry to inform you that the SILVA Release 138 is delayed further and we have decided to release the SSU and LSU datasets separately to compensate for the delay. We estimate the SSU datasets to be available in October and the LSU datasets by the end of the year. This news article will give you some background information on the release.  
[go to Archive ->](#)

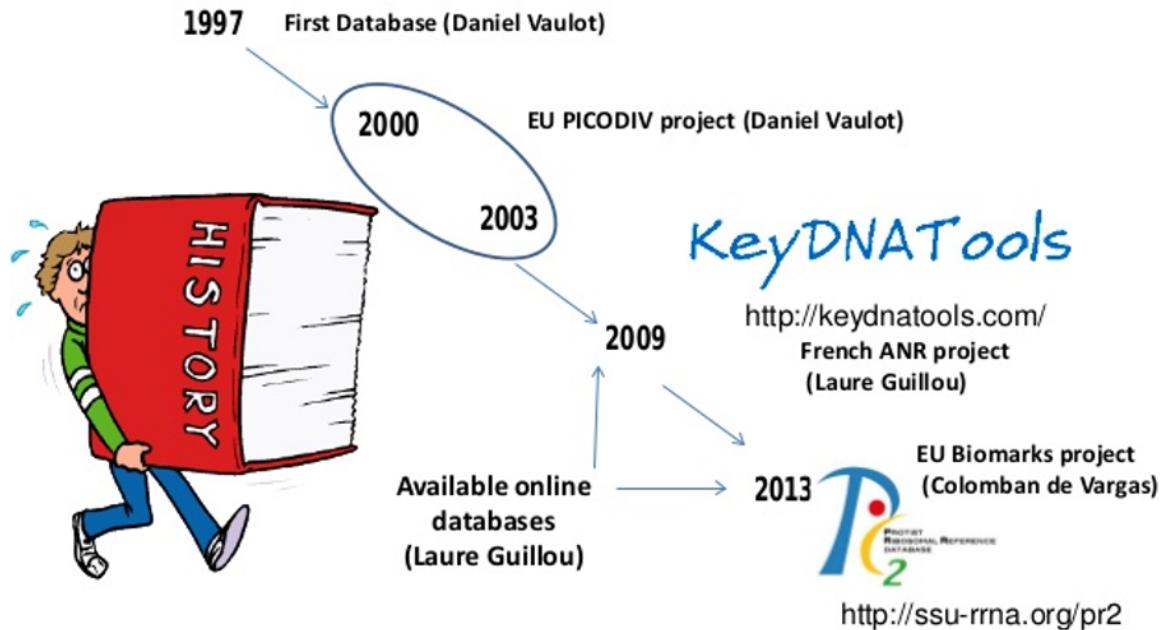
**User satisfaction survey**  
SILVA is now part of the German Network for Bioinformatics Infrastructure de.NBI.  
To evaluate and improve our quality of service we need your feedback. Please help us by participating in this short → [survey](#).

**de.NBI**  
GERMAN NETWORK FOR BIOINFORMATICS INFRASTRUCTURE

# The PR<sup>2</sup> database



# History



Published online 27 November 2012

Nucleic Acids Research, 2013, Vol. 41, Database issue D597-D604  
doi:10.1093/nar/gks1160

## The Protist Ribosomal Reference database (PR<sup>2</sup>): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy

Laure Guillou<sup>1,2,\*</sup>, Dipankar Bachar<sup>3,4</sup>, Stéphane Audic<sup>1,2</sup>, David Bass<sup>5</sup>, Cédric Berney<sup>5</sup>, Lucie Bittner<sup>1,2</sup>, Christophe Boutte<sup>1,2</sup>, Gaétan Burgaud<sup>6</sup>, Colomban de Vargas<sup>1,2</sup>, Johan Decelle<sup>1,2</sup>, Javier del Campo<sup>7</sup>, John R. Dolan<sup>8</sup>, Micah Dunthorn<sup>9</sup>, Bente Edvardsen<sup>10</sup>, Maria Holzmann<sup>11</sup>, Wiebe H.C.F. Kooistra<sup>12</sup>, Enrique Lara<sup>13</sup>, Noan Le Bescot<sup>1,2</sup>, Ramiro Logares<sup>7</sup>, Frédéric Mahé<sup>1,2</sup>, Ramon Massana<sup>7</sup>, Marina Montresor<sup>12</sup>, Raphael Morard<sup>1,2</sup>, Fabrice Not<sup>1,2</sup>, Jan Pawłowski<sup>11</sup>, Ian Probert<sup>14,15</sup>, Anne-Laure Sauvadet<sup>1,2</sup>, Raffaele Siano<sup>16</sup>, Thorsten Stoeck<sup>9</sup>, Daniel Vaulot<sup>1,2</sup>, Pascal Zimmermann<sup>17</sup> and Richard Christen<sup>3,4,\*</sup>

# Team

## The PR2 Team



### Core team



Daniel Vaultot  
NTU, Singapore  
Marine Phytoplankton



Javier del Campo  
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Laure Guillou  
CNRS, France  
Marine parasites



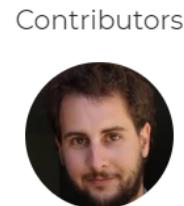
Frédéric Mahé  
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Diatoms



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Ciliates



Tristan Biard  
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Radiolaria



Vittorio Boscaro  
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Ciliates



Johan Decelle  
CEA, France  
Plastids

### Scientific Committee



Fabien Burki  
Uppsala U., Sweden  
Tree of eukaryotes,  
phylogenomics, metabarcoding,  
long-reads, protists, diversity



Bente Edvardsen  
U. Oslo, Norway  
Haptophyta



Martin Kolisko  
CAS, Czech Republic  
Apicomplexa



Ramon Massana  
ICM, Spain  
Stramenopiles



Fabrice Not  
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Radiolaria



Bente Edvardsen  
U. Oslo, Norway  
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Ciliates



Solenn Mordret  
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Dinoflagellates



Margot Tragin  
IFREMER, France  
Chlorophyta

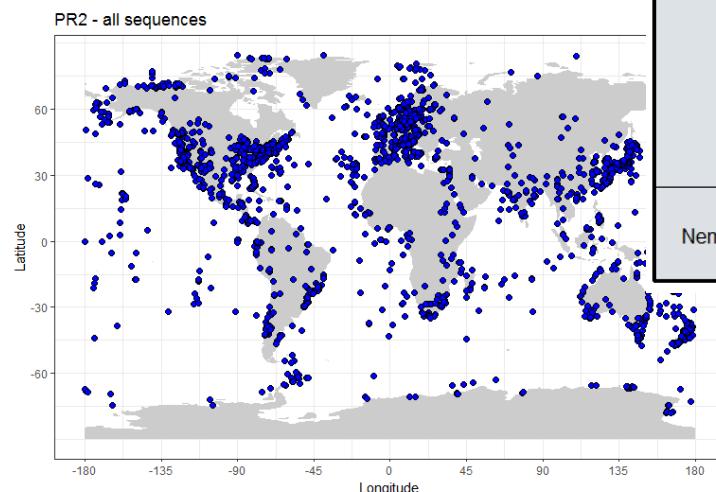
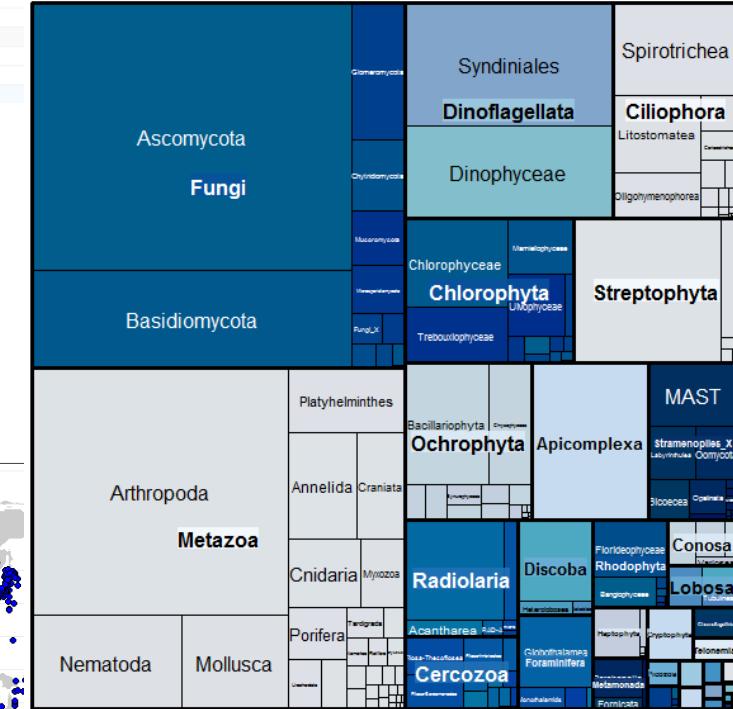


Wei-Ting Chen  
NTO U., Taiwan  
Ciliates

# Key features - Version 4.12.0 (08-2019)

- **Unified taxonomy** (8 ranks from kingdom to species)
- 177 934 sequences of nuclear 18S rRNA
- 6 010 sequences of plastid 16S rRNA (PhytoRef)
- **Quality control** (e.g. > 500 bp., N < 20, no "NNN")
- **Metadata** (e.g. coordinates, environment)
- Available as flat file or as **R package**
- Web site: <https://pr2-database.org/>

| taxo_id | kingdom   | superfamily | division   | class             | order    | family       | genus       |
|---------|-----------|-------------|------------|-------------------|----------|--------------|-------------|
| 947     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 948     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 949     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 950     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 951     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 952     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 953     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Parameciidae | Paramecium  |
| 955     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Penicula_X   | Parameculla |
| 956     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Penicula_X   | Penicula_XX |
| 957     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Stokesiidae  | Stokesia    |
| 958     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Stokesiidae  | Stokesia    |
| 959     | Eukaryota | Alveolata   | Ciliophora | Oligohymenophorea | Penicula | Urocentridae | Urocentrum  |
| 960     | Eukaryota | Alveolata   | Ciliophora |                   |          |              |             |
| 961     | Eukaryota | Alveolata   | Ciliophora |                   |          |              |             |
| 962     | Eukaryota | Alveolata   | Ciliophora |                   |          |              |             |
| 963     | Eukaryota | Alveolata   | Ciliophora |                   |          |              |             |
| 964     | Eukaryota | Alveolata   | Ciliophora |                   |          |              |             |
| 965     | Eukaryota | Alveolata   | Ciliophora |                   |          |              |             |



Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., Boutte, C. et al. 2013. The Protist Ribosomal Reference database ( PR<sup>2</sup>): a catalog of unicellular eukaryote Small Sub-Unit rRNA sequences with curated taxonomy. Nucleic Acids Res. 41:D597–604.



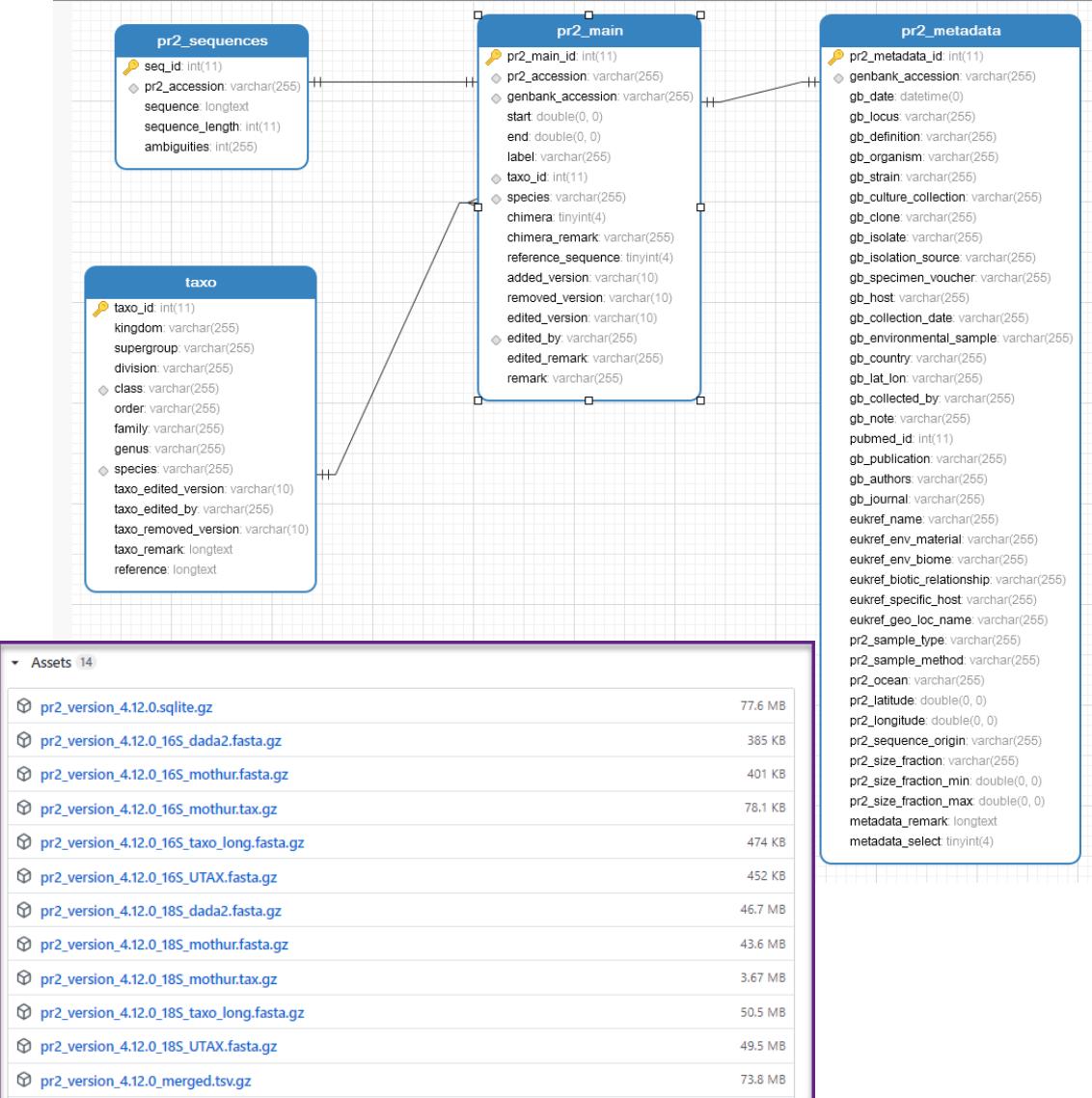
# Management



- MySQL database
- R scripts for:
  - importing
  - exporting
  - validating

# Data provided

- metabarcoding (dada2, QIIME)
- fasta (phylogeny)
- R package



<https://github.com/pr2database/pr2database/releases>

# R package

pr2database 4.12.0 [Get started](#) Reference Articles ▾

## Get started

Daniel Vaultot

The PR2 database is provided as a R package called **pr2database**. This page pro

## Installation

Install from the GitHub web site using the devtools package

```
install.packages("devtools")
devtools::install_github("pr2database/pr2database")
```

## The R pr2database package

Daniel Vaultot edited this page 4 days ago · 7 revisions

The PR2 database is now provided as a R package

## Installation

1

Install from the GitHub web site using the devtools package

```
install.packages("devtools")
devtools::install_github("vaulot/pr2database")
```

## Selecting sequences from a specific taxon

2

Let us select all the available sequences for the Mamiellophyceae *Ostreococcus*

```
# Filter only the sequences for which the column genus contains Ostreococcus
pr2_ostreo <- pr2 %>% dplyr::filter(genus == "Ostreococcus")

# Select only the columns of interest
pr2_ostreo <- pr2_ostreo %>% dplyr::select( genbank_accession, species,
pr2_sample_type, gb_strain, gb_clone,
pr2_latitude, pr2_longitude,
sequence_length, sequence )
```

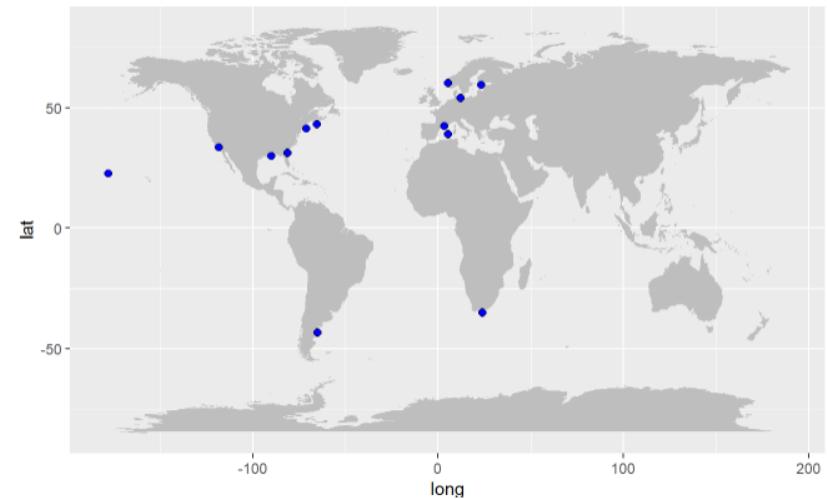
## Drawing a map of sequence locations

3

```
library(maps)
world <- map_data("world")

ggplot() +
  geom_polygon(data = world, aes(x=long, y = lat, group = group), fill="grey") +
  coord_fixed(1.3) +
  geom_point(data=pr2_ostreo, aes(x=pr2_longitude, y=pr2_latitude), fill="blue", size=2, shape=21) +
  ggtitle("Ostreococcus")
```

### Ostreococcus



# Annotation - Contributions

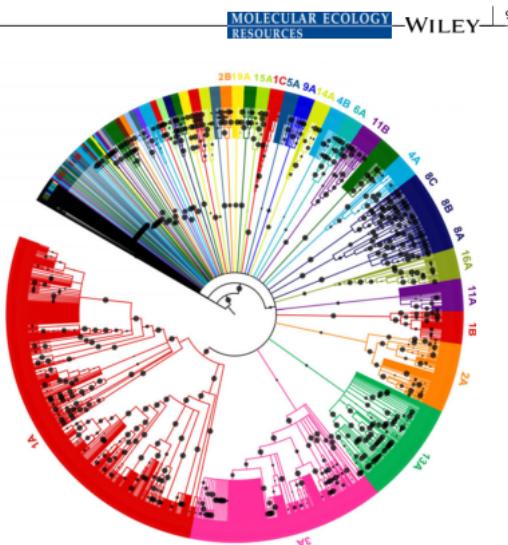
Received: 3 November 2017 | Revised: 15 February 2018 | Accepted: 24 February 2018  
DOI: 10.1111/1755-0998.12781

RESOURCE ARTICLE

**DINOREF: A curated dinoflagellate (Dinophyceae) reference database for the 18S rRNA gene**

Solenn Mordret<sup>1</sup> | Roberta Piredda<sup>1</sup> | Daniel Vaulto<sup>2</sup> | Marina Montresor<sup>1</sup> |  
Wiebe H. C. F. Kooistra<sup>1</sup> | Diana Sarno<sup>1</sup>

MORDRET ET AL.

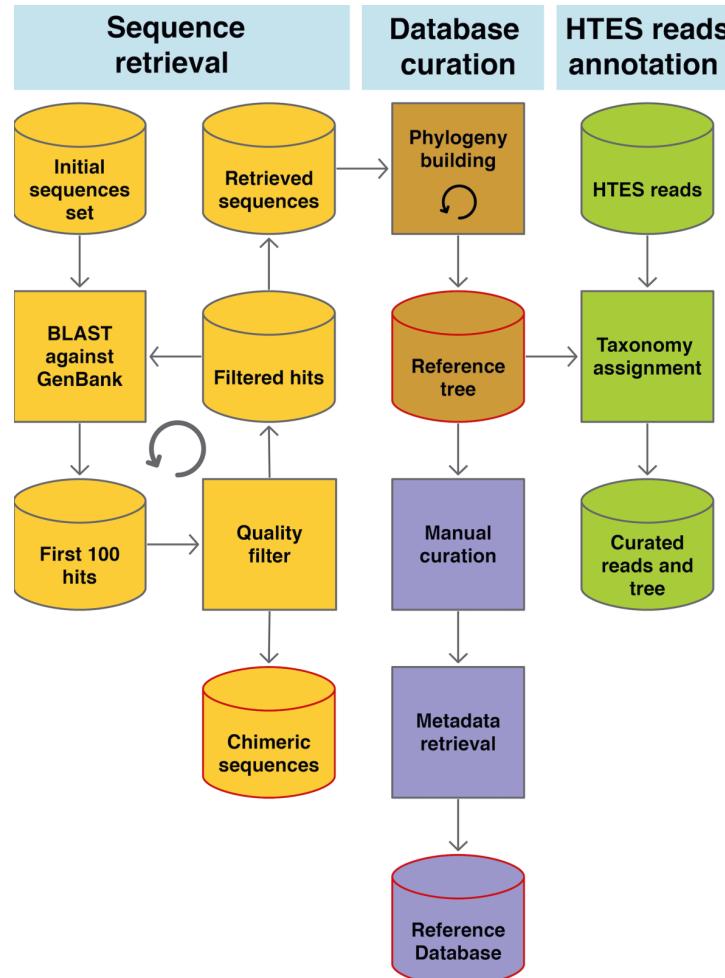


**FIGURE 2** Consensus phylogenetic tree (raxML, GTR model) based on 1,540 unique 18S rRNA sequences in the DINOREF. Alignment of 2,153 bp with three sequences of Ciliates (U97109; X56165 and X03772) and three sequences of Apicomplexa (M97703; AF236097 and AF291427) used as outgroup. Clades are ordered according to their size and are supported by bootstrap values  $\geq 50\%$ ; black dots are proportional to bootstrap values. The colours of the Superclades and clades correspond to those in Table 1. Clades within each Superclade have been marked (A, B, C, etc.), along the outer rim of the tree, corresponding to their assignment in this figure. The Superclades "Uncertain Naked Dinophyceae" and "Uncertain Thecate Dinophyceae" have not been marked and neither have the small clades on the upper left of the tree. The tree can be visualized on iToL version 3—Interactive Tree of Life (Letunic and Bork, 2016, at <https://itol.embl.de/tree/1932052318357911479398328>) in which all clades are marked.

## Groups with updated annotations

| Division      | Class                               | Who  | Date | Status | Version |
|---------------|-------------------------------------|--|------|--------|---------|
| Alveolata     | Apicomplexa                         | J. del Campo   | 2019 | Done   | 4.12    |
| Alveolata     | Ciliates                            | W. Ting, C. Bachy                                      | 2017 | Done   | 4.7     |
| Alveolata     | Ciliates                            | V. Boscaro, L. Santoferrara, E. Gentekaki and Q. Zhang | 2018 | Done   | 4.11    |
| Alveolata     | Dinoflagellates                     | S. Mordret, D. Sarno                                   | 2018 | Done   | 4.9     |
| Alveolata     | Dinoflagellates (Suessiales)        | J. del Campo   | 2020 | Done   | 5.0     |
| Rhizaria      | Collodaria                          | T. Biard   | 2015 | Done   | 2.0     |
| Chlorophyta   |                                     | M. Tragin, A. Lopes dos Santos                         | 2015 | Done   | 3.0     |
| Haptophyta    |                                     | B. Edvardsen   | 2015 | Done   | 4.0     |
| Stramenopiles |                                     | R. Massana   | 2019 | Done   | 4.12    |
| Stramenopiles | Diatoms ( <i>Chaetoceros</i> )      | C. Gaonkar   | 2019 | Done   | 4.12    |
| Stramenopiles | Diatoms ( <i>Thalassiosirales</i> ) | L. Arsenieff   | 2020 | Done   | 5.0     |
| Stramenopiles | Bolidophyceae                       | D. Vaulto  | 2017 | Done   | 4.6     |
| Stramenopiles | Pelagophyceae                       | D. Vaulto  | 2017 | Done   | 4.6     |
| Stramenopiles | Pelagophyceae                       | A.M. Cabello   | 2020 | Done   | 5.0     |
| Stramenopiles | Chrysophyceae                       | D. Vaulto  | 2020 | Done   | 5.0     |

# Annotation - EukRef (J. del Campo)



## Workshop Roscoff 2018

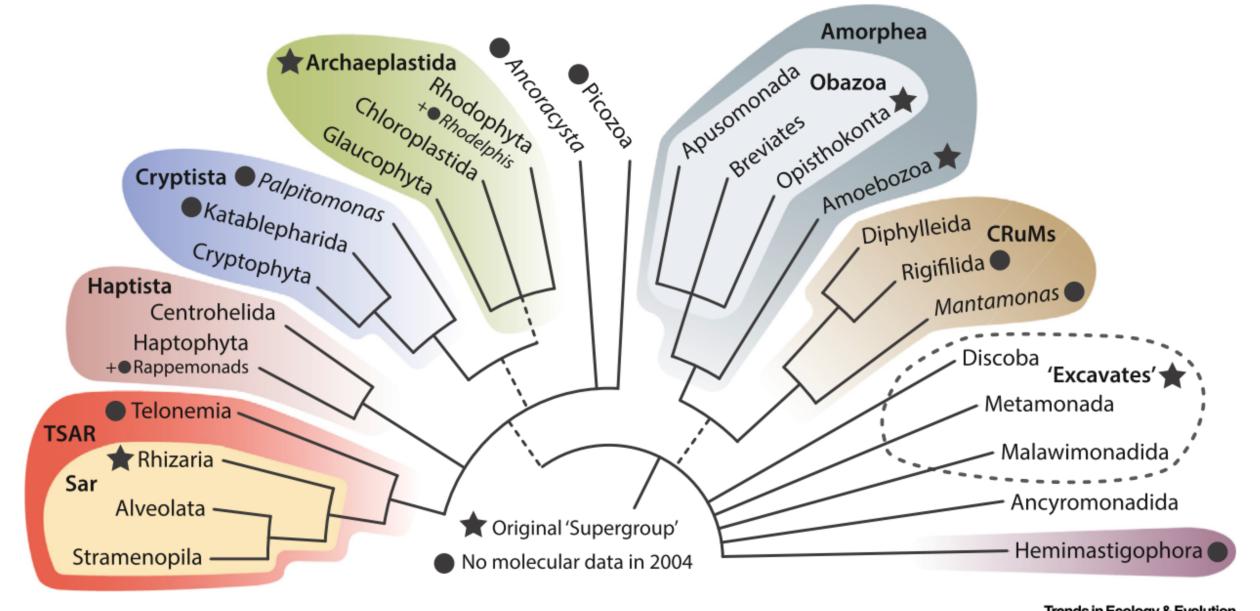
Javier del Campo  
Last updated on 2019-11-29 · eukref



Our third workshop took place at the **Station Biologique Roscoff** in Roscoff, France from **November 5 to 9 2018**. This effort brought together taxonomists with expertise in individual lineages that span the eukaryotic tree of life to curate reference 18S rDNA sequences of these lineages by incorporating knowledge of phylogenetic, morphological, and/or environmental contextual data. **This third edition was particularly (but not exclusively) focused on Diatoms, Dinoflagellates, and Green Algae.** During the workshop,

# Version 5.0.0 - July 2020

- Taxonomy goes from 8 to 9 levels
  - kingdom -> domain
  - division / **subdivision** / class
- New sequences
  - 18S nuclear: 300,000 Silva and Genbank
    - not yet integrated into PR<sup>2</sup>
    - assigned with dada2
  - 18S nucleomorph: 250
  - 16S mitochondria
- Groups reannotated
  - Dinoflagellates
  - Diatoms, Chrysophyceae, Pelagophyceae
  - Foraminifera



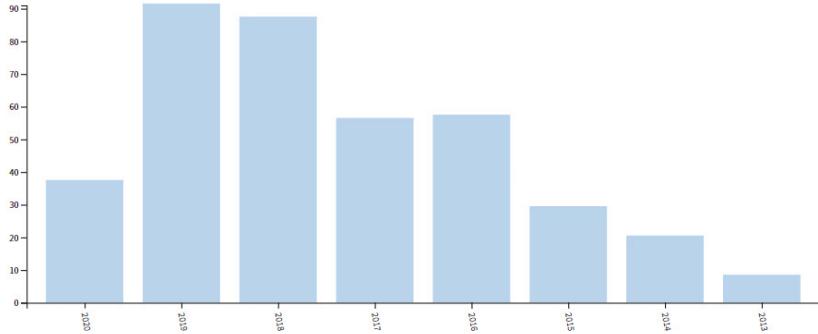
Adl SM. et al.. 2019. Revisions to the Classification, Nomenclature, and Diversity of Eukaryotes. *Journal of Eukaryotic Microbiology* 66:4–119.

Burki F., Roger AJ., Brown MW., Simpson AGB. 2019. The New Tree of Eukaryotes. *Trends in Ecology & Evolution*

A word cloud centered around the word "diversity". The word "diversity" is the largest and most prominent word, rendered in a large green font. Surrounding it are numerous other words related to biology, ecology, and environmental science, each associated with a small cluster of text. The words include "microbial eukaryotes", "biogeography", "genetic diversity", "bacterial communities", "abundance", "biodiversity dynamics", "bacteria dna", "biodiversity reveals", "environmental dna", "ribosomal-rna sequences", "phylogenies", "sequences", "seawaters", "ocean", "protists", "ecology", "database", "protozoa", "dispersal", "identification", "coastal waters", "variability", "assemblages", "plankton", "classification", "water", "protist diversity", "rare biosphere", "communities", "patterns", "morphology", "rare", "bacterial", "ribosomal-rna", "marine", "community", "deep-sea", "picoplankton", "community structure", "phytoplankton", "evolution", "extracellular dna", "ribosomal-rna gene", and "microbial communities". The words are colored in various shades of green, purple, blue, and yellow.

Using PR<sup>2</sup>

# More than 380 papers citing PR<sup>2</sup>



microbial eukaryotes  
 genetic diversity  
 abundance bacteria dna  
**biiodiversity** reveals environmental dna  
**ribosomal-rna sequences**  
 phylogeny database protozoa  
 dispersal identification coastal waters  
 variability assemblages  
**diversity** rdna  
 communities patterns morphology  
 rare bacterial  
 ribosomal-rna marine community deep-sea  
 community structure phytoplankton picoplankton  
 evolution extracellular dna  
**ribosomal-rna gene**  
 microbial communities

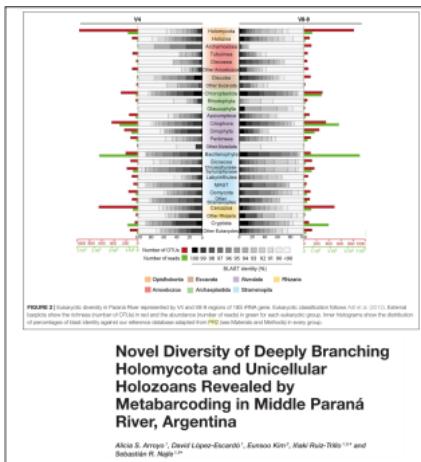
# Metabarcoding

- Marine
- Freshwater
- Soil
- Applied domains

- Gutters of Paris

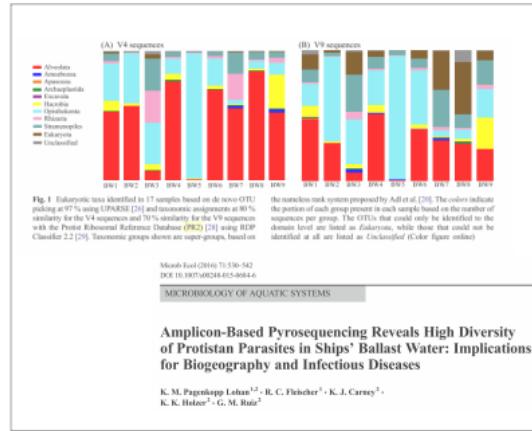
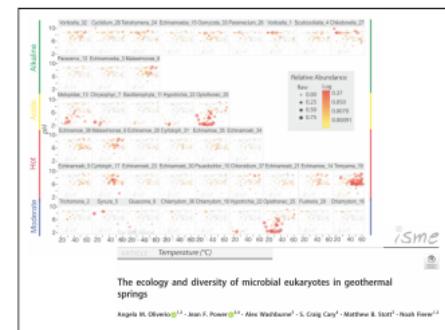
- Human microbiome

- Forensics



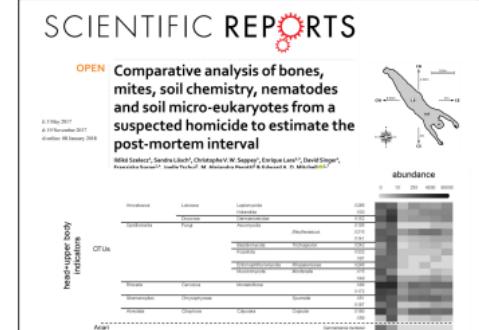
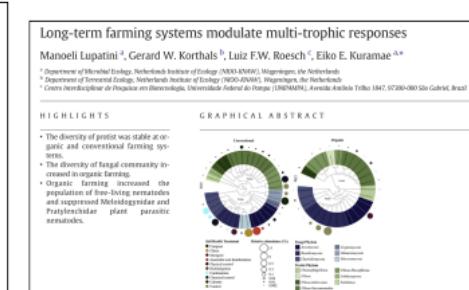
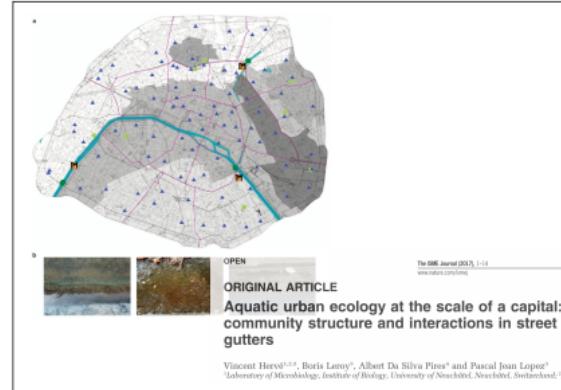
**Novel Diversity of Deeply Branching Holomycota and Unicellular Holozoa Revealed by Metabarcoding in Middle Paraná River, Argentina**

Alicia S. Arevalo<sup>1</sup>, David López-Escobar<sup>2</sup>, Esteban Kett<sup>1</sup>, Matías Rubí-Tripp<sup>1,3</sup> and Sebastián R. Aguirre<sup>1,4\*</sup>



**Amplicon-Based Pyrosequencing Reveals High Diversity of Protistan Parasites in Ships' Ballast Water: Implications for Biogeography and Infectious Diseases**

K. M. Pagenstecher Lehman<sup>1,2</sup>, R. C. Fletcher<sup>1</sup> · K. J. Carney<sup>2</sup> ·  
K. C. Heber<sup>3</sup> · G. M. Ruiz<sup>1</sup>



# Primer database

## In silico analysis

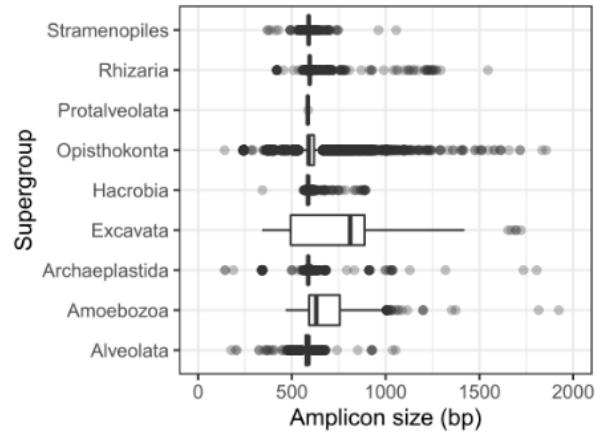


### Contributors

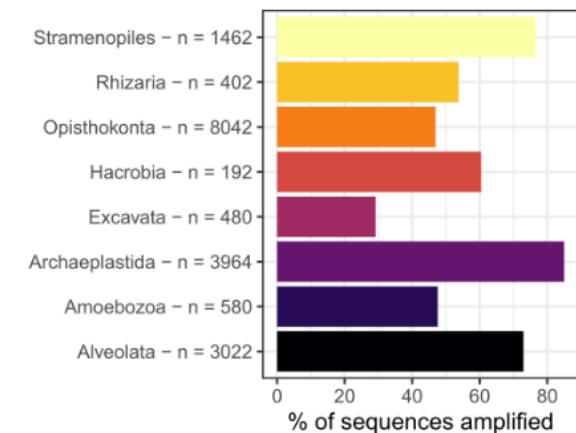
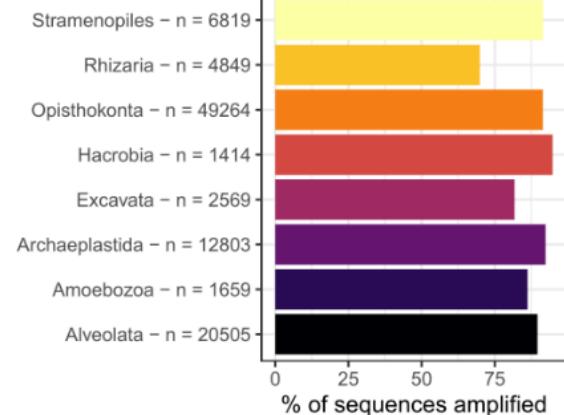
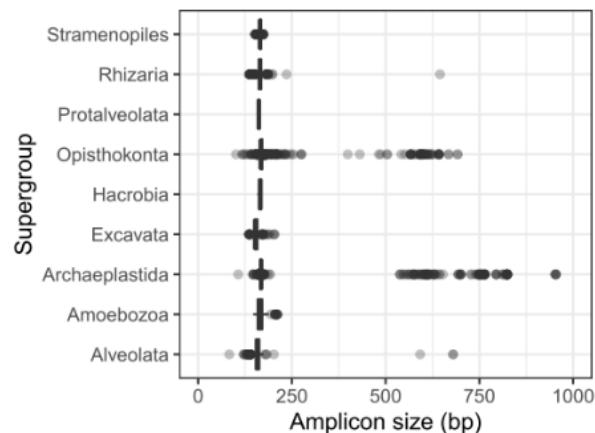
- Stefan Geisen: [S.Geisen@nioo.knaw.nl](mailto:S.Geisen@nioo.knaw.nl)
- Frédéric Mahé: [frederic.mahe@cirad.fr](mailto:frederic.mahe@cirad.fr)
- Daniel Vaulot: [vaulot@gmail.com](mailto:vaulot@gmail.com)
- Enrique Lara: [enrique.lara@rjb.csic.es](mailto:enrique.lara@rjb.csic.es)

| primer_id | name     | synonyms | sequence                | sequence revcomp        | direction |
|-----------|----------|----------|-------------------------|-------------------------|-----------|
| 71        | PF1      |          | TGCGCTACCTGGTTGATCCTGCC | GGCAGGATCAACCAGGTAGCGCA | fwd       |
| 78        | EukA     |          | AACCTGGTTGATCCTGCCAGT   | ACTGGCAGGATCAACCAGGTT   | fwd       |
| 81        | Euk328F  |          | ACCTGGTTGATCCTGCCAG     | CTGGCAGGATCAACCAGGT     | fwd       |
| 138       | 18SV1V2F |          | ACCTGGTTGATCCTGCCA      | TGGCAGGATCAACCAGGT      | fwd       |

Primer set – V4 04

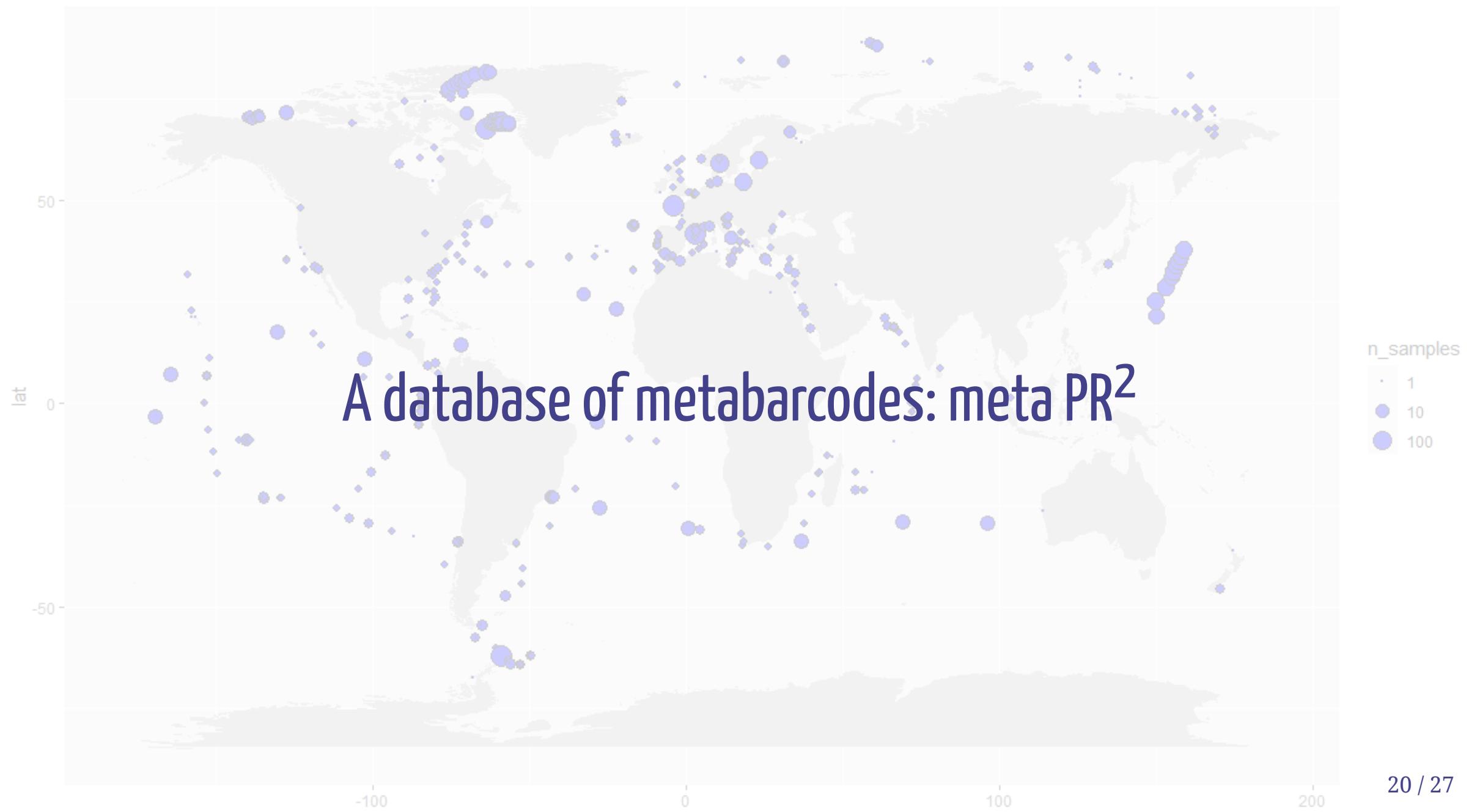


Primer set – V9 29



<https://github.com/pr2database/pr2-primers/wiki/18S-rRNA-primers>

Geisen, Vaulot et al. A user guide to environmental protistology: primers, metabarcoding, sequencing, and analyses. bioRxiv <https://doi.org/10.1101/850610>.

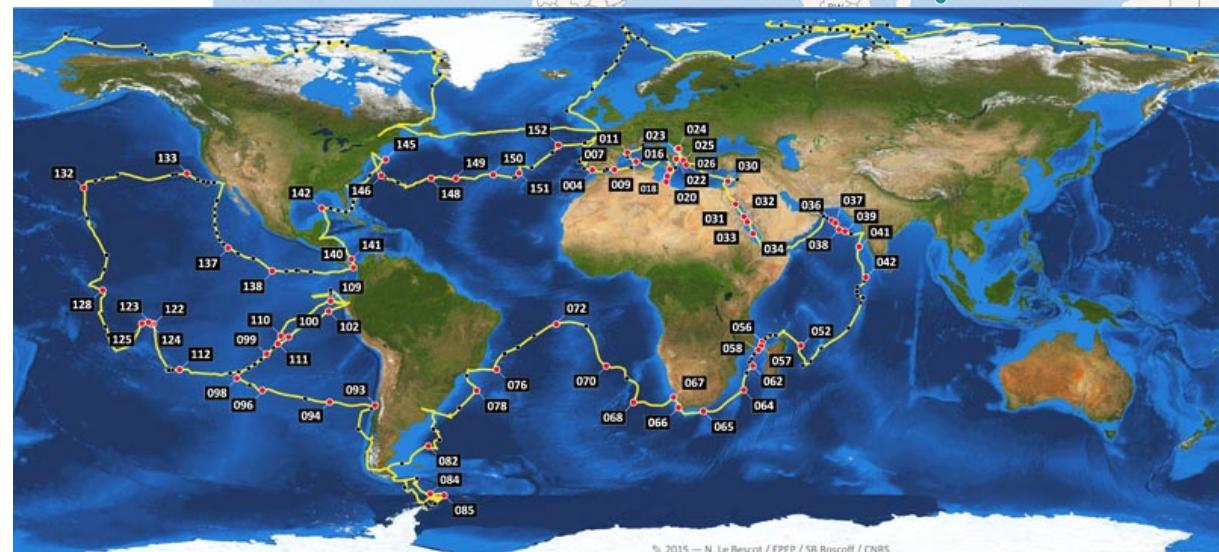
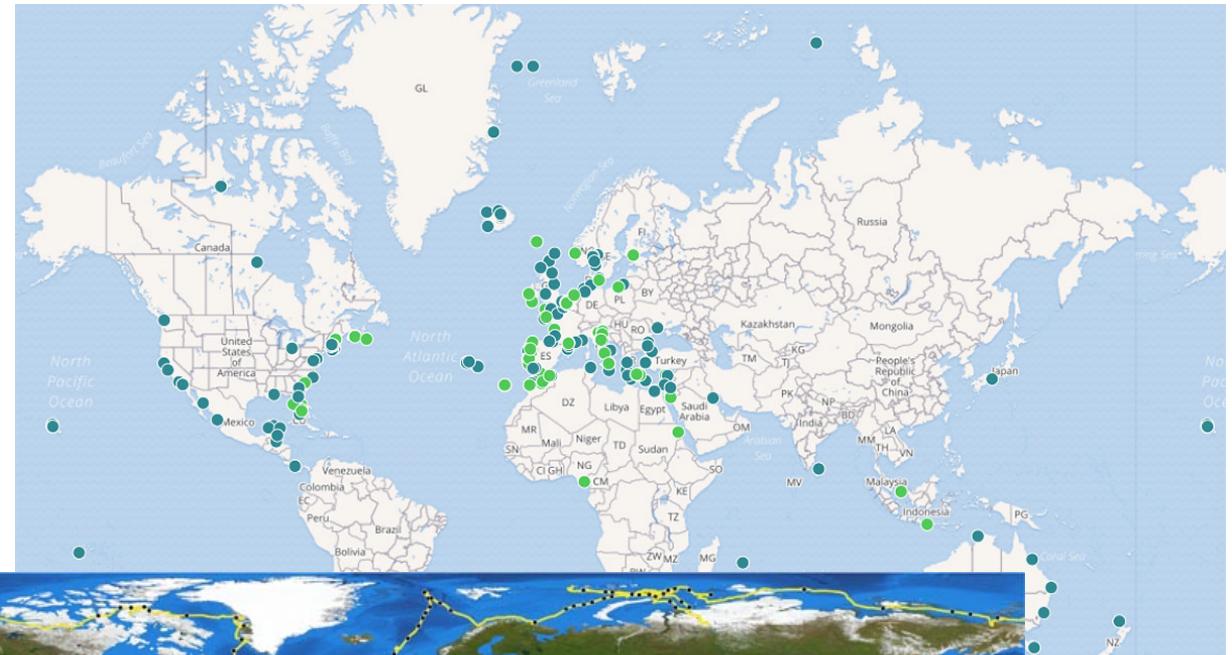


# Many metabarcoding data sets available

- Ocean Sampling Day (OSD)
- Malaspina
- Tara Oceans
- individual studies

But hard to use together...

- Processed with different pipelines
- Different levels of similarity
- Different reference databases
- Metadata lacking



## Download public data

- Raw sequences (fastq)
- Metadata

The screenshot shows the NCBI SRA Run Selector interface. At the top, the accession number PRJEB1843 is entered in the search bar. Below the search bar, a table titled "Common Fields" displays various metadata entries:

|             |                         |
|-------------|-------------------------|
| acc         | ERR266040               |
| Alias       | BiomarksV4Vampyrellids1 |
| Assay Type  | AMPLICON                |
| AvgSpotLen  | 389                     |
| Bases       | 1077051                 |
| BioProject  | PRJEB1843               |
| BioSample   | SAMEA1904855            |
| Bytes       | 711870                  |
| Center Name | SBR ROSCOFF             |

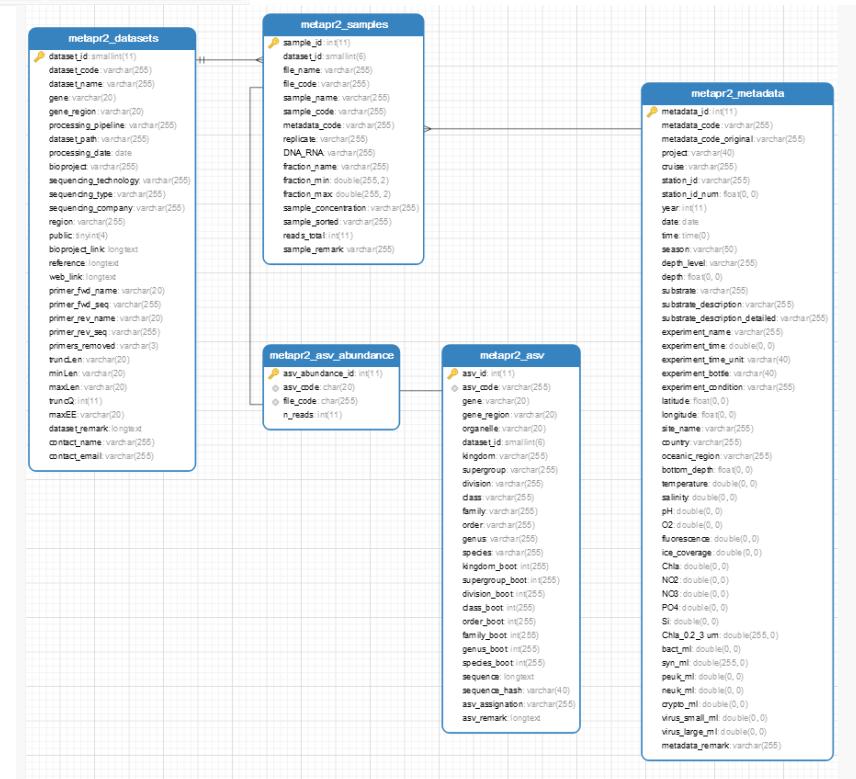
Below the table, there is a summary table with columns: Select, Runs, Bytes, Bases, and Download. The "Selected" row shows 0 runs, 0 bytes, and 0 bases. There are also links for "Metadata" and "Accession List".

## Reprocess

- Amplicon Sequence Variant (*dada2*)
- Different datasets can be merged

## Stored in MySQL database

- Processed with R scripts



## DADA2: High-resolution sample inference from Illumina amplicon data

Benjamin J Callahan<sup>1</sup>, Paul J McMurdie<sup>2</sup>, Michael J Rosen<sup>3</sup>, Andrew W Han<sup>2</sup>, Amy Jo A Johnson<sup>2</sup> & Susan P Holmes<sup>1</sup>

We present the open-source software package DADA2 for modeling and correcting Illumina-sequenced amplicon errors (<https://github.com/benjneb/dada2>). DADA2 infers sample sequences exactly and resolves differences of as little as 1 nucleotide. In several mock communities, DADA2 identified more real variants and output fewer spurious sequences than other methods. We applied DADA2 to vaginal samples from a cohort of pregnant women, revealing a diversity of previously undetected *Lactobacillus crispatus* variants.



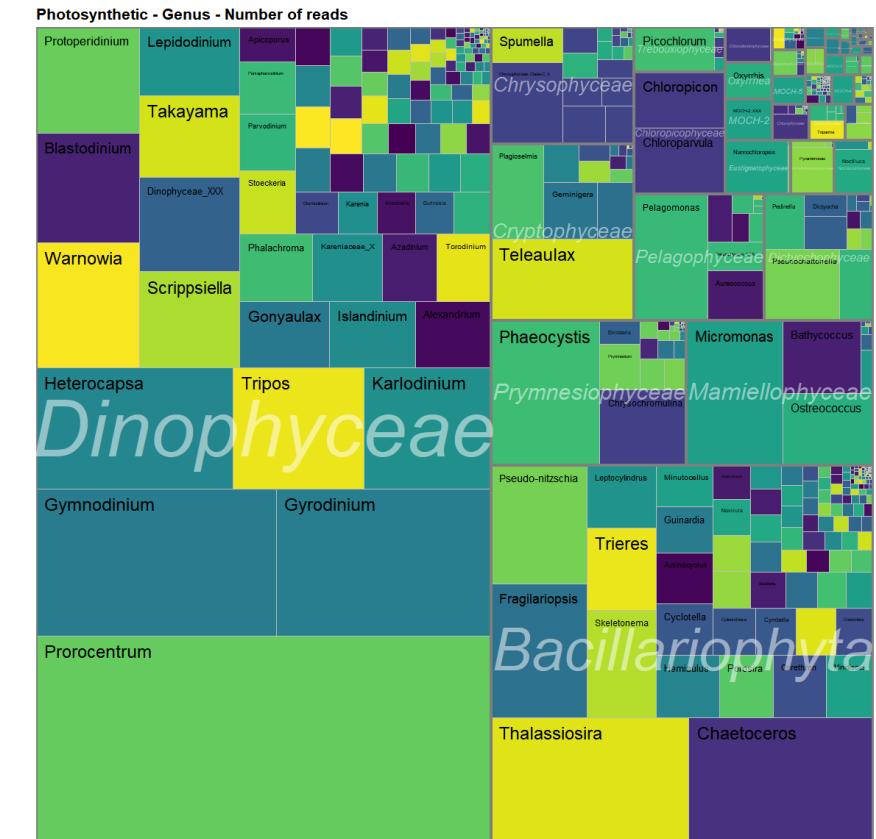
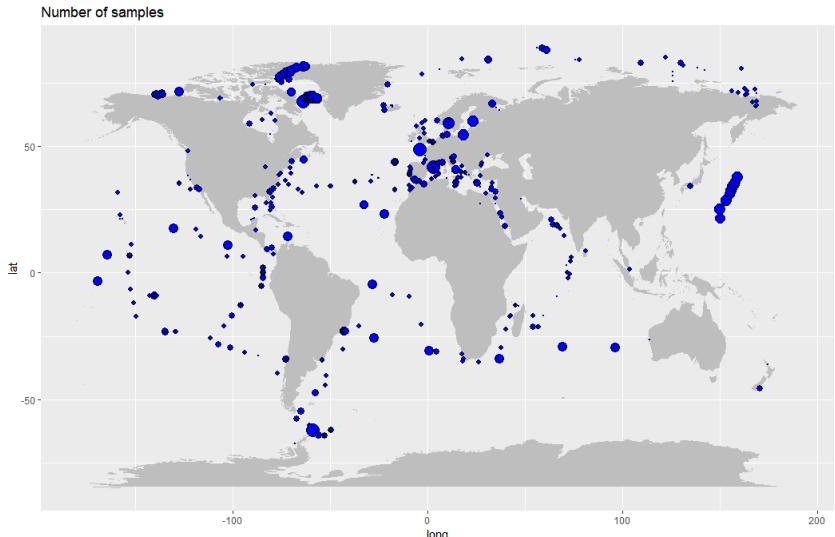
# Status of the database

Datasets included : 32

- V4
  - OSD
  - Malaspina
  - Polar regions
- V9
  - Tara Oceans

Samples: 5,094

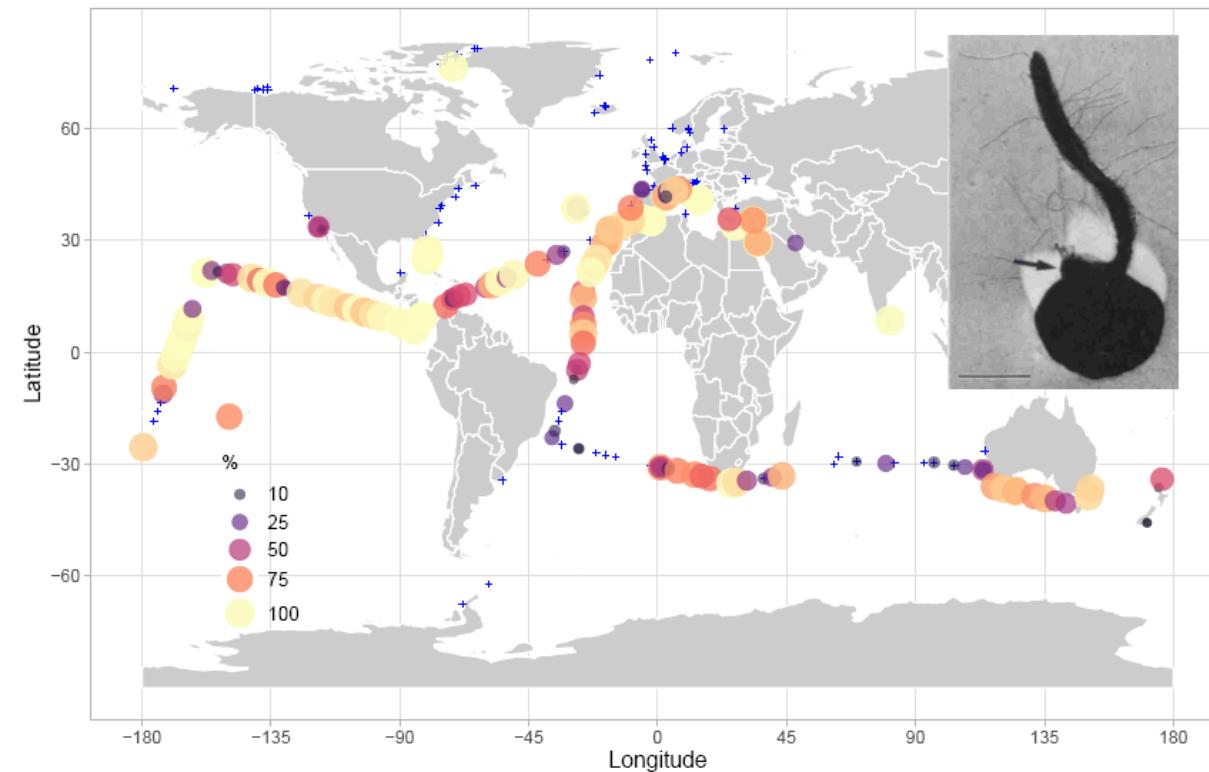
ASVs: 126,669



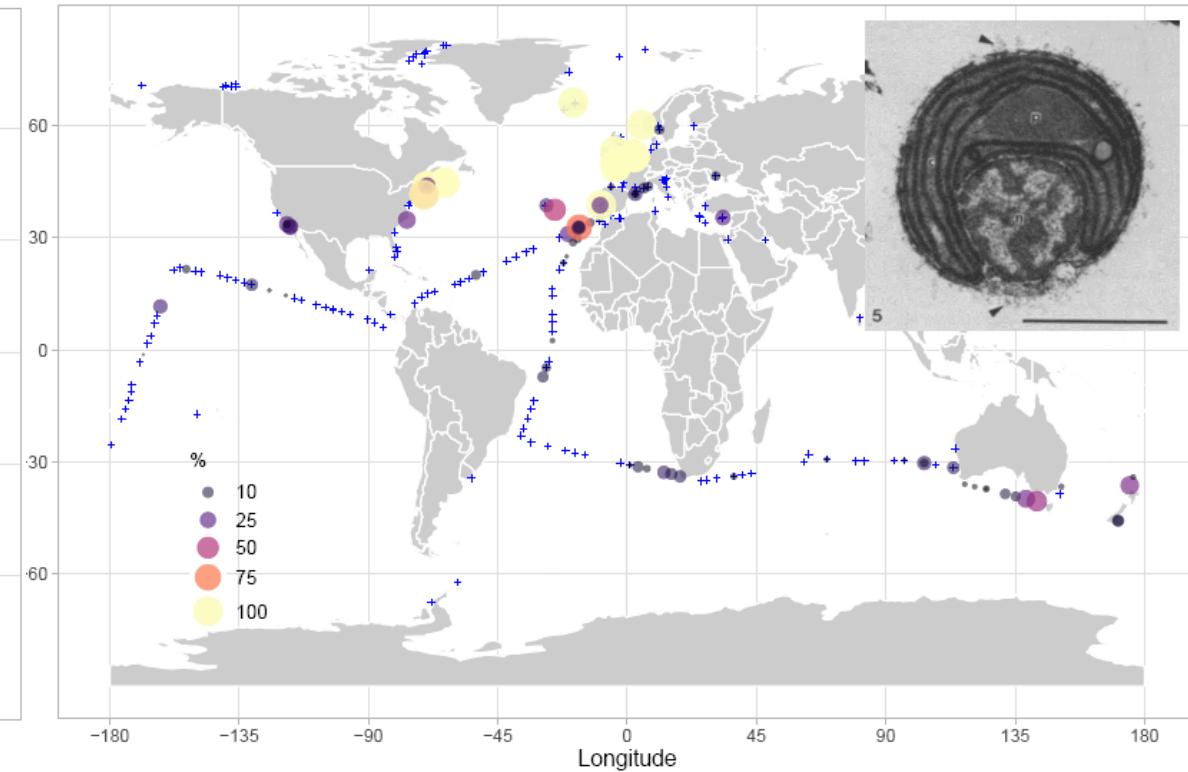
# Pelagophyceae

Key picophytoplankton group in oceanic waters.

Pelagomonas - % of Pelagophyceae



Aureococcus - % of Pelagophyceae



Cabello AM. et al. 2018. Pelagophyte assemblages of the global ocean display low intraspecific diversity. in prep.

Andersen RA., Saunders GW., Paskind MP., Sexton J. 1993. Ultrastructure and 18S rRNA gene sequence for *Pelagomonas calceolata* gen. and sp. nov. and the description of a new algal class, the Pelagophyceae classis nov. Journal of Phycology 29:701–715.

# What is next

- Full rRNA operon
- Annotation of specific groups
  - Contributors
  - EukRef
- Metadata
- Phenotypes

# Acknowledgments

- Biomarks (EU)
- Moore Foundation (EukRef)
- CNRS
- Sorbonne Université
- Nanyang Technological University

# pr2-database.org

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## PR<sup>2</sup> database

A reference 18S rRNA sequence database



- expert curated taxonomy
- metadata such as geo-localisation
- use to annotate metabarcodes

PR2 is on GitHub | 34

release 4.12.0 date 08 August 2019 downloads 11k

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