

# The Protist Ribosomal Reference database ecosystem

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

PR<sup>2</sup> Zoom meeting - 2022-11-09



# Outline

- PR<sup>2</sup>
- PR<sup>2</sup> primers
- metaPR<sup>2</sup>
- Open Discussion



figure: adapted from Adl et al. 2012  
text: Geijeranda

# Key features

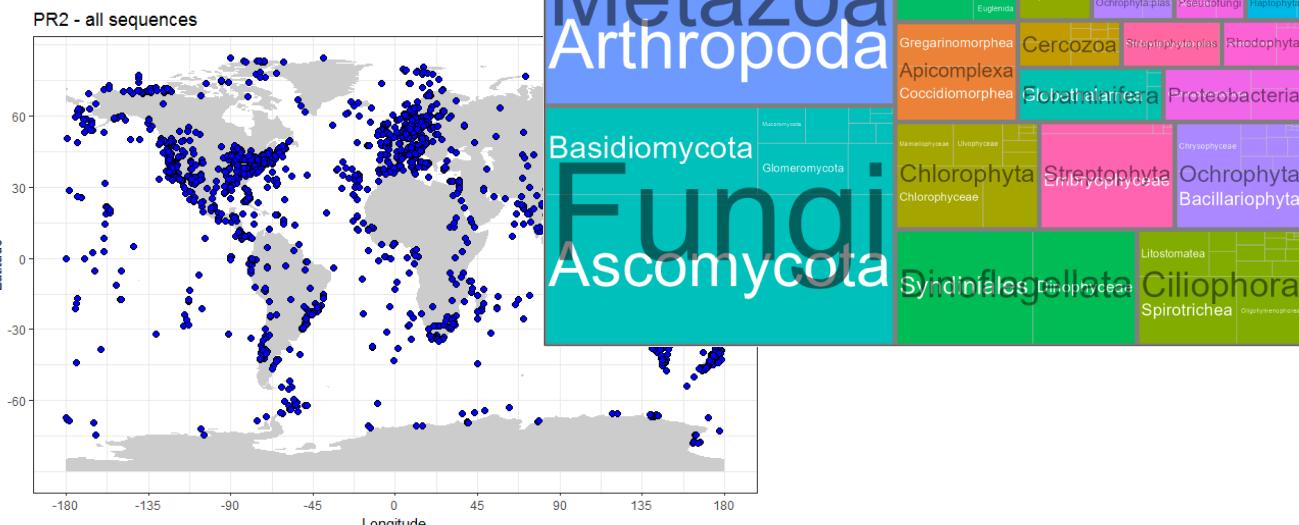


- **Unified taxonomy** (8 ranks from kingdom to species)
- 197 602 sequences
  - nuclear 18S rRNA
  - plastid 16S rRNA (PhytoRef)
  - bacteria and archaea 16S rRNA
- **Quality control** (e.g. > 500 bp., N < 20, no "NN")
- **Metadata** (e.g. coordinates, environment)
- **Version 4.14.0 released in May 2021**
- Web site: <https://pr2-database.org/>

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

- Current version : 4.14.0
- Last update : 25 June 2021
- DOI [10.5281/zenodo.5031733](https://doi.org/10.5281/zenodo.5031733)
- Download: <https://github.com/pr2database/pr2database/releases>  
downloads 21k
- Contributors

taxo_id	kingdom	superfamily	division	class	order	family	genus	species
947	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_polyacryum
948	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_primeurelia
949	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_putrinum
950	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_schewiakoffi
951	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_sp.
952	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_tetraurelia
953	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Paramedidae	Paramecium	Paramecium_woodruffi
955	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Peniculidae	Paranassula	Paranassula_sp.
956	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Peniculidae	Penicula	Penicula_XX_sp.
957	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Stokesidae	Stokesia	Stokesia_sp.
958	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Stokesidae	Stokesia	Stokesia_vernalis
959	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Urocentridae	Urocentrum	Urocentrum_sp.
960	Eukaryota	Alveolata	Ciliophora	Oligohymenophorea	Penicula	Urocentridae	Urocentrum	Urocentrum_turbo
961	Eukaryota	Alveolata	Ciliophora	Oligohymenop				
962	Eukaryota	Alveolata	Ciliophora	Oligohymenop				
963	Eukaryota	Alveolata	Ciliophora	Oligohymenop				
964	Eukaryota	Alveolata	Ciliophora	Oligohymenop				
965	Eukaryota	Alveolata	Ciliophora	Oligohymenop				



# Annotation - Contributions



## Groups with updated annotations

Division	Class	Group	Who	Date	Version
Alveolata	Apicomplexa		J. del Campo	2019	4.12, 4.14
Alveolata	Ciliates		W. Ting, C. Bachy	2017	4.7
Alveolata	Ciliates		V. Boscaro, L. Santoferrara, E. Gentekaki and Q. Zhang	2018	4.11
Alveolata	Dinoflagellates		S. Mordret, D. Sarno	2018	4.9
Alveolata	Dinoflagellates	Suessiales	J. del Campo	2021	4.13
Chlorophyta			M. Tragin, A. Lopes dos Santos	2015	3.0
Excavata			J. del Campo	2021	4.14
Haptophyta			B. Edvardsen	2015	4.0
Prasinodermophyta			D. Vaulot	2021	4.13
Rhizaria	Colloformia		T. Biard	2015	2.0
Rhizaria	Foraminifera		R. Morard	2021	
Rhizaria	Radiolaria		M. Mendez Sandin.	2021	
Stramenopiles			R. Massana	2019	4.12
Stramenopiles	Cafeteria		Alex Schoenlie	2021	4.14
Stramenopiles	Labyrinthulomycetes		J. del Campo	2021	4.14
Stramenopiles	Diatoms	Chaetoceros	C. Gaonkar	2019	4.12
Stramenopiles	Diatoms	Thalassiosirales	L. Arsenieff	2021	4.13
Stramenopiles	Bolidophyceae		D. Vaulot	2017	4.6
Stramenopiles	Pelagophyceae		A.M. Cabello	2021	4.13
Stramenopiles	Chrysophyceae		D. Vaulot	2021	4.13

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

## RESOURCE ARTICLE

WILEY MOLECULAR ECOLOGY RESOURCES

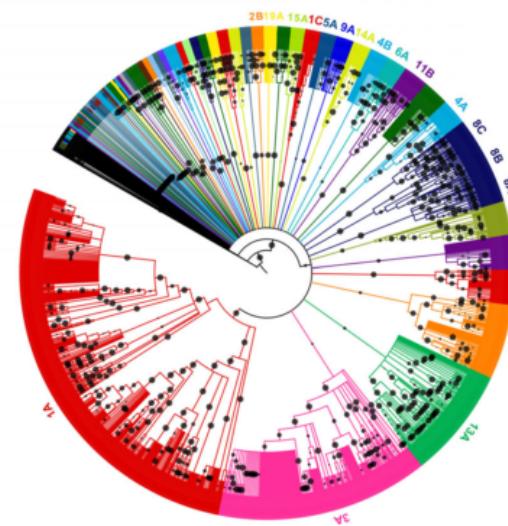
### DINOREF: A curated dinoflagellate (Dinophyceae) reference database for the 18S rRNA gene

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

MORDRET ET AL.

MOLECULAR ECOLOGY RESOURCES WILEY

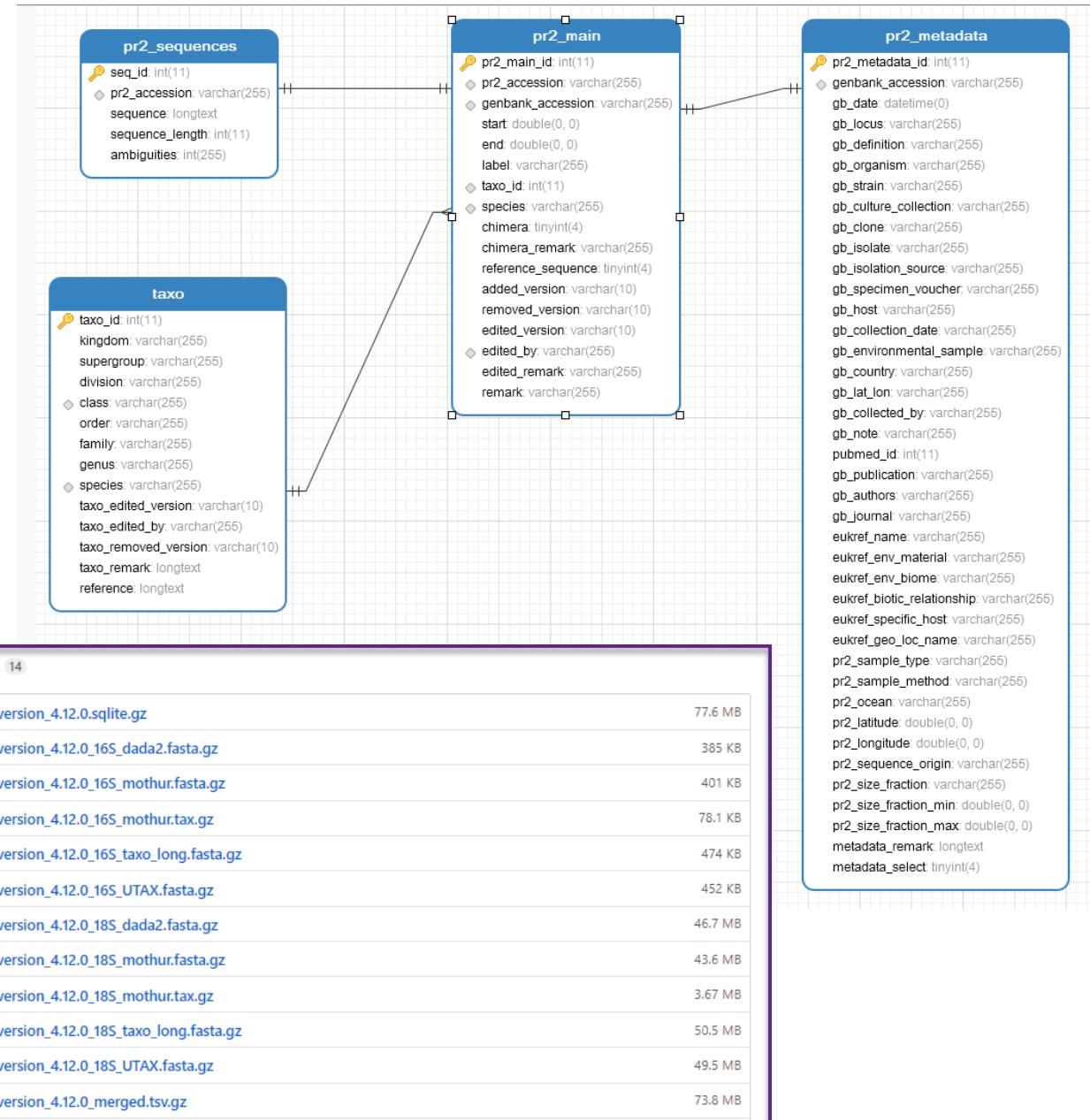
- Superclades**
- # 1 - Gonyaulacales
  - # 2 - Dinophysiales
  - # 3 - Suessiales
  - # 4 - Thoracosphaeraceae
  - # 5 - Amphidomataceae
  - # 6 - Kryptoperidiniaeae
  - # 7 - genera Penitapharsodium-Ensiculifera
  - # 8 - Peridiales sensu stricto
  - # 9 - Heterocapsaceae
  - # 10 - Podolampadaceae
  - # 11 - Prorcentrales
  - UTD - Uncertain Thecate Dinophyceae
  - # 12 - genus Akashiwo
  - # 13 - Gymnodinales sensu stricto
  - # 14 - Kareniaceae
  - # 15 - genus Gyrodinium
  - # 16 - genus Amphidinium
  - # 17 - Torodinales
  - # 18 - Tovelliaceae
  - # 19 - genus Blastodinium
  - # 20 - Ptychodiscales
  - UND - Uncertain Naked Dinophyceae
  - OUTGROUPS



**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 was 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.

# Management

- MySQL database
- R scripts for:
  - importing
  - exporting
  - validating
- Data provided as
  - text files (for dada2, mothur)
  - fasta (phylogeny)
  - R package



# R package



pr2database 4.14.0 [Home](#) Get started Reference Articles Changelog

## Get started

Daniel Vaulot

The PR2 database is provided as a R package called `pr2database`. This page provides instruction to install and use the package.

### Installation

Install from the GitHub web site using the `devtools` package

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

\* installing \*source\* package 'pr2database' ...  
\*\* R  
\*\* data  
\*\*\* moving datasets to lazyload DB  
\*\* byte-compile and prepare package for lazy loading  
\*\* help  
\*\*\* installing help indices  
 converting help for package 'pr2database'  
 finding HTML links ... fini  
 pr2.html  
\*\* building package indices  
\*\* testing if installed package can be loaded  
\*\*\* arch - i386  
\*\*\* arch - x64  
\* DONE (pr2database)  
In R CMD INSTALL

### The R pr2database package

Daniel Vaulot 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

lat

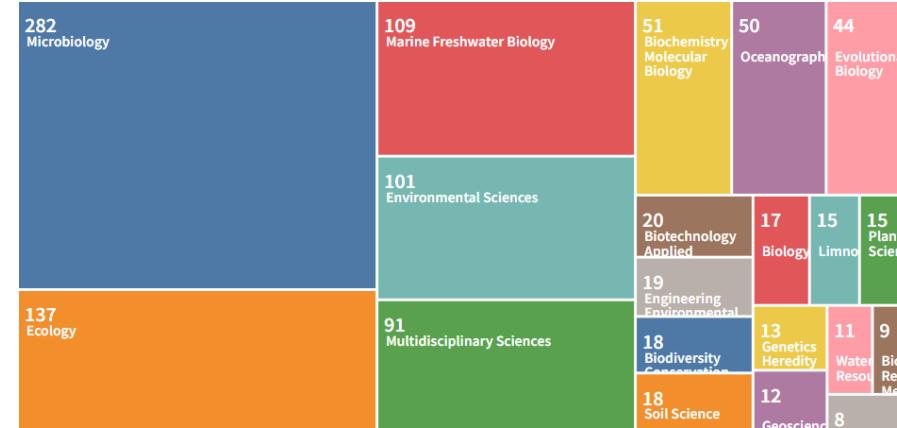
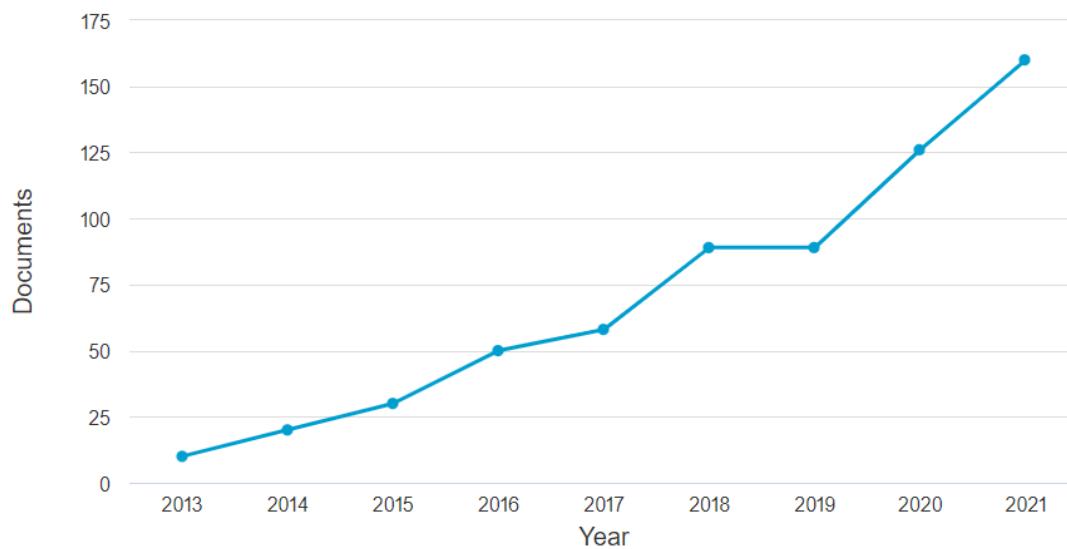
long

# More than 700 papers citing PR<sup>2</sup>



🏆 As of January/February 2022, this [highly cited paper](#) received enough citations to place it in the top 1% of the academic field of **Biology & Biochemistry** based on a highly cited threshold for the field and publication year.

Documents by year



microbial eukaryotes biogeography  
genetic diversity bacterial communities  
biodiversity dynamics environmental dna  
ribosomal-rna sequences reveals  
diversity  
communities patterns morphology rare bacterial  
ribosomal-rna marine community deep-sea  
community structure phytoplankton evolution  
ribosomal-rna gene extracellular dna picoplankton  
microbial communities

# What's next for pr2-database

- Web interface (end 2022)
- Version 4.15 (early 2023) - update of some groups
- Full operon
- Functional annotation
  - Trophic mode (photo, hetero, mixo...)
  - Size group (pico, nano, micro...)

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

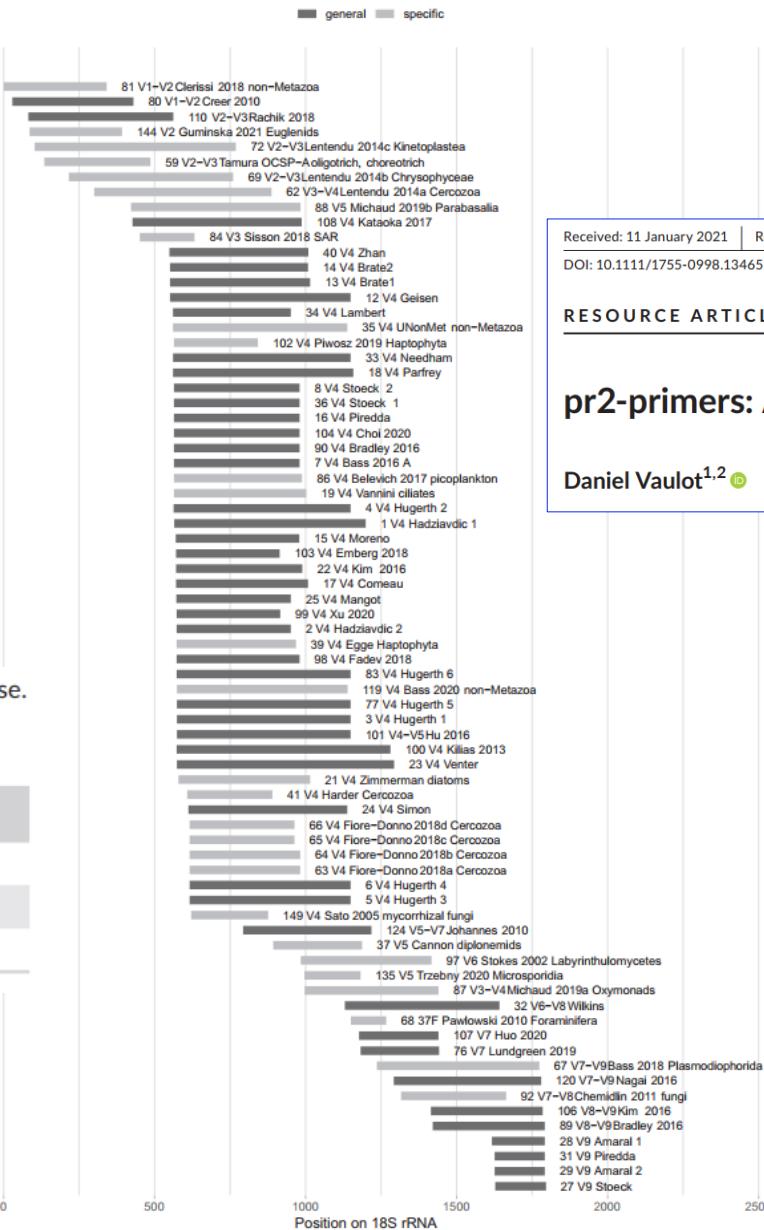
# 18S rRNA primers



- Wide diversity of primers and sets
- No database for protists
- Taxonomic specificity of primers?

**TABLE 1** Summary of primers listed in the pr2-primers database. General primers target all eukaryotes and specific primers only certain taxonomic groups

Direction	General primers	Specific primers
fwd	55	89
rev	53	88
Total	108	177



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DOI: 10.1111/1755-0998.13465

## RESOURCE ARTICLE

MOLECULAR ECOLOGY  
RESOURCES WILEY

## pr2-primers: An 18S rRNA primer database for protists

Daniel Vaulot<sup>1,2</sup> | Stefan Geisen<sup>3,4,5</sup> | Frédéric Mahé<sup>6,7</sup> | David Bass<sup>8,9</sup>

# pr2-primers database



The PR2 primer database

## Primers

column	definition
primer_id	primer id in pr2-primers database
name	original name of the primer
synonyms	synonyms found in the literature
sequence	
sequence_revcomp	reverse complement of the sequence
direction	forward (fwd) or reverse (rev)
start_yeast	start of primer relative to FU970071
end_yeast	end of primer relative to FU970071
specificity	is the primer specific of a group
reference	original reference where primer was first defined
doi	link to original paper

[Download primers](#)

### Columns to show:

- primer\_id
- gene
- organelle
- direction
- name
- synonyms
- sequence
- sequence\_revcomp
- length
- start\_yeast
- end\_yeast
- specificity
- reference
- doi
- doi\_html

About    Primers    Primer sets    Amplification - overview    Amplification - details    Test your primer set

Show 25 entries

Search:

primer_id	gene	organelle	direction	name	sequence	length	start_yeast	specificity	reference
123	16S rRNA	plastid	fwd	Pla491F	GAGGAATAAGCATCGGCTAA	20		plastid	Fuller et al. (2007)
124	16S rRNA	plastid	rev	PP936R	CCTTGAGTTCAYYCTTGC	20		plastid	<a href="https://biomarks.eu/pp936r">https://biomarks.eu/pp936r</a>
212	16S rRNA	plastid	rev	OXY1313R	CTTCAYGYAGGCAGTTGCAGC	22			West et al. (2001)
213	16S rRNA	plastid	fwd	OXY107F	GGACGGGTGAGTAACCGTGR	21			West et al. (2001)
71	18S rRNA	nucleus	fwd	PF1	TGCGCTACCTGGTTGATCCTGCC	23	-5		Keeling, 2002
78	18S rRNA	nucleus	fwd	EukA	AACCTGGTTGATCCTGCCAGT	21	0		Medlin et al. 1988
81	18S rRNA	nucleus	fwd	Euk328F	ACCTGGTTGATCCTGCCAG	19	1		Moon et al. 2001
138	18S rRNA	nucleus	fwd	18SV1V2F	ACCTGGTTGATCCTGCCA	18	1	non-Metazoa	Clerissi et al. (2018)
220	18S rRNA	nucleus	fwd	NSF4/18	CTGGTTGATYCTGCCAGT	18	3		Hendriks et al. (1989)
168	18S rRNA	nucleus	fwd	Pbr1	GGTTGATCCTGCCAGTAGTC	20	5	Plasmodiophora	Niwa et al. 2011
169	18S rRNA	nucleus	rev	Pbr1r	GACTACTGGCAGGATCAACC	20	5	Plasmodiophora	Niwa et al. 2011
109	18S rRNA	nucleus	fwd	SF2Dark	GTTGATCCTGCCAGTAGTGT	20	6	Myxomycetes	Fiore-Donno (2016)

# In silico analysis against PR<sup>2</sup>



The PR2 primer database

- Mismatches #
- Mismatches position
- Amplicon size

Precomputed results for primer sets

Against PR2 sequence database

Primer set  
004 - V4 Hugersh\_2 - general

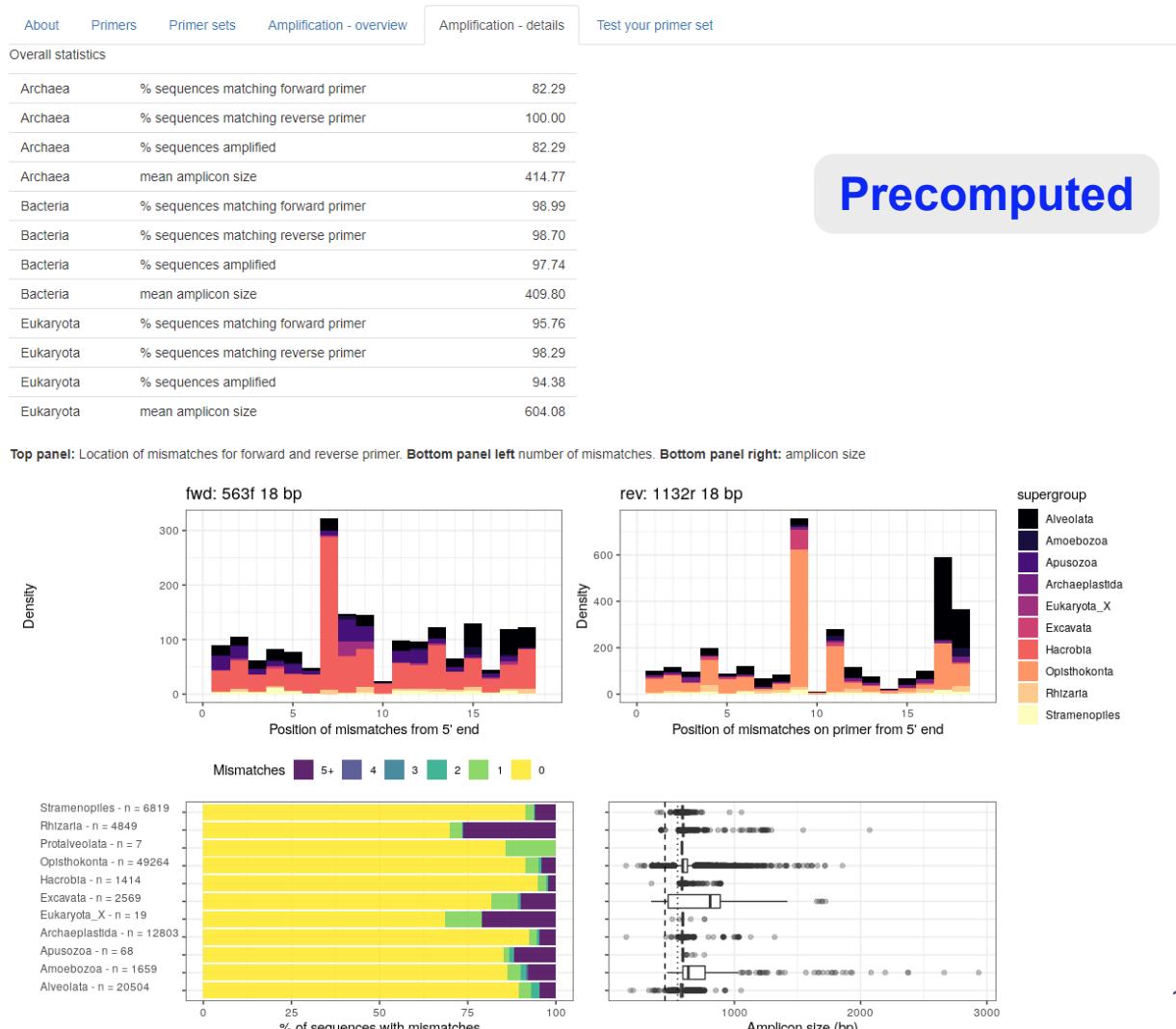
Kingdom  
Eukaryota

Supergroup  
All

Division  
All

Class  
All

Update plot



Precomputed

# Test your own



The PR2 primer database

**Test your primer/probe**

Primer/Probe is tested against the PR2 database.

Use only IUPAC characters ( ACGTTRYSWKMBDHVN ).

**Length of primers:** between 15 and 30 bp.

**Primer/probe (5' -> 3')**  
CTTCGAGCCCCAACTTT

**Max mismatches**  
 0    1    2

**Type**  
 primer fwd    primer rev/probe

**Run**

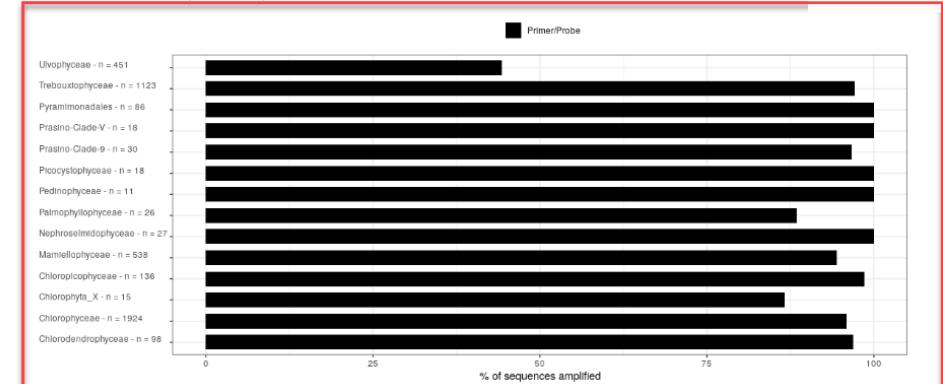
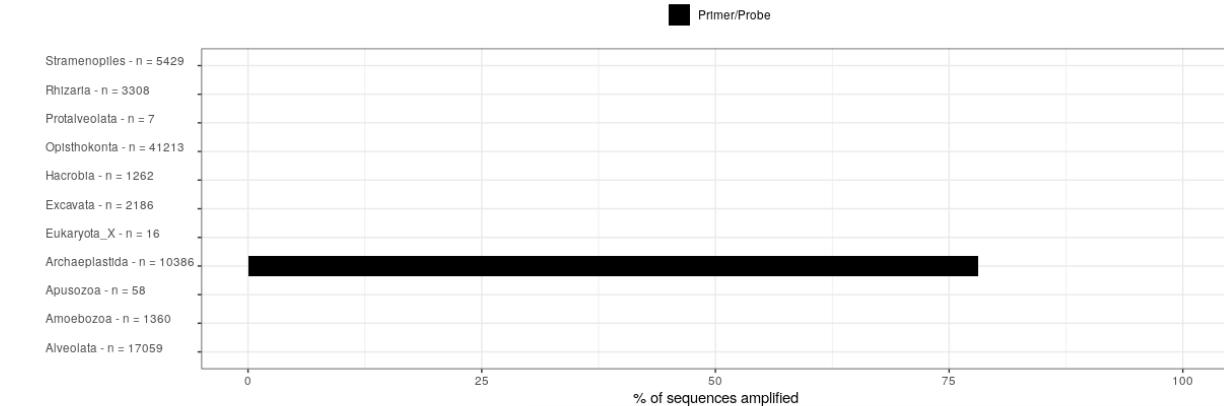
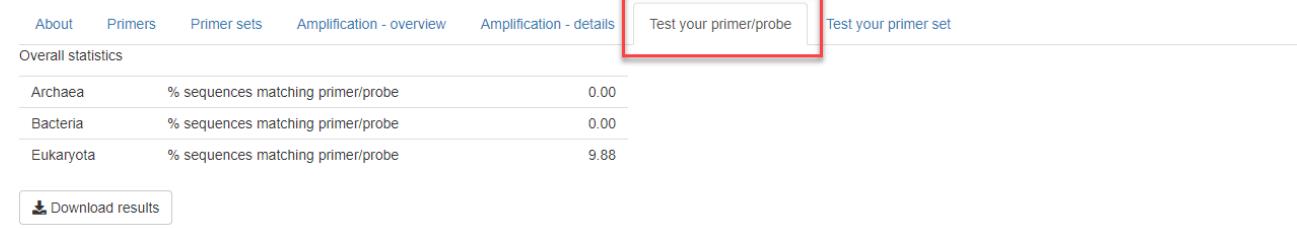
**Kingdom**  
Eukaryota

**Supergroup**  
All

**Division**  
All

**Class**  
All

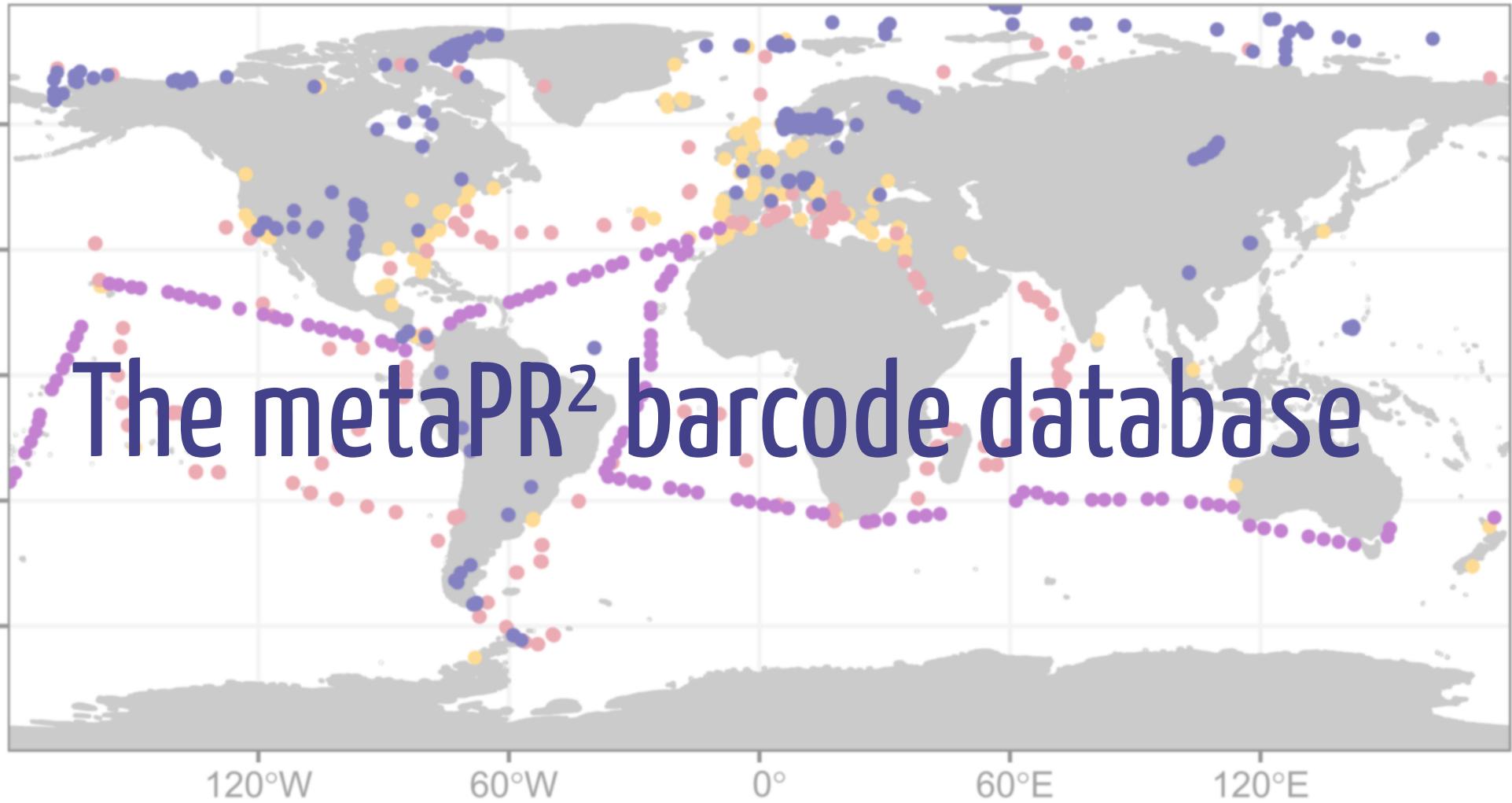
**Update plot**



# What's next for pr2-primers

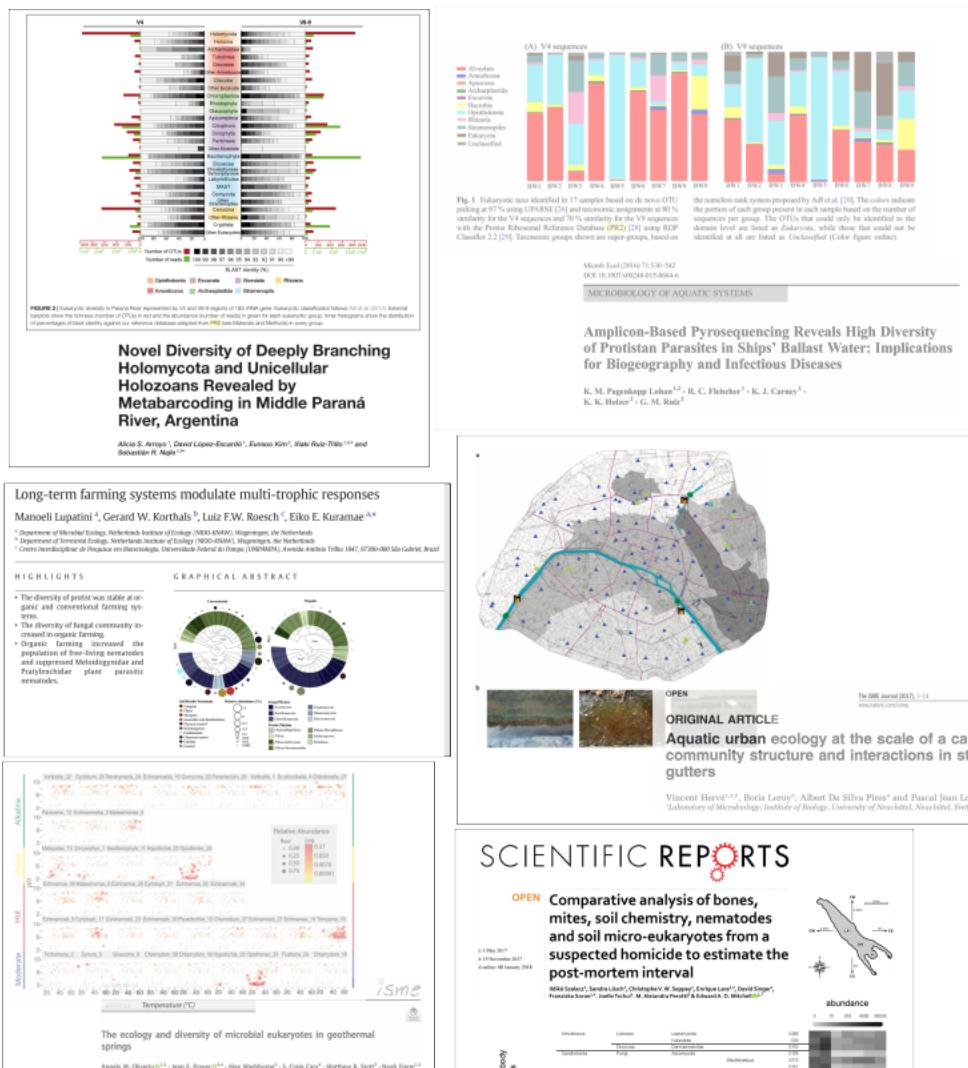
- Update PR<sup>2</sup> from 4.12.0 to 4.14.0
- Add more primers
- ITS/28S primers

# The metaPR<sup>2</sup> barcode database



# Motivation

- In the last decade, many metabarcoding studies
- Data hard to compare:
  - Different primers
  - Different processing
  - Different similarity levels
- Processed data usually not available
- Metadata not available
- Few global datasets used (*Tara*, *Malaspina*)
- These datasets only temperate and tropical marine



# Strategy



- Scan papers and build database
- Start from raw data (fastq) available from GenBank SRA
- Use dada2 pipeline producing ASVs
  - Different datasets are comparable
- Annotate taxonomy with PR<sup>2</sup>
- Integrate metadata
  - Latitude and longitude
  - Depth
  - Substrate (water, ice, soil)
- Data stored in MySQL database
- Develop web interface using R shiny

Received: 7 February 2022 | Revised: 26 May 2022 | Accepted: 20 June 2022

DOI: 10.1111/1755-0998.13674

RESOURCE ARTICLE

MOLECULAR ECOLOGY  
RESOURCES WILEY

## metaPR<sup>2</sup>: A database of eukaryotic 18S rRNA metabarcodes with an emphasis on protists

Daniel Vaulot<sup>1</sup> | Clarence Wei Hung Sim<sup>2</sup> | Denise Ong<sup>2</sup> | Bryan Teo<sup>2</sup> | Charlie Biwer<sup>3</sup> | Mahwash Jamy<sup>3</sup> | Adriana Lopes dos Santos<sup>2</sup>

DADA2: Fast and accurate sample inference from amplicon data with single-nucleotide resolution



# metapr2 version 1.0

- Datasets: 41

- Tara Oceans (reprocessed with dada2)
- Malaspina
- Ocean Sampling Day - 2014 & 2015
- Arctic datasets
- Deep Sea
- Lakes, Rivers, Soils

- Samples: 4,150

- ASVs: 90,000



The metaPR2 database

Select datasets

41 items selected

Search: [ ]

Select All   Deselect All

- Antarctic\_Fildes\_Bay\_2013
- Antarctic\_Fildes\_Bay\_2015\_18S\_V4
- Antarctic\_Fildes\_Bay\_2015\_18S\_V4\_sorted
- Arct\_Baffin\_Bay\_2013
- Arctic\_Beaufort\_Sea\_MALINA\_2014
- Arctic\_Nansen\_Basin\_2012
- Arctic\_Nares\_Strait\_2014
- Arctic\_Ocean\_Central\_2012
- Arctic\_Ocean\_P80\_2012
- Arctic\_Ocean\_Survey\_2005\_2011
- Arctic\_White\_Sea\_2013\_2015
- Baltic\_Sea\_2012\_2013
- Baltic\_Sea\_Gdansk\_2012
- Chukotk\_SeaICESCAPE\_2010
- European\_coast\_Biomarks\_2009
- Italy\_Naples\_2011
- Lake\_Balkal\_2013
- Lake\_Chaochu\_2014\_2015
- Lake\_Chevreuse\_2012
- Lake\_Fuxian\_2015
- Lake\_Garda
- Lakes\_Argentina
- Lakes\_mountain\_2013
- Lakes\_Scandinavia
- Malaspina\_surface\_2010\_2011

About   Datasets   Treemap   Map   Barplot   Diversity   Query   Download   Help

Quick dataset selection.

Dataset groups

marine\_global\_V4   oceanic   coastal   rivers   lakes   soils   arctic   antarctic   temperate   tropical   time series

Show 10 entries

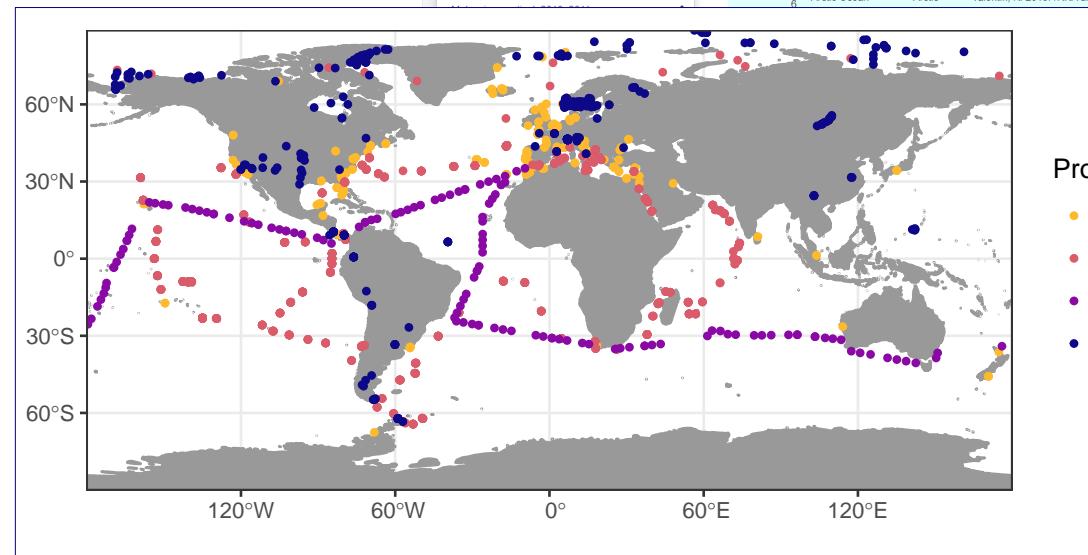
Search: [ ]

dataset_id	dataset_name	region	paper_reference	sample_number	asv_number	n_reads_mean	selected
11	Antarctic Fildes Bay - 2013	Southern Ocean	Luo, W. et al. Molecular diversity of microbial eukaryotes in sea water from Fildes Peninsula, King George Island, Antarctica. <i>Polar Biol.</i> (2015)	10	69	13631	true
16	Antarctic Fildes Bay 2015 18S V4	Southern Ocean	Trefault, N., De la Iglesia, R., Moreno-Pino, M., Lopes dos Santos, A., Gómez-Ribeiro, C., Parada-Pozo, G., Cristi, A., Marie, D., & Vaultol, D. (2021). Annual phytoplankton dynamics in coastal waters from Fildes Bay, Western Antarctic Peninsula. <i>Scientific Reports</i> , 11(1), 1368.	123	685	48261	true
18	Antarctic Fildes Bay 2015 18S V4 sorted	Southern Ocean	Trefault, N., De la Iglesia, R., Moreno-Pino, M., Lopes dos Santos, A., Gómez-Ribeiro, C., Parada-Pozo, G., Cristi, A., Marie, D., & Vaultol, D. (2021). Annual phytoplankton dynamics in coastal waters from Fildes Bay, Western Antarctic Peninsula. <i>Scientific Reports</i> , 11(1), 1368.	60	280	31615	true
9	Arctic Nansen Basin - 2012	Arctic Ocean	Meffies, K., von Appen, W.-J., Kilias, E., Nicolaus, A. & Nittrouer, E.-M. Biogeography and Photosynthetic Biomass of Arctic Marine Pico-Eukaryotes during Summer of the Record Sea Ice Minimum 2012. <i>PLoS One</i> 11, 20 pp. (2016)	17	328	13700	true
42	Arctic Nares Strait - 2014	Arctic Ocean	Kalenitchenko, D., Joli, N., Polvin, M., Tremblay, J.-G., Lovejoy, C. 2019. Biodiversity and Species Change in the Arctic Ocean: A View Through the Lens of Nares Strait. <i>Frontiers in Marine Science</i> 6:1-96-17.	247	1510	36626	true
6	Arctic Ocean	Arctic	Stecher, A., Neuhaus, S., Lange, B., Frickerhaus, S., Beszteri, B., Kroth, P.G. & Valentini, K. 2015. rRNA and rDNA based	8	182	36628	true
36				182	7136	7136	true
24				270	6704	6704	true
45				978	73933	73933	true
17				380	23990	23990	true

Project

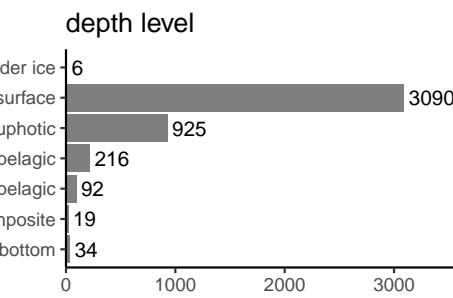
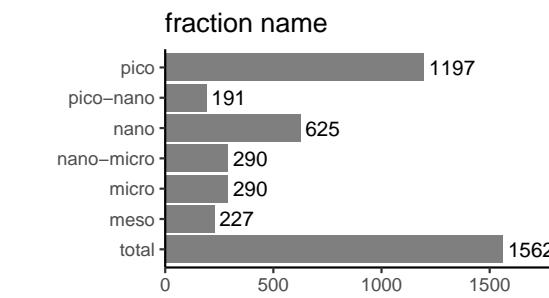
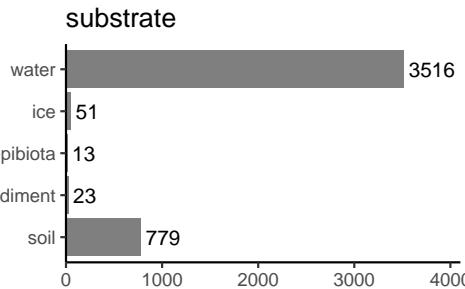
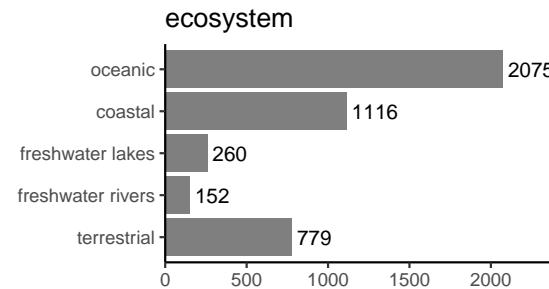
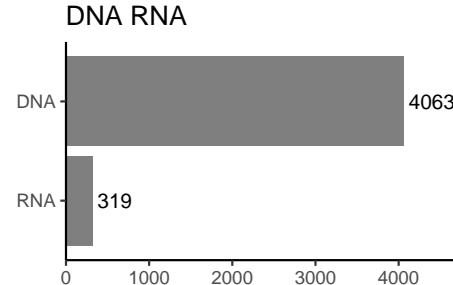
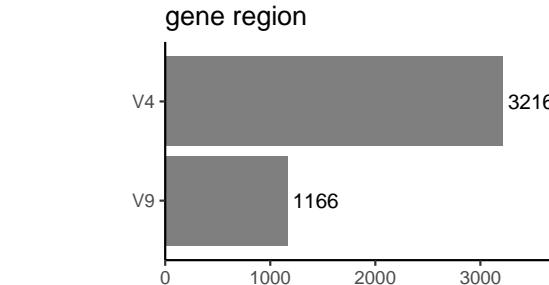
- OSD
- Tara
- Malaspina
- Other

Previous [1] 2 3 4 5 Next

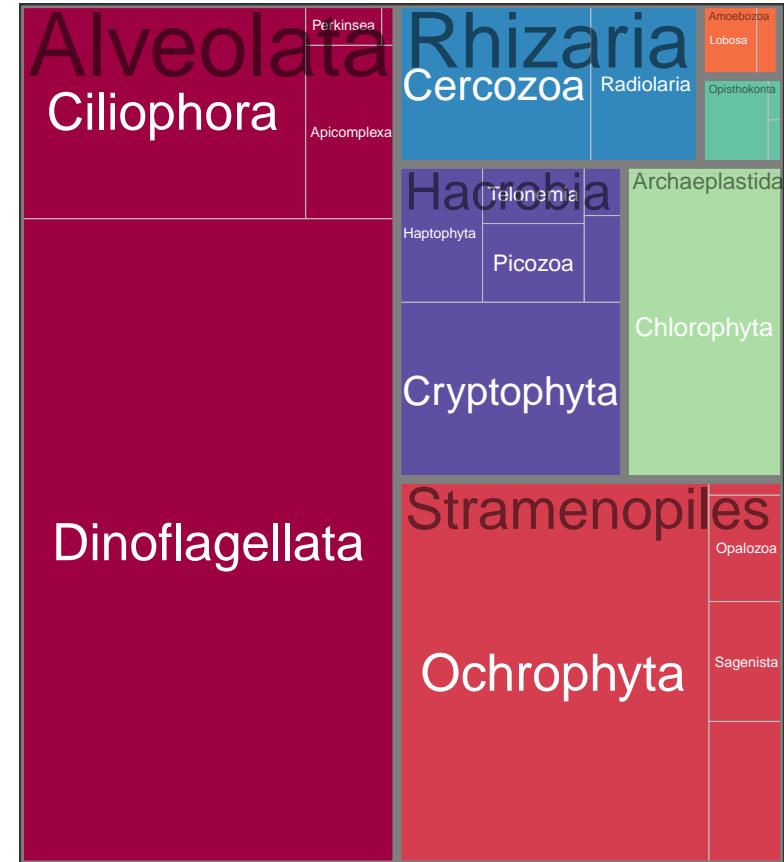


# Samples and ASVs

Number of samples



Reads

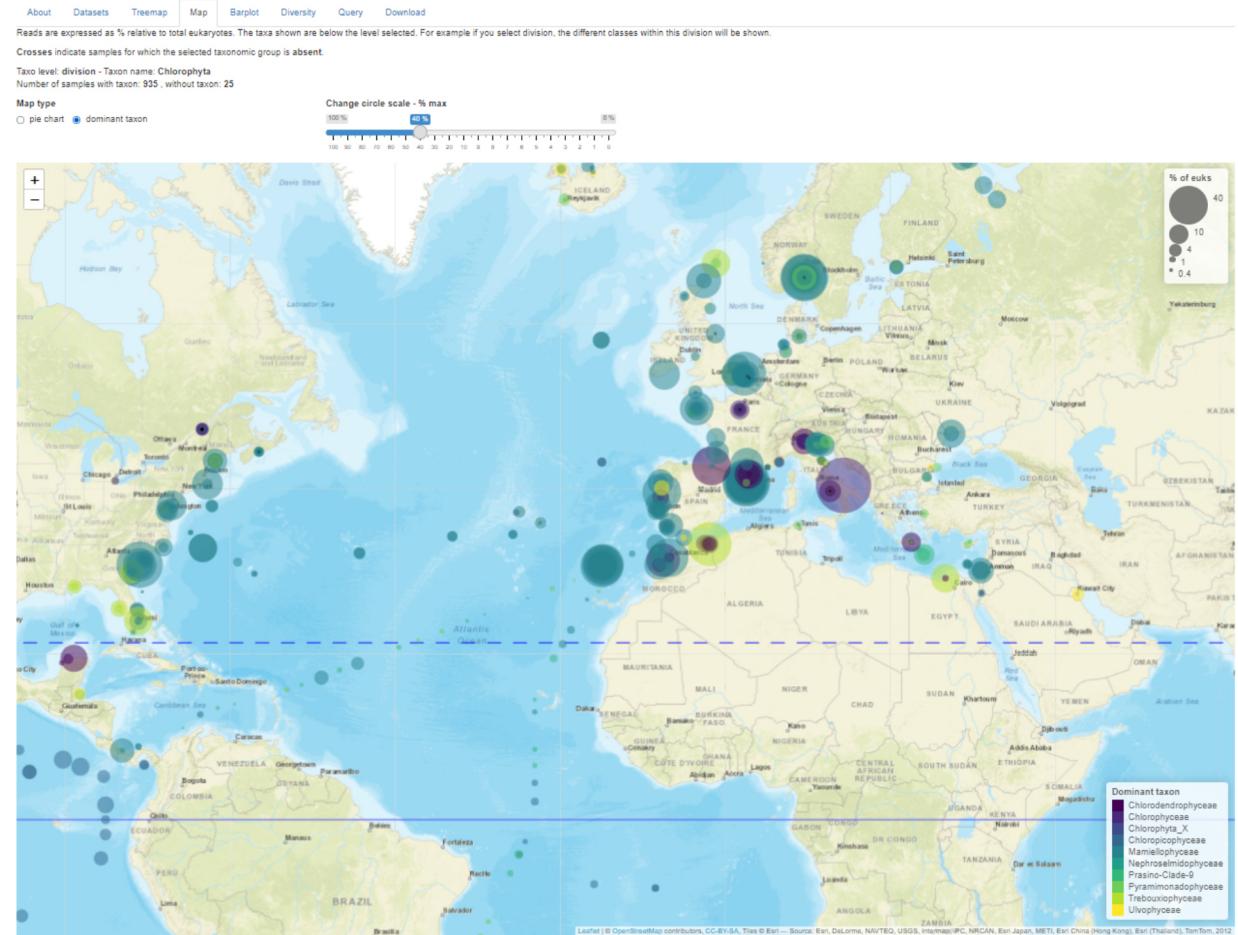
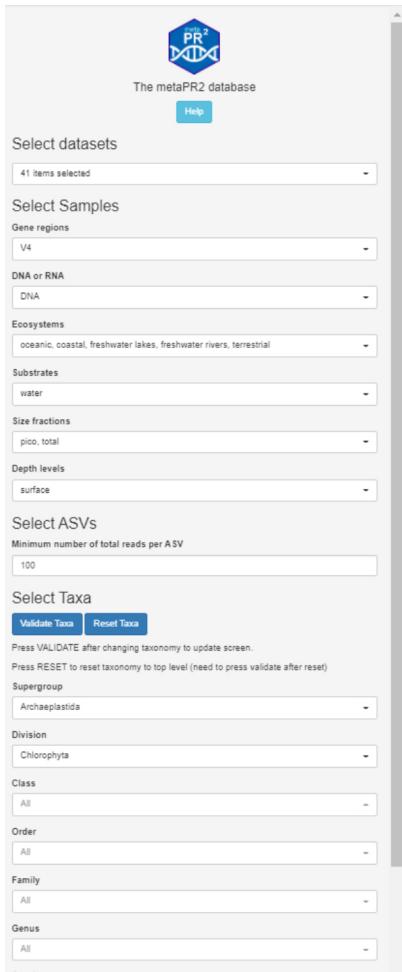


Protists only

# Web interface

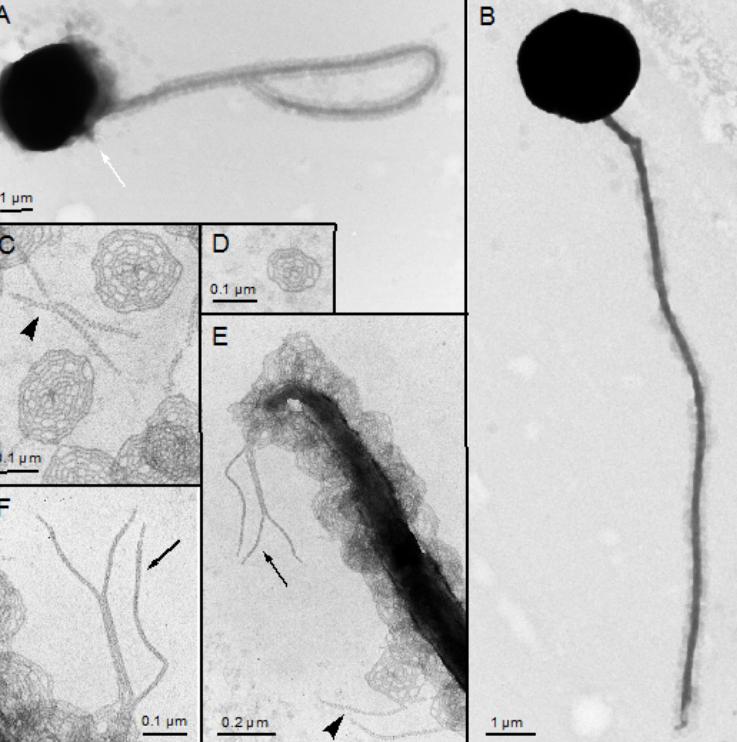


- Built with R shiny
    - Available also as R package
  - Panels
    - Datasets
    - Treemaps
    - Maps
    - Barplots
    - Diversity
    - Query
    - Download





# Biogeography



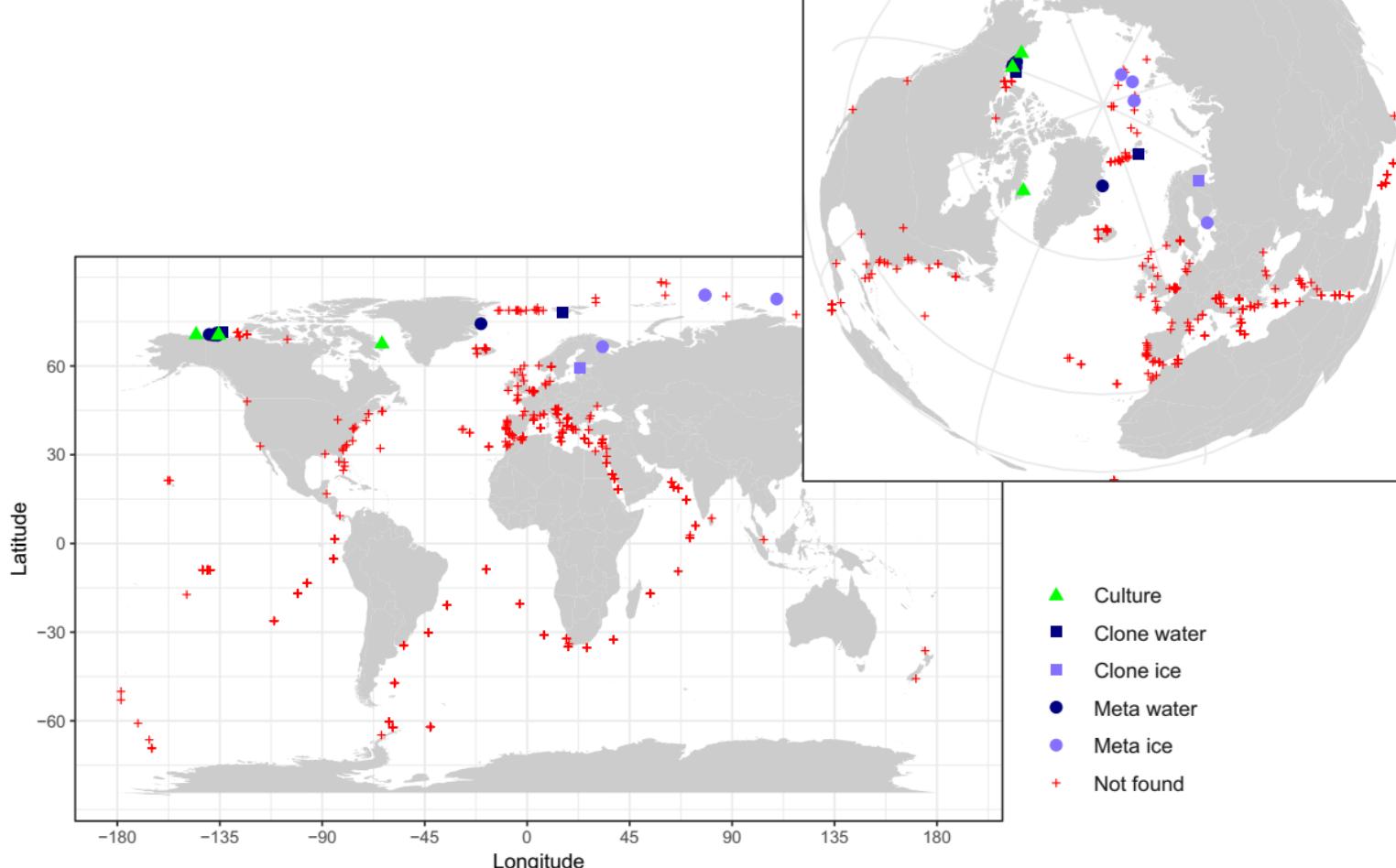
*J. Phycol.* 56, 37–51 (2020)  
© 2019 Phycological Society of America  
DOI: 10.1111/jpy.12932

MANTONIELLA BEAUFORTII AND MANTONIELLA BAFFINENSIS SP. NOV. (MAMIELLALES, MAMIELLOPHYCEAE), TWO NEW GREEN ALgal SPECIES FROM THE HIGH ARCTIC<sup>1</sup>



Sheree Yau<sup>2,3</sup>

Integrative Marine Biology Laboratory (BIOM), CNRS, UMR7232, Sorbonne Université, Banyuls sur Mer, France



# What's next for metapr2

- Datasets

- Version 2.0 (nov. 2022) :
    - **18 new datasets**
    - Clustered ASVs
    - Bug fixes and improved visualization
    - New panels (taxonomy)

- Planned

- More datasets (soils, freshwater, microbiomes)
  - Heatmaps



# Team

## Core team



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## pr2-database

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## Scientific Committee



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phylogenomics, metabarcoding,  
long-reads, protists, diversity



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## pr2-primers



Stefan Geisen



Fred Mahé



David Bass

# Open discussion

## Evolution of PR2

- Web interface
- Number of taxonomic levels
- Long sequences

## Funding needed, where to find ?

- Cloud resources
- Web and script development
- Workshops

## How to get help on annotation ?

- Community call
- Workshop (2 weeks ?)

## Project structure

- Core team
- Scientific committee