

Optimization of diatom DNA metabarcoding for freshwater biomonitoring

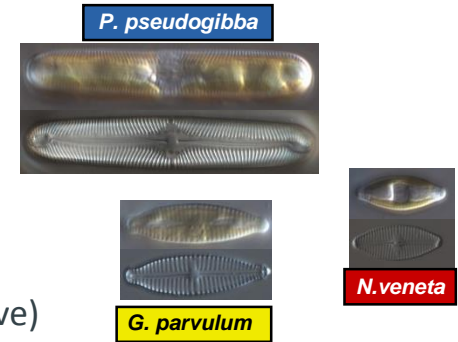
Application to Mayotte streams monitoring network



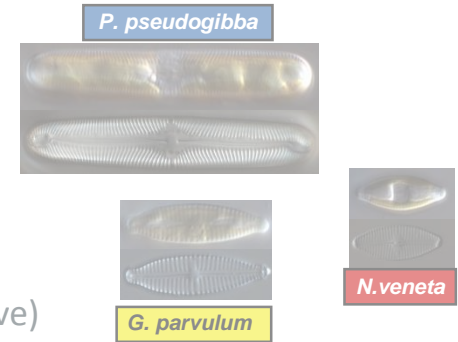
V. Vasselon, I. Domaizon, F. Rimet, K. Tapolczai, A. Bouchez

❖ Benthic diatoms are good biological indicators (Gerhardt et al. 2002)

- Widely distributed in aquatic ecosystems
- Some species are sensitive to physical and chemical factors
- Diatom community is representative of water quality status
- Used for water quality assessment (WFD - Water Framework Directive)



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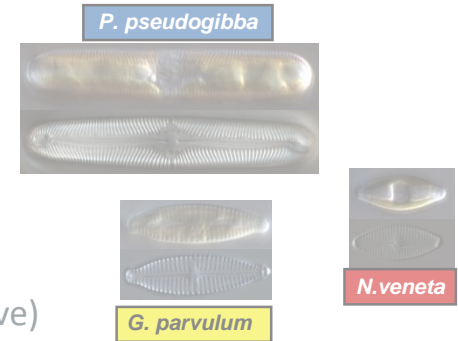
- ❖ **Different approaches to evaluate the diatom community composition**

Microscopy

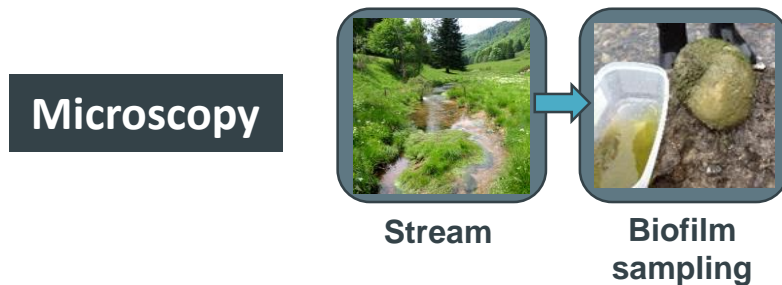


Stream

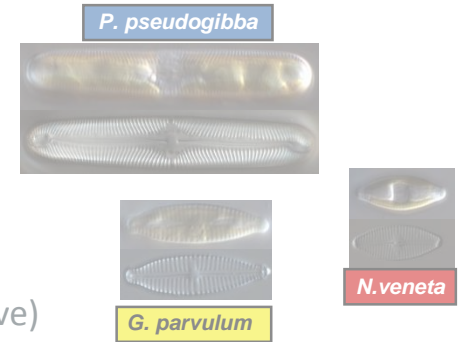
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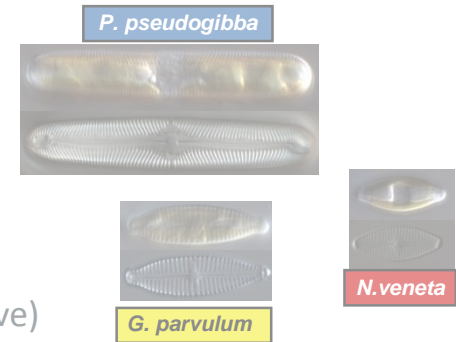
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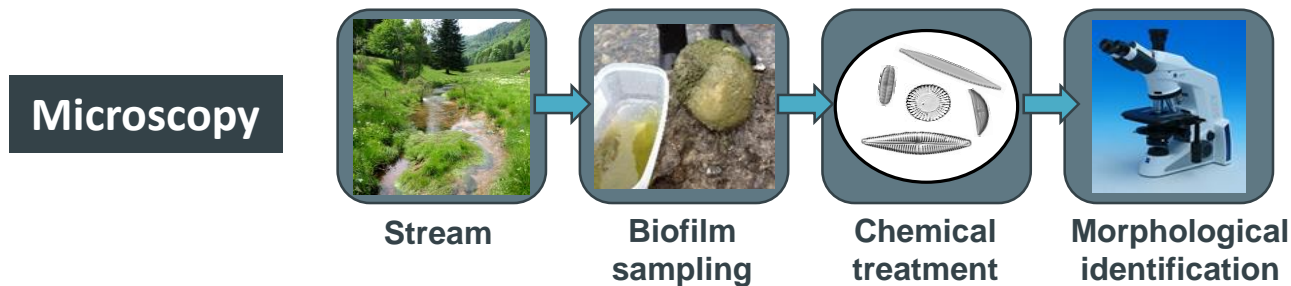
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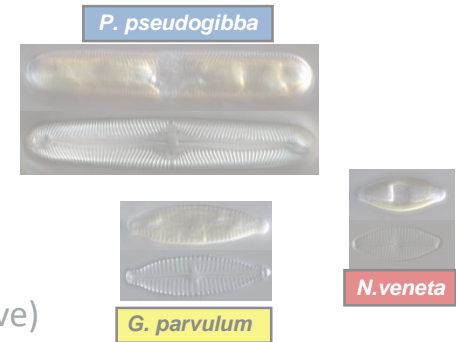
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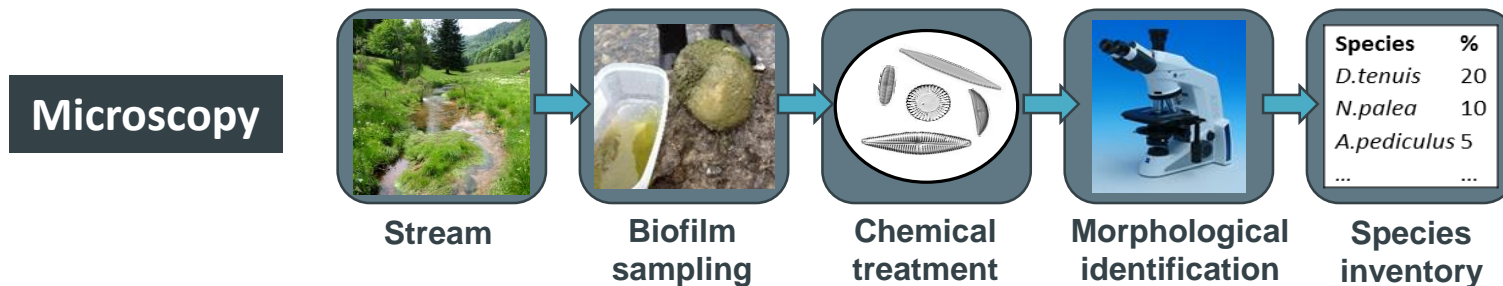
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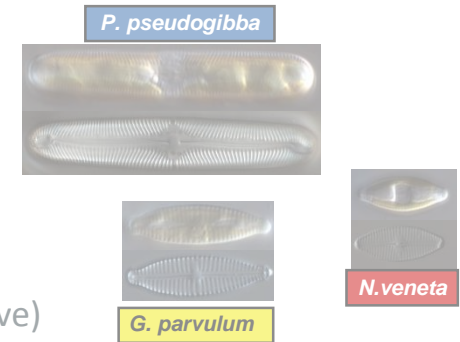
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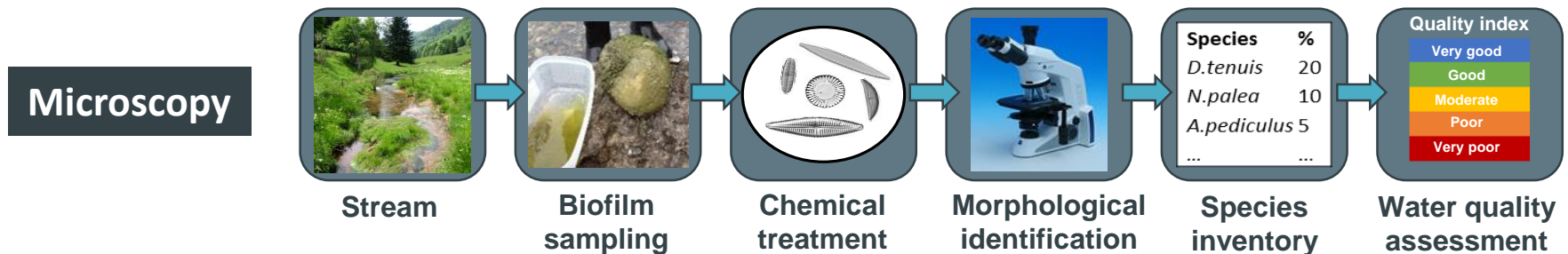
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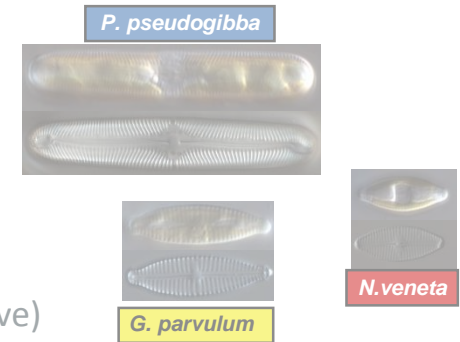
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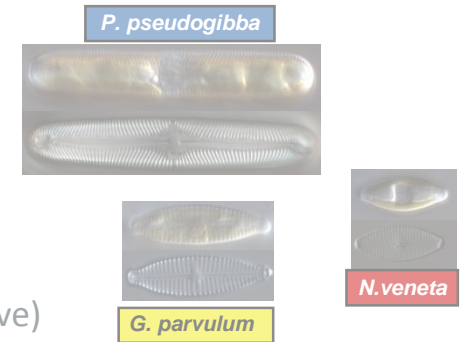
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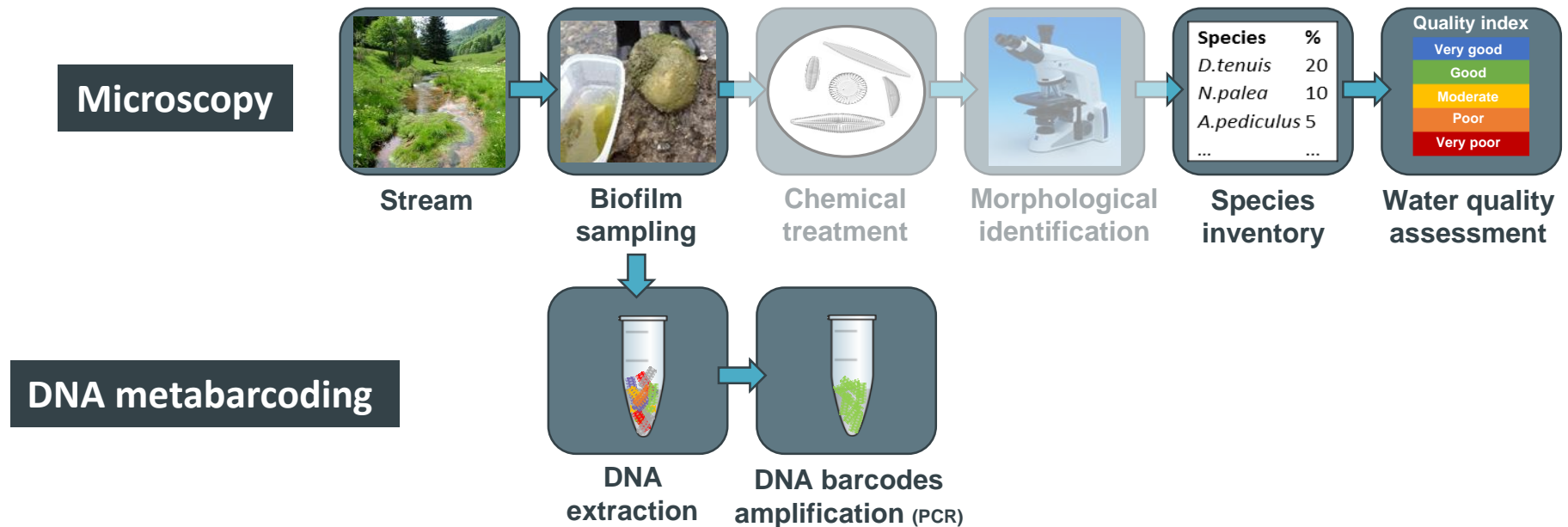
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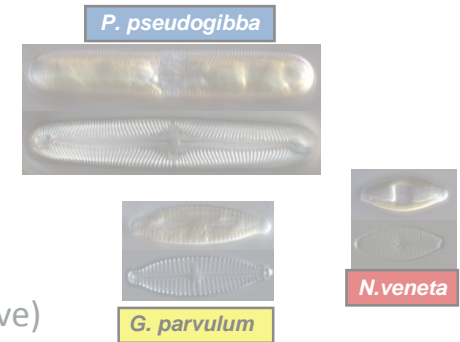
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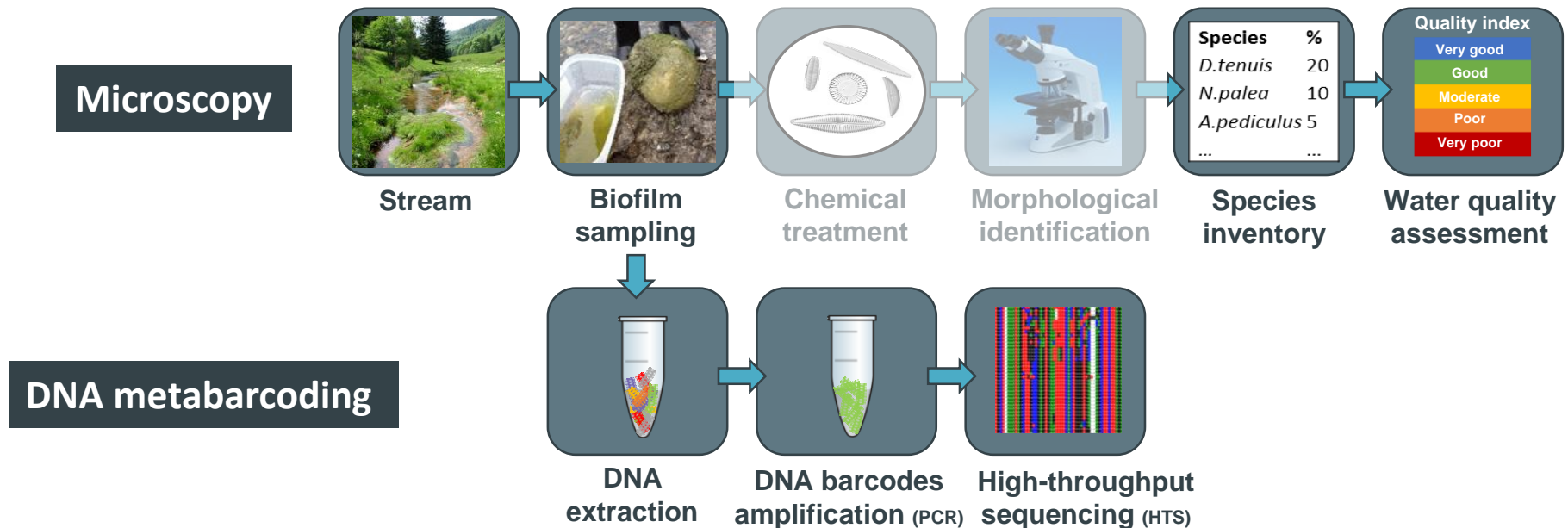
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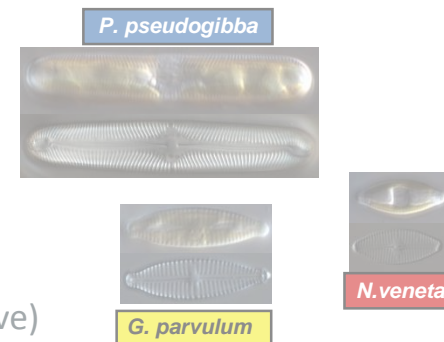


Benthic diatom for water quality assessment

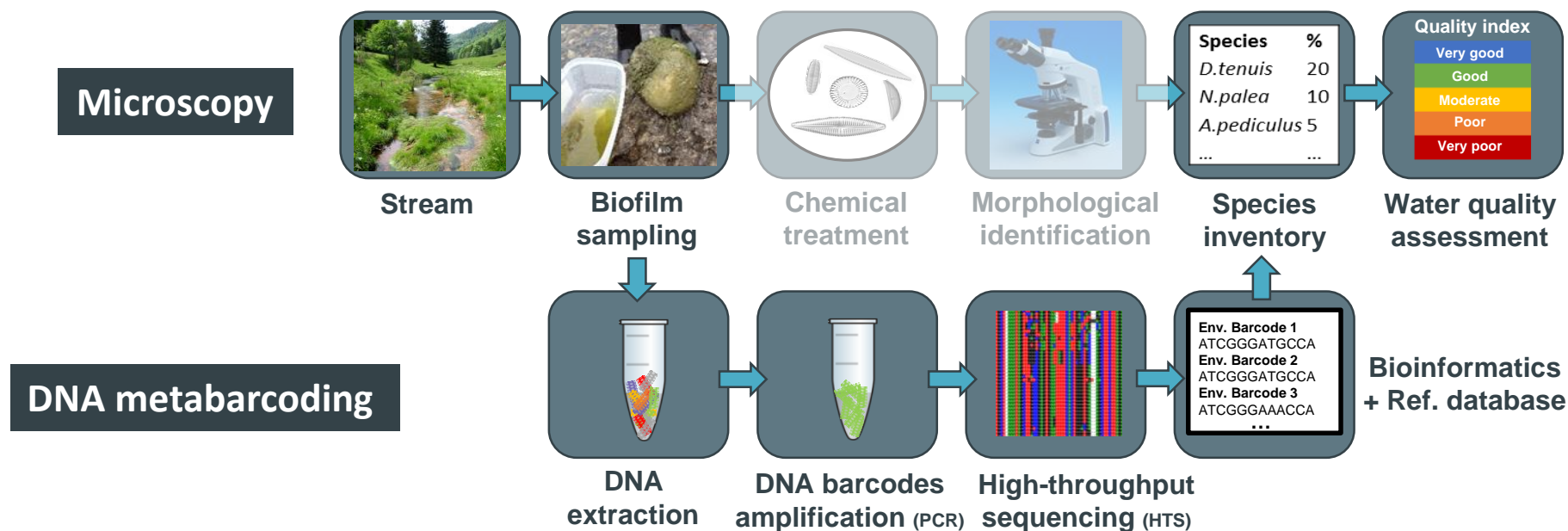


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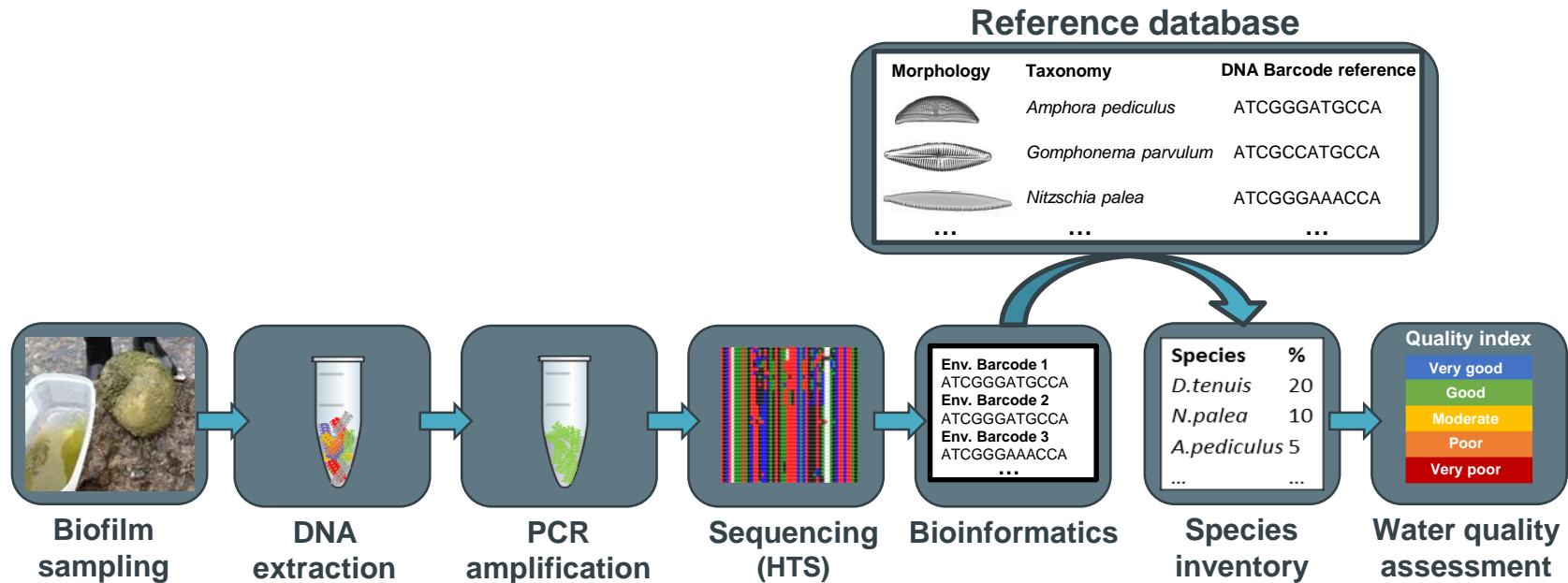
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Development of DNA metabarcoding



❖ A powerful approach



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Incompleteness of the database
Taxonomic knowledge's
Database curation



Sampling method ?
Sample preservation
Free DNA
Cross contamination

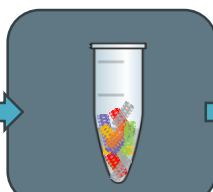
Choice of DNA barcode
Primers efficiency
Amplification errors
PCR inhibitors
Primers specificity

Reference database

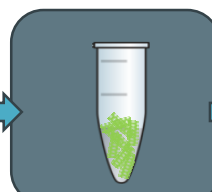
Morphology	Taxonomy	DNA Barcode reference
	<i>Amphora pediculus</i>	ATCGGGATGCCA
	<i>Gomphonema parvulum</i>	ATCGCCATGCCA
	<i>Nitzschia palea</i>	ATCGGGAACCA
...



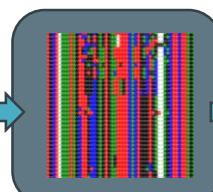
Biofilm
sampling



DNA
extraction



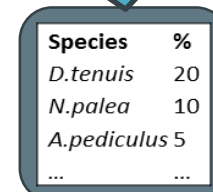
PCR
amplification



Sequencing
(HTS)



Bioinformatics



Species
inventory



Water quality
assessment



Extraction efficiency
Diatom silica wall
DNA quality/purity



Mistagging
Library preparation
Sequencing technology



Trimming
Alignment
Clustering



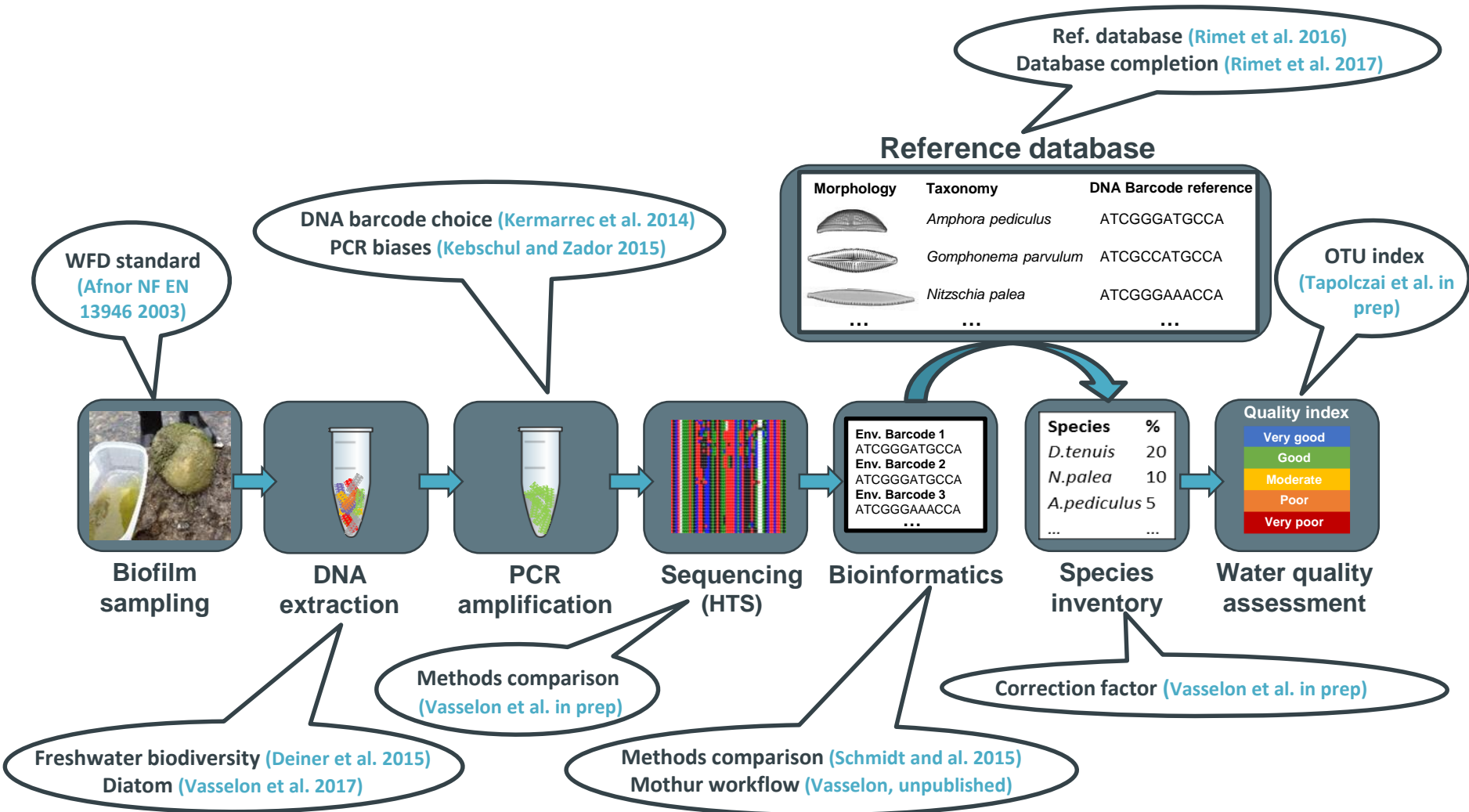
Taxonomic assignment
Gene copy number variation
Index calculation



Development of DNA metabarcoding



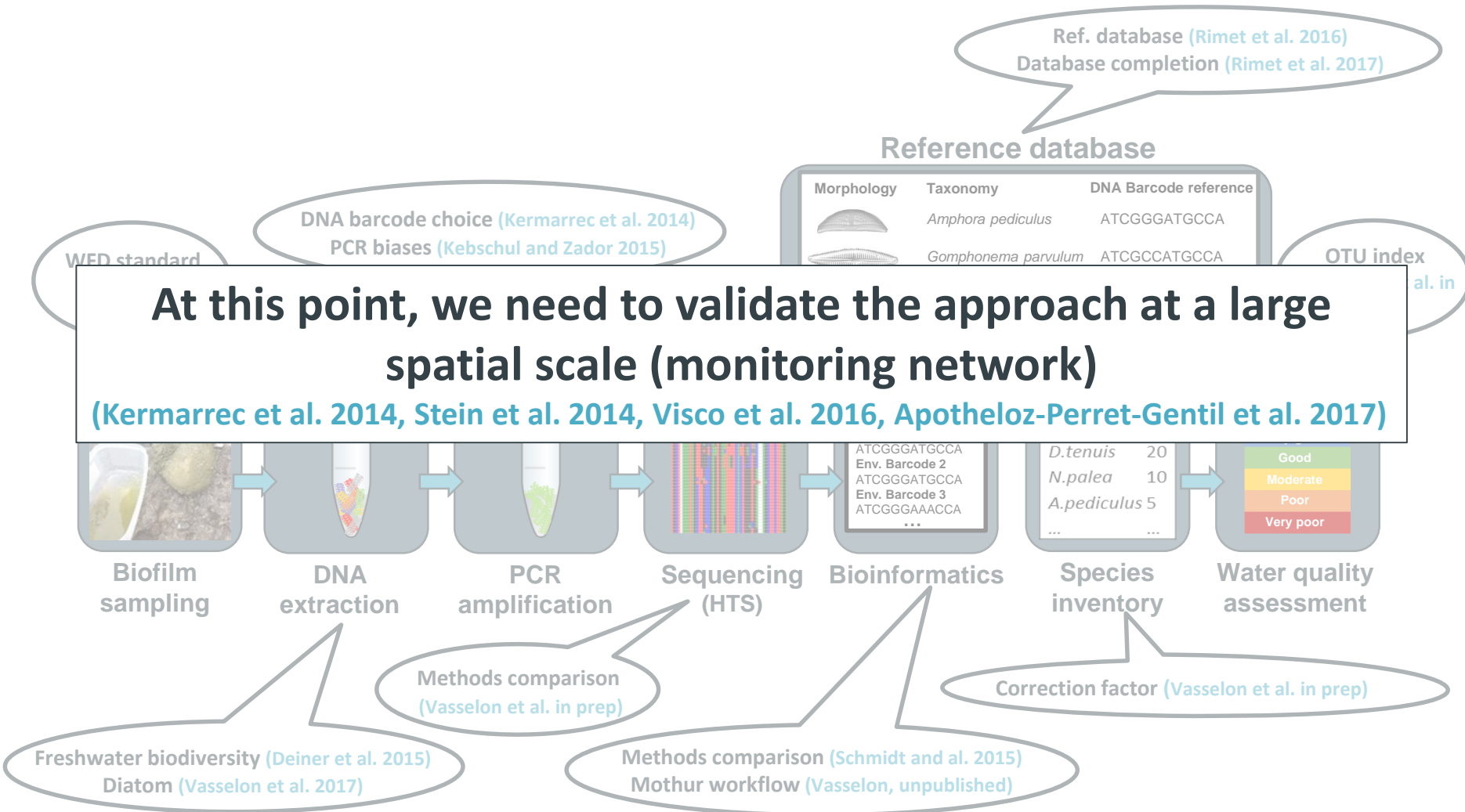
❖ A powerful approach which has been optimized at each step of the process



Development of DNA metabarcoding



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❖ Mayotte tropical Island

- French department since 31 March 2011 (between Mozambique and Madagascar)
- WFD must be applied to **Mayotte streams**

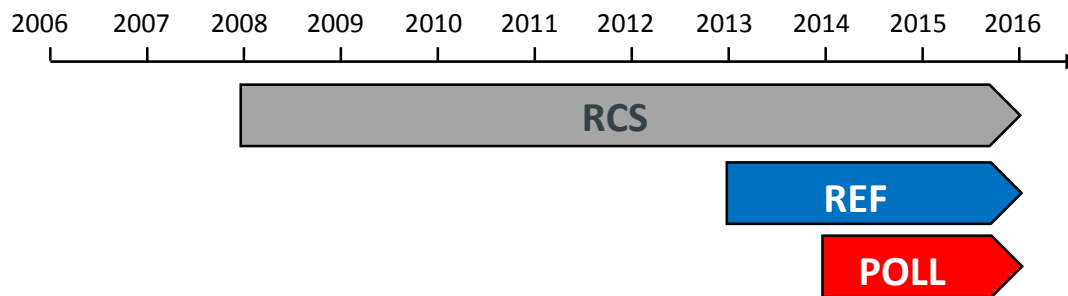


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❖ Mayotte freshwater biomonitoring network :

- Regular biomonitoring network (RCS)
- Reference (REF) and polluted (POLL) networks are considered to have a water quality gradients

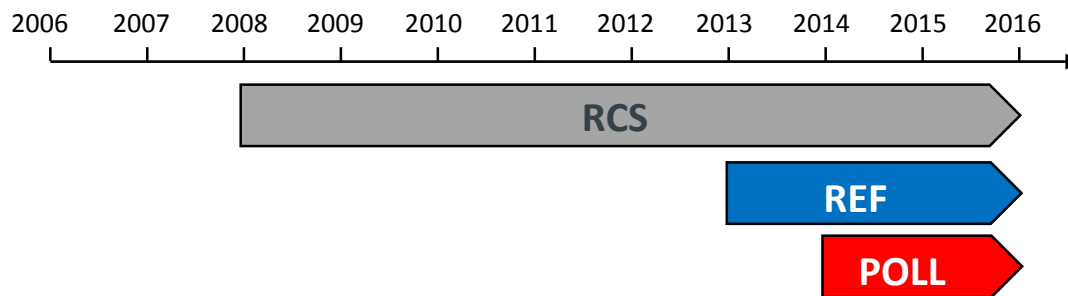


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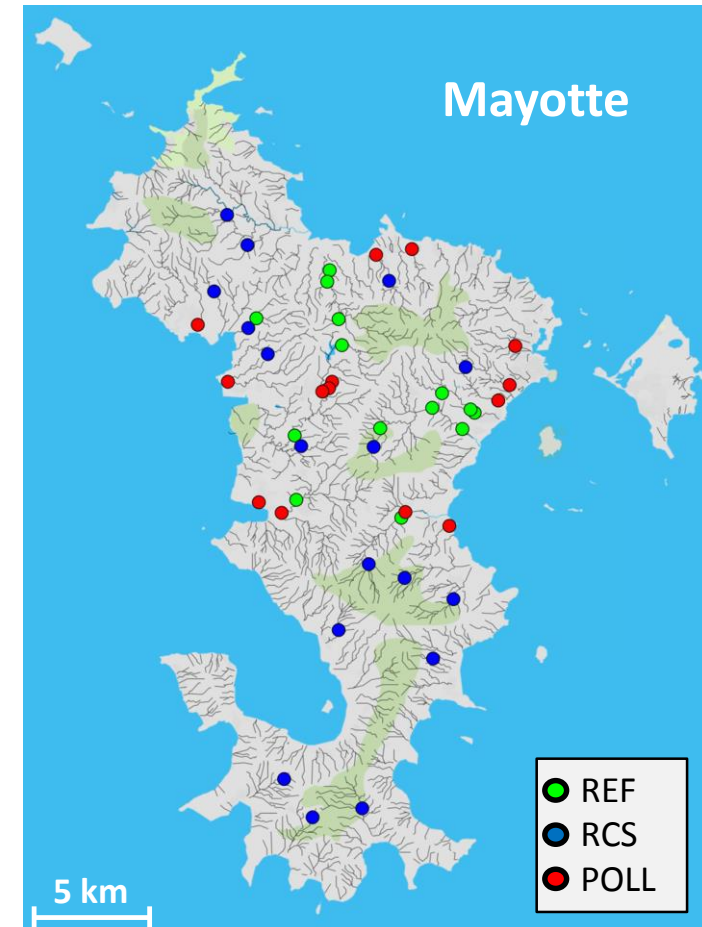


Objective : development of biomonitoring tool for benthic diatoms

- Classical approach (based on microscopy and the WFD)
- **DNA metabarcoding approach**

❖ Sampling of Mayotte's streams

- Sampling campaign realized in 2014 and 2015
- 45 sites for a total of 80 samples
- Sites from the 3 monitoring networks (RCS, REF, POLL)

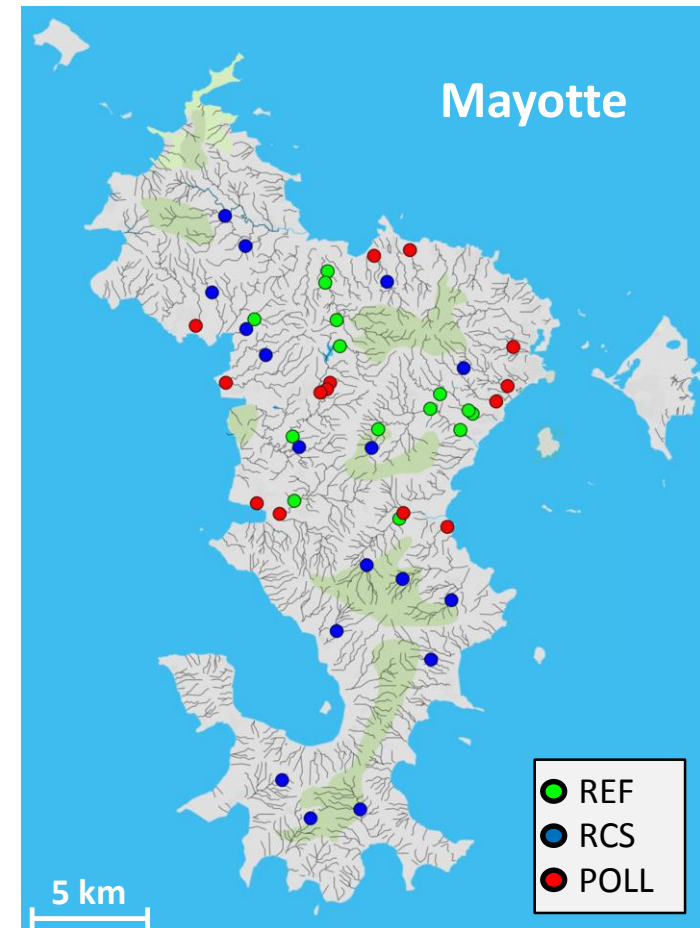


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❖ Diatoms communities analysis

- Morphological identification
- DNA metabarcoding approach performed using
 - SA-gen method for DNA extraction
 - ***rbcL* DNA barcode (312 bp)**
 - PGM-Ion torrent for HTS
 - R-syst::diatom database



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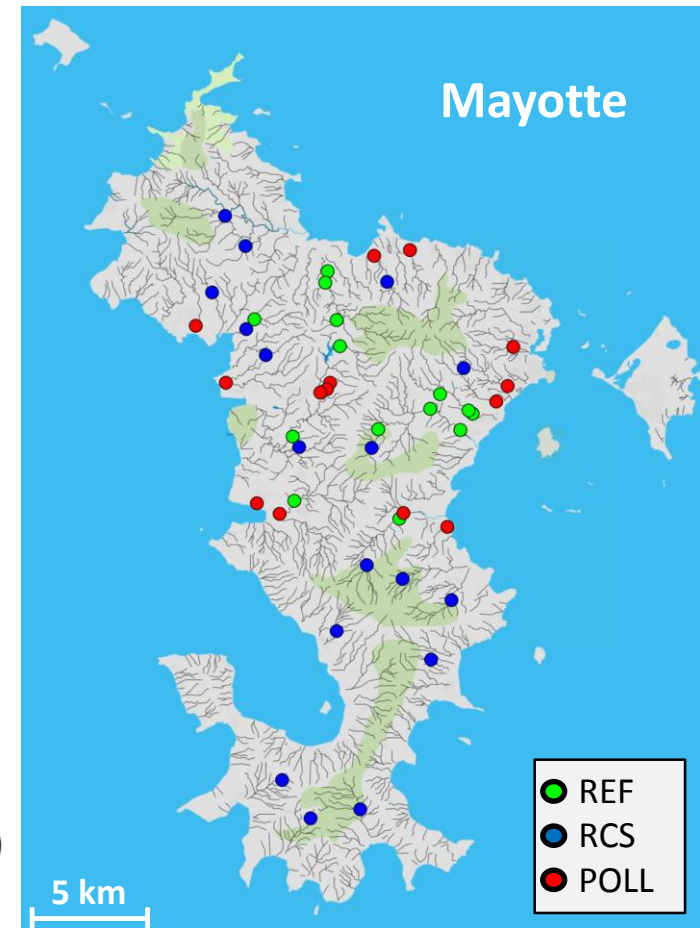
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❖ Comparison morphological vs. molecular approaches

- Community structure (Bray Curtis dissimilarity + Mantel test)
- Diatom taxonomic composition
- Specific Pollution-Sensitivity Index (SPI) calculation



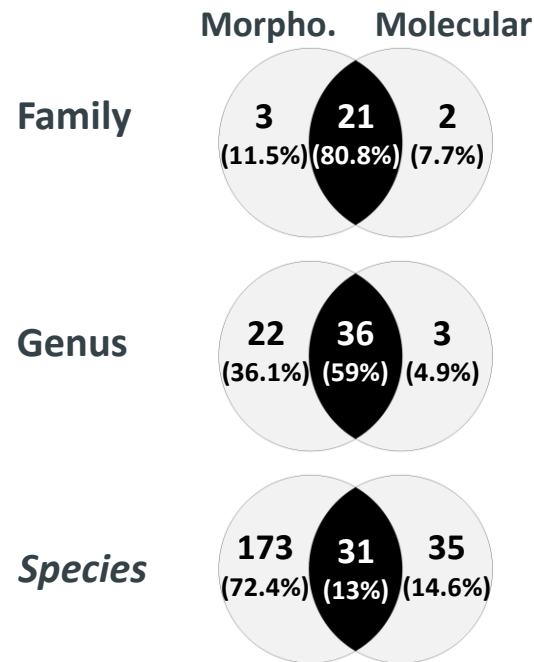
❖ Comparison of molecular (OTUs) and morphological (diatom species)

Mantel's test using Bray-Curtis dissimilarity indices ($r = 0.43$, $p = 0.01$) => **similar community structure**

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❖ Correspondence between morphological and molecular diatom inventories

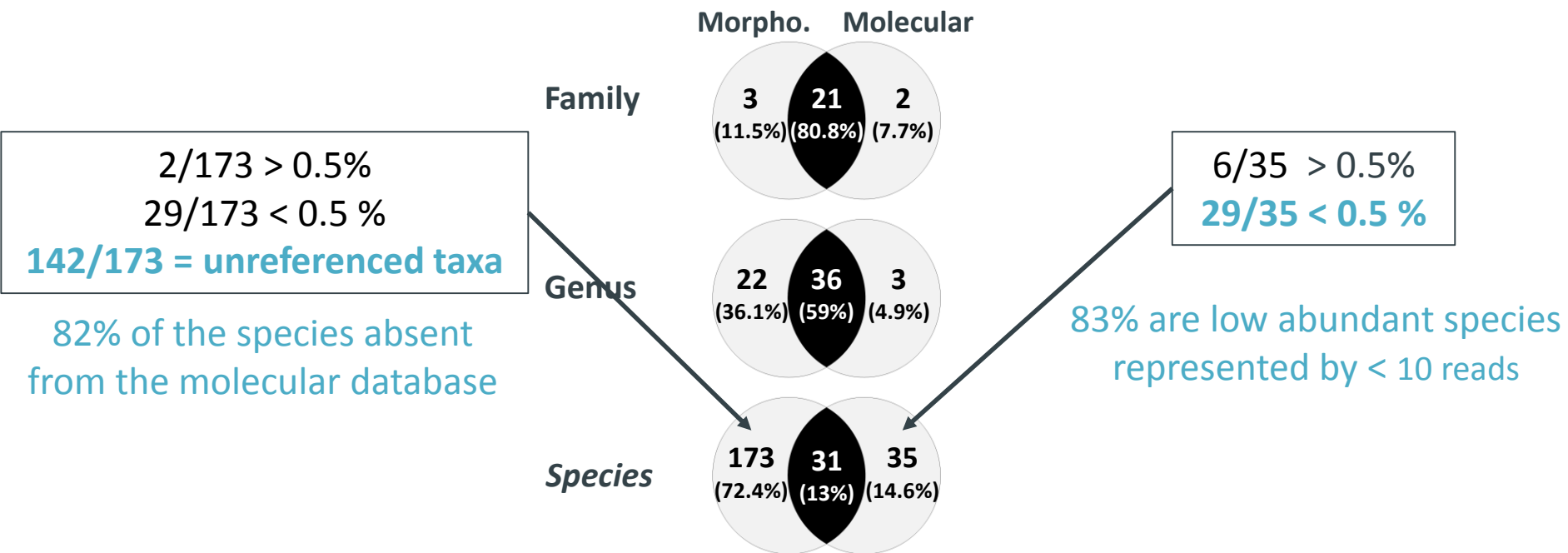


Low correspondence at the species level

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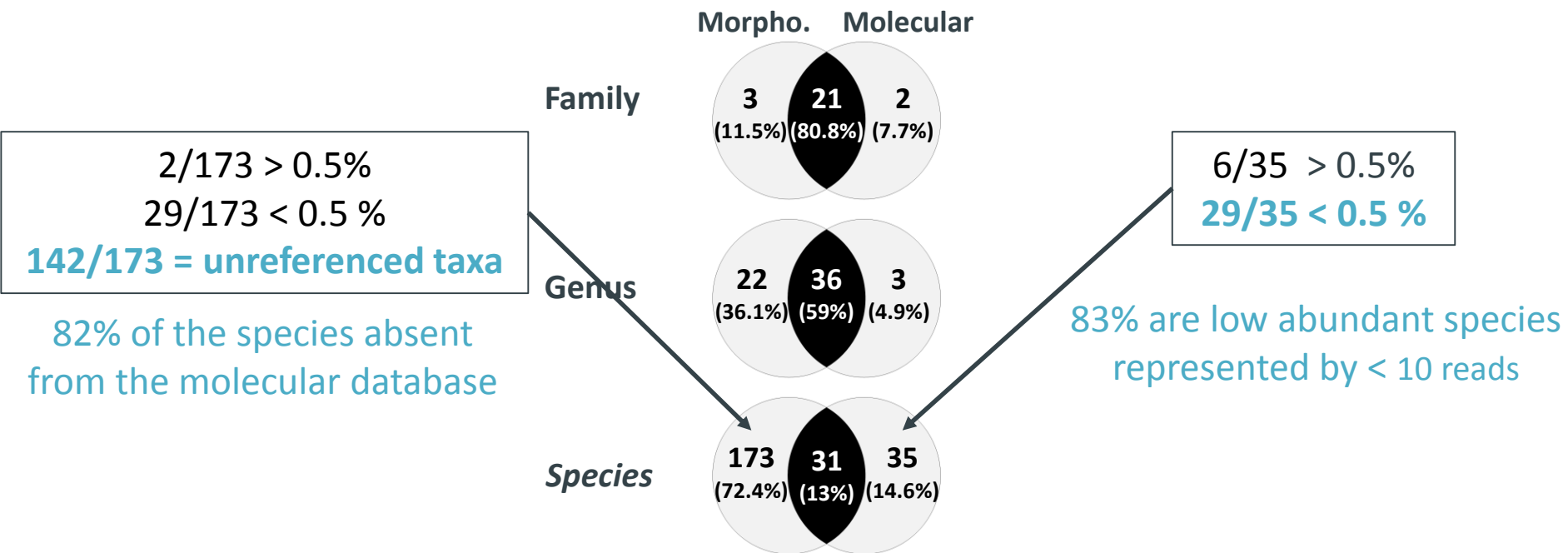
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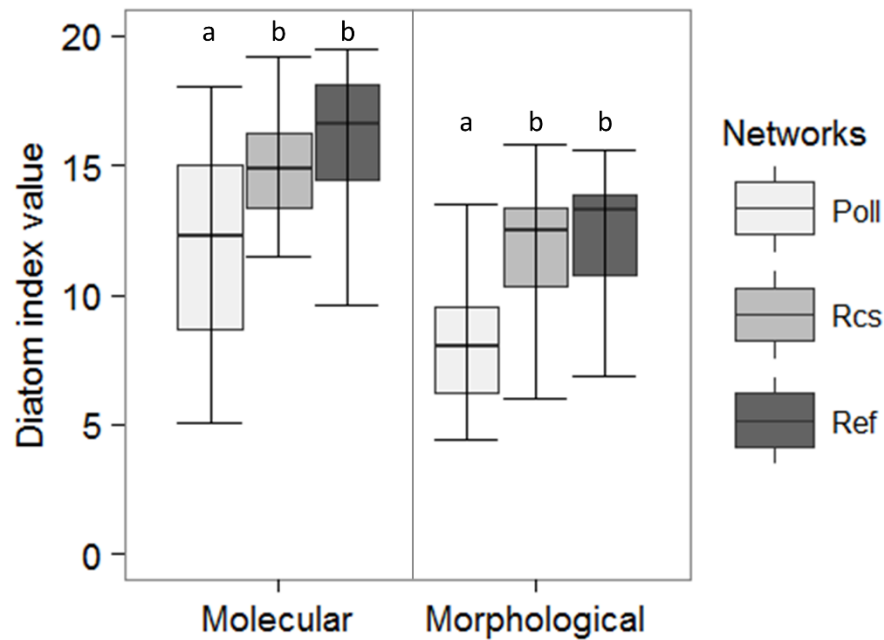
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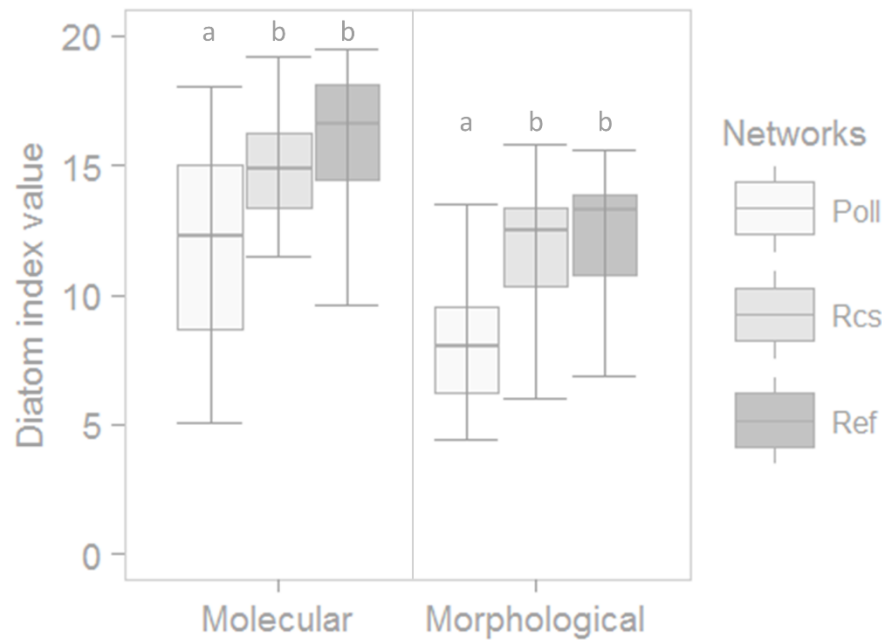
Major bias: incompleteness of the reference database
Problem for water quality assessment ?

❖ Calculation of molecular and morphological water quality indices (SPI – Specific Pollution Index)

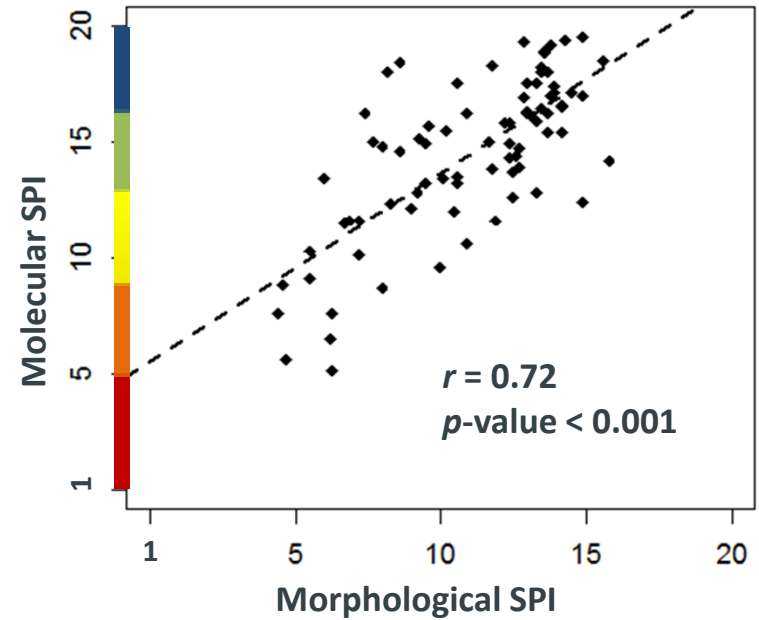


Possible to discriminate samples from polluted network with both approaches

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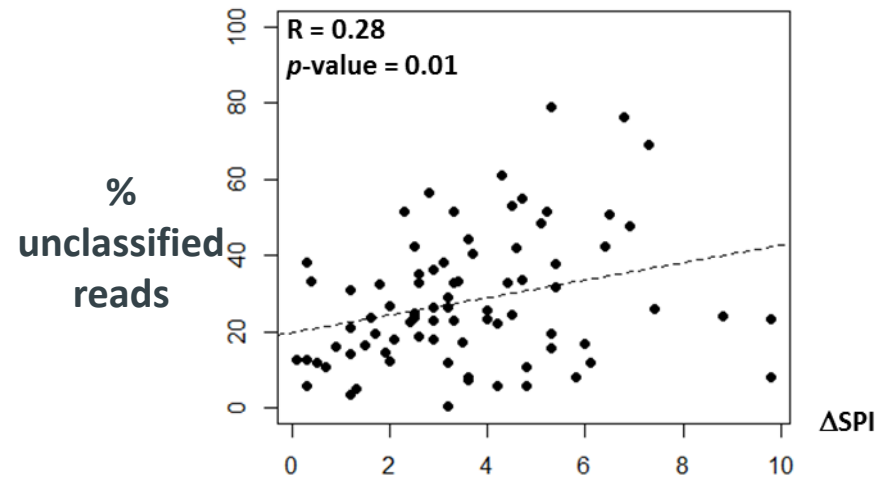
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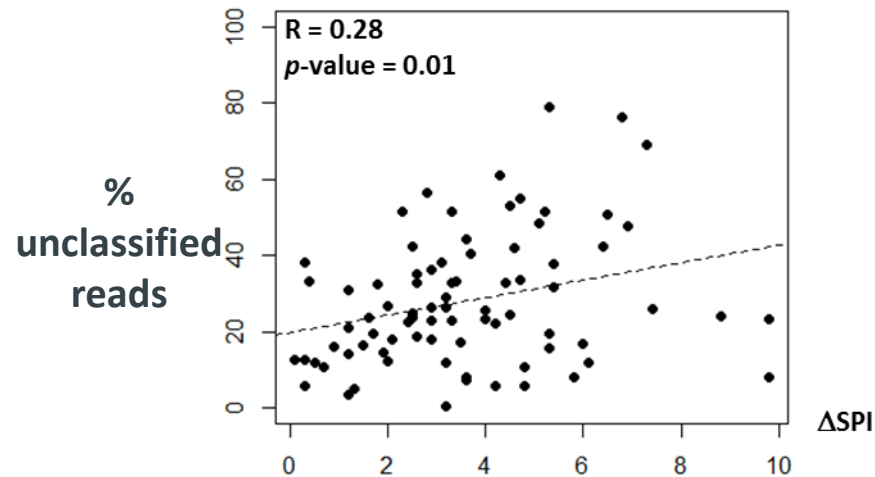
Good correlation between molecular and morphological SPI

But molecular SPI \approx 3.6 points higher

❖ Source of difference between molecular and morphological SPI

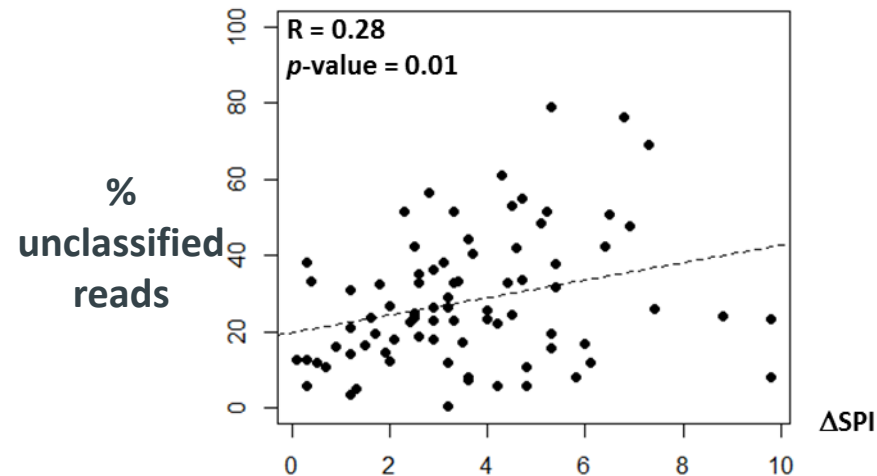


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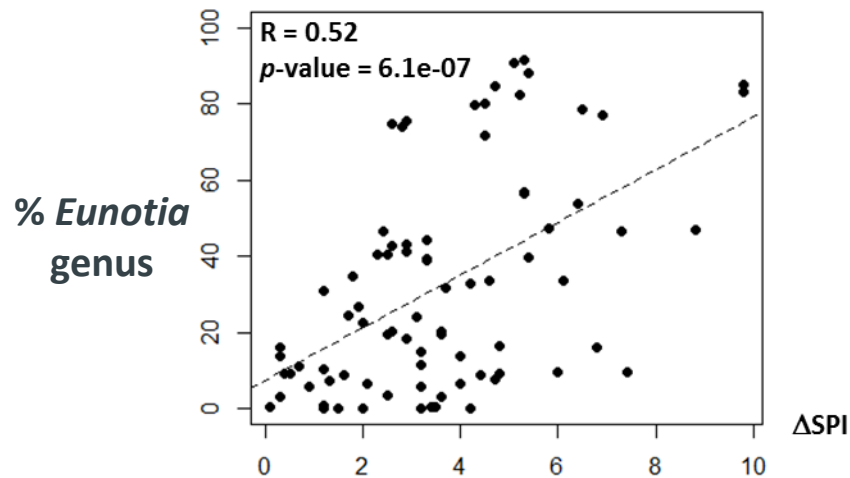


Incompleteness of the reference
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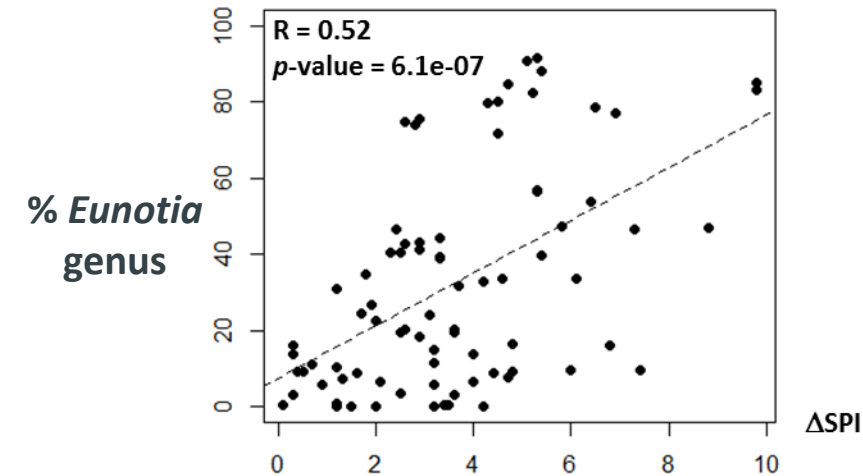
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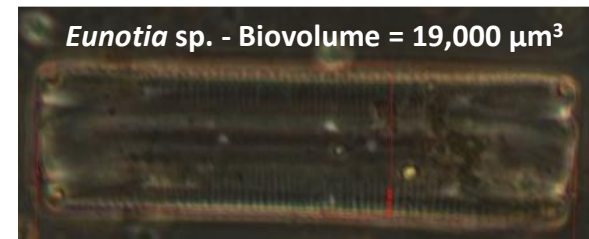
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But not the major bias in Mayotte's molecular data

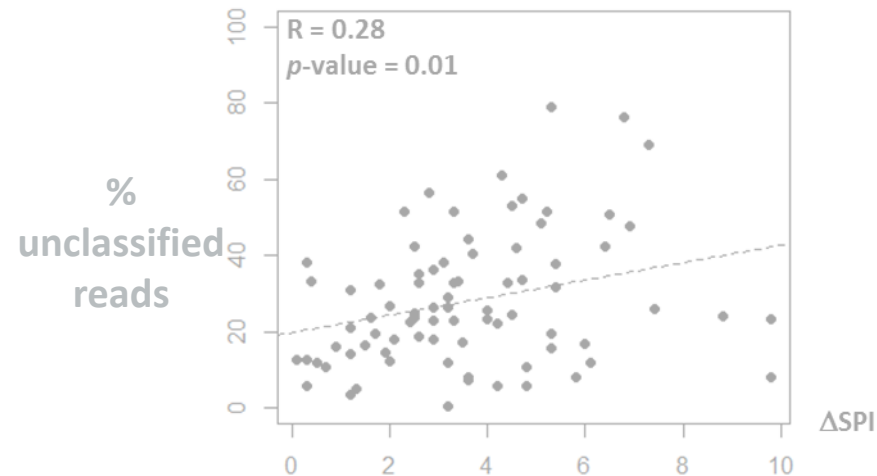


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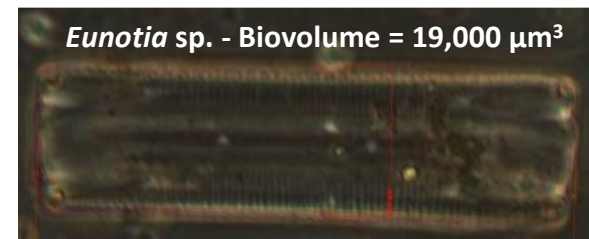
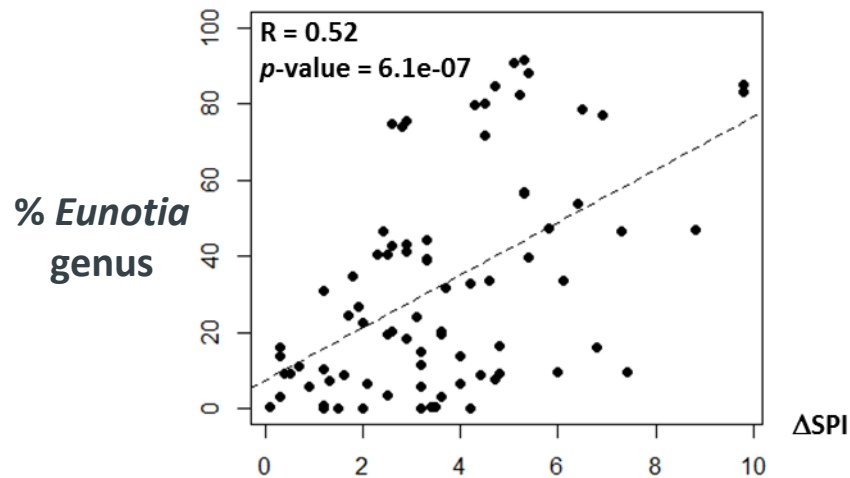


Microscopy -> low abundant
Molecular -> abundant

❖ Source of difference between molecular and morphological SPI



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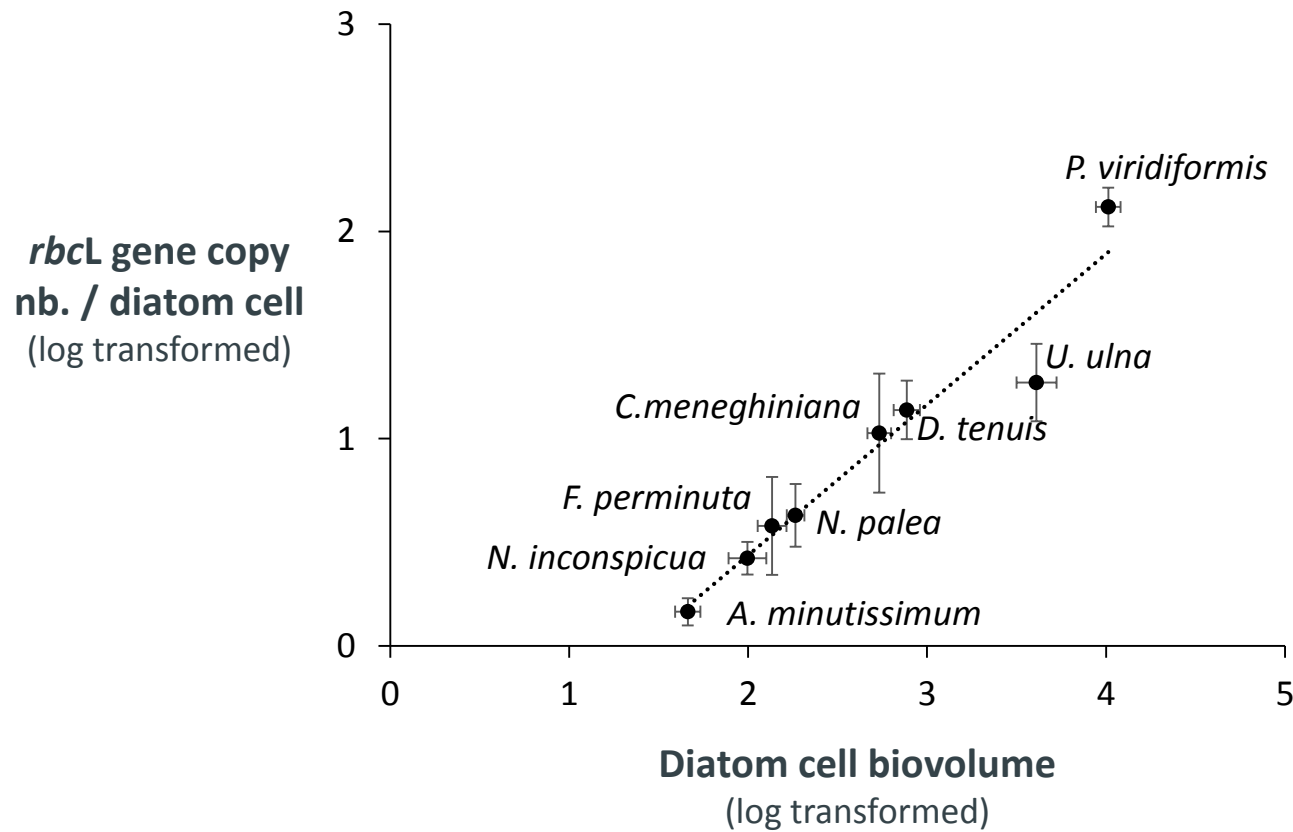
**Diatom with high biovolume are over-estimated
=> A need to develop a correction factor**

❖ Development of a Correction Factor (CF) based on diatom biovolume

- Link between cell biovolume and *rbcL* gene copy number ?
qPCR on 8 diatom species (pure culture) with various biovolume

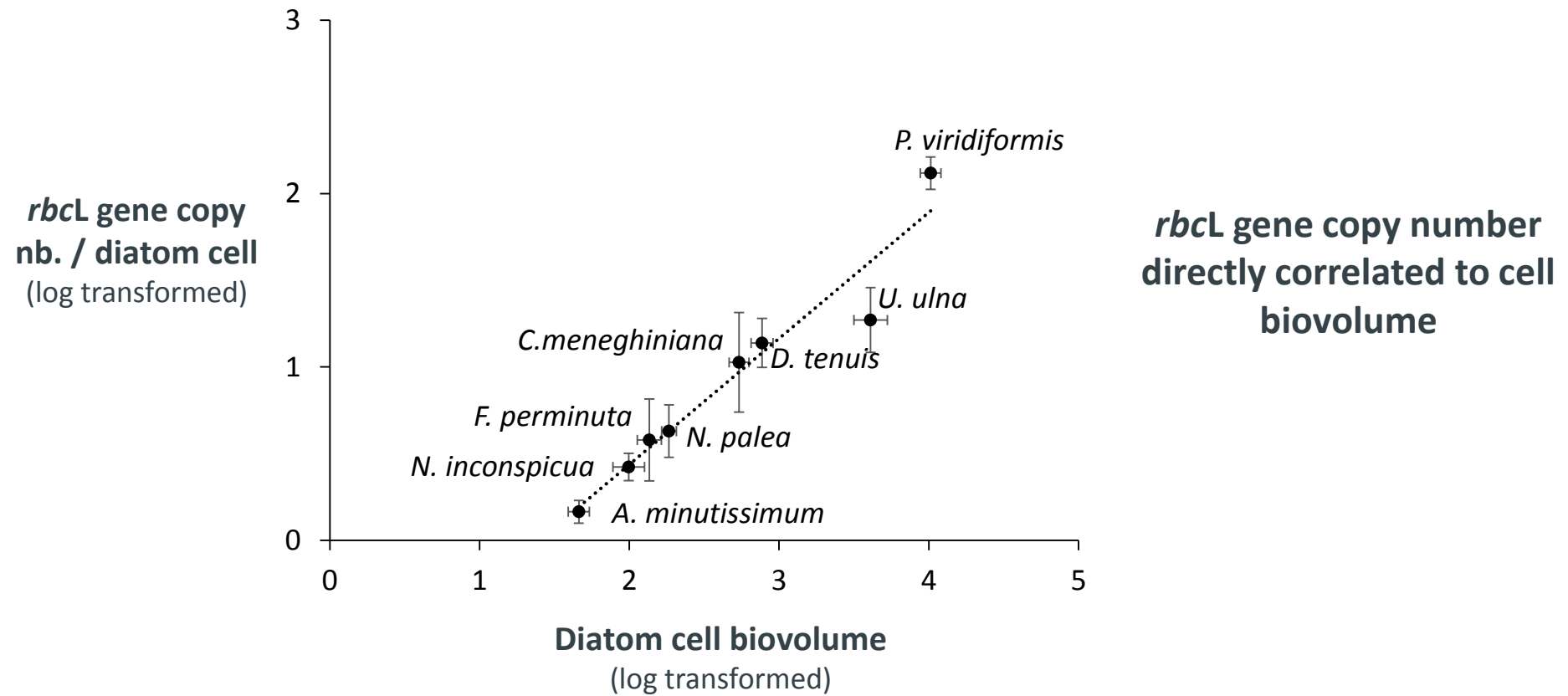
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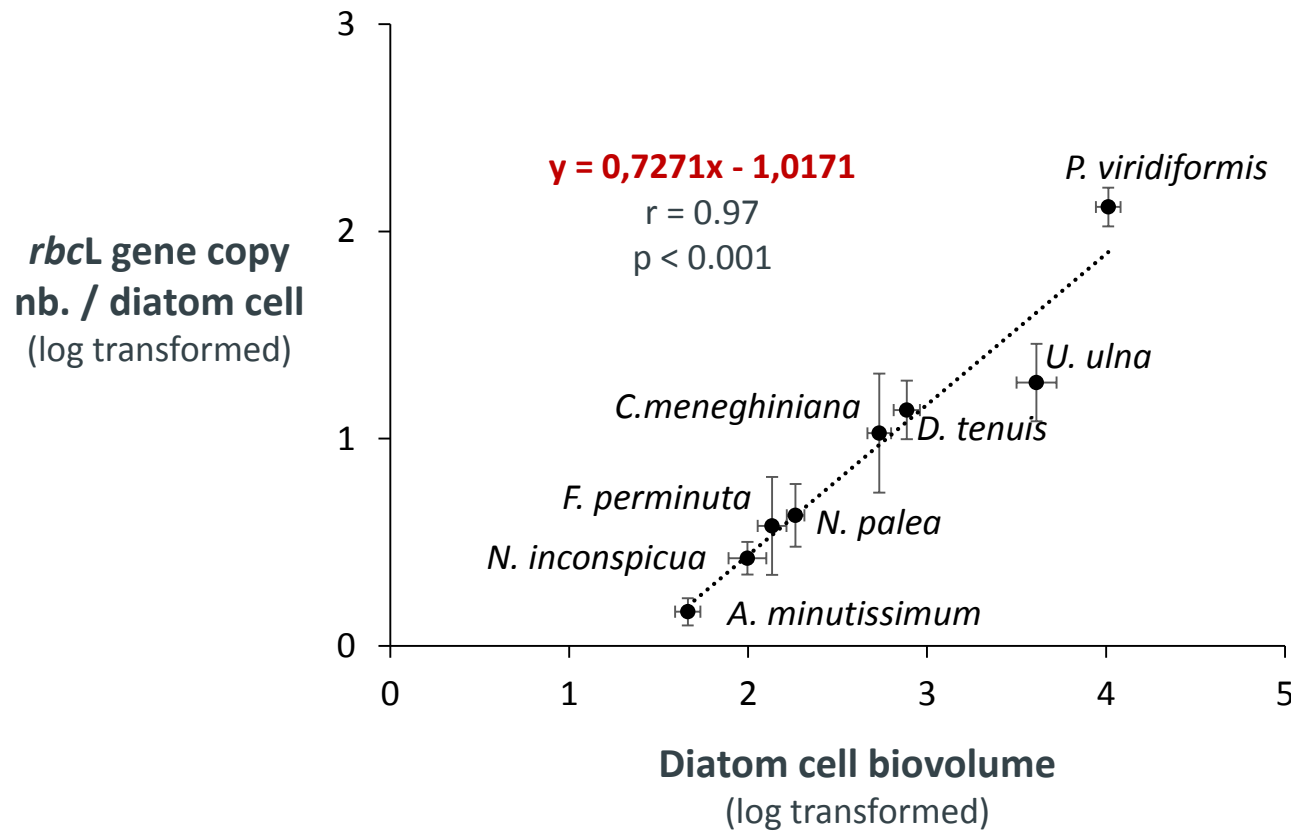
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rbcL gene copy number directly correlated to cell biovolume

Equation of the linear model used to create a CF for HTS molecular data

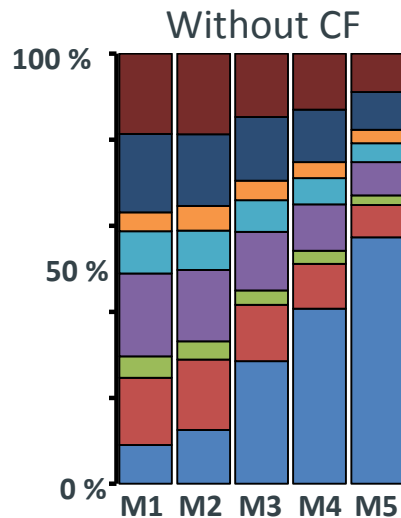
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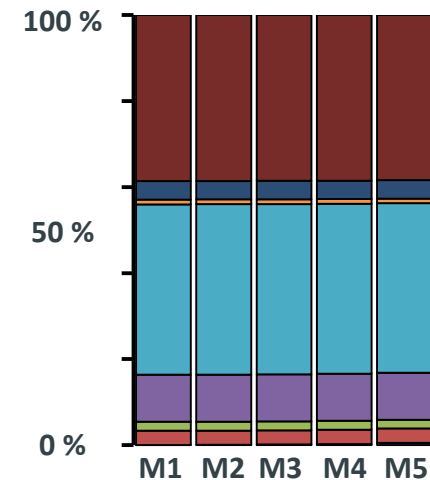
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Molecular inventories (HTS)

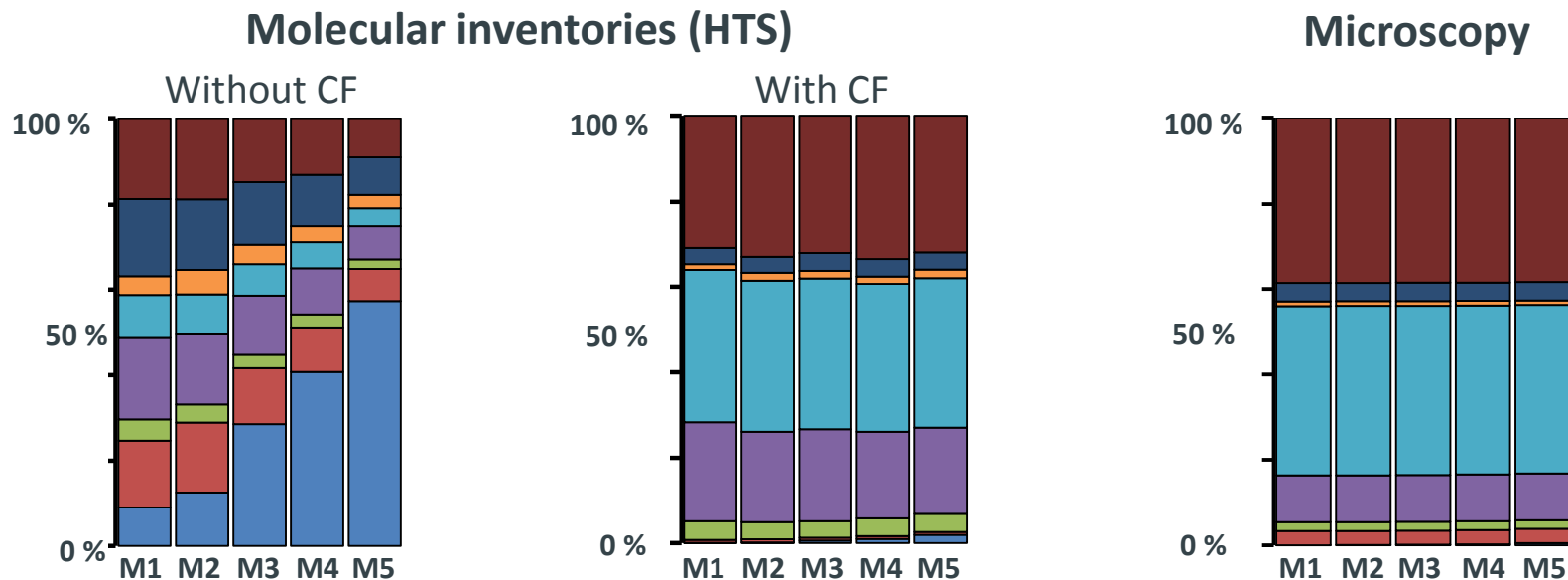


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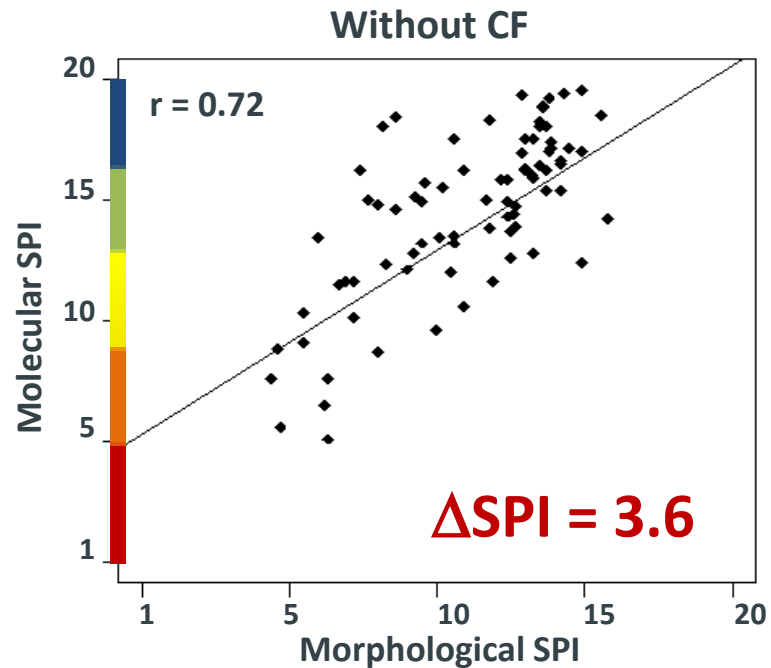
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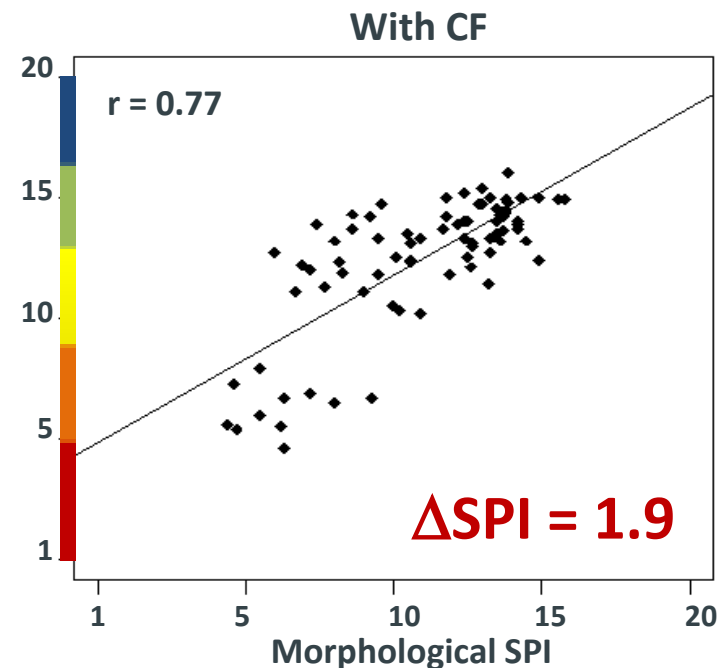
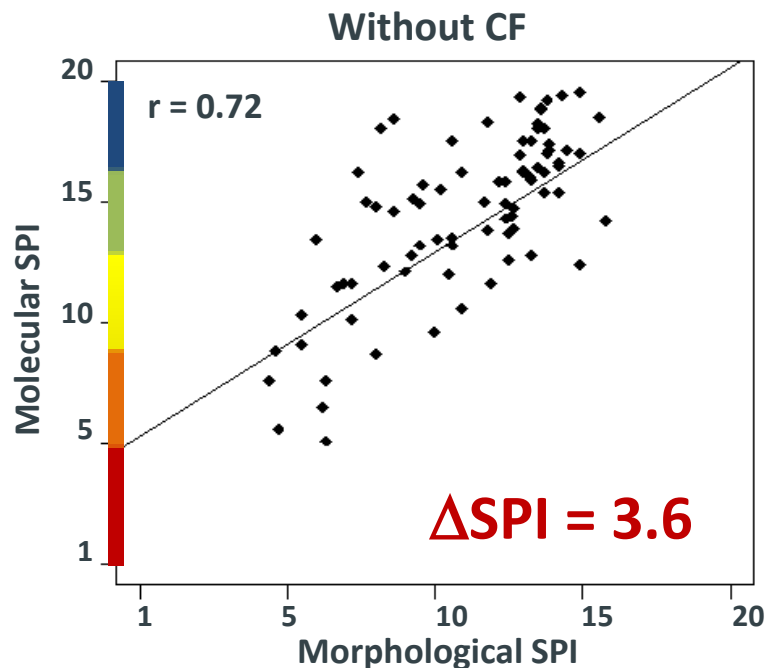


**CF increased congruence between molecular
and morphological diatom inventories**

❖ Application of the CF on Mayotte molecular inventories



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Good correction of *Eunotia* genus
CF reduce significantly the difference between the
two approaches (47%)

- ❖ **Incorporation of DNA metabarcoding in monitoring network helps improving the molecular approach by identifying the major biases**
 - Reference database incompleteness (endemic and tropical species)
 - Quantification bias linked to diatoms cell biovolume

- ❖ **The DNA metabarcoding can be used for water quality assessment (SPI)**
 - Good estimation of water quality status
 - Correction factors allow increasing the congruence with morphological SPI

- ❖ **What is next ?**
 - Continue the development : completion of reference database, validation of standards, explore new molecular quality index, ...
 - Move towards a progressive integration of DNA metabarcoding into monitoring network and the WFD

**Thank you for your
attention**

Some people think scientists exclaim

Eureka!



When doing experiments.

But they're way more likely to say...

Bollocks!



oh...Shit!



F*ck!



Arse!



Stupid piece-
of-crap machine!



I hate
Science!



twisteddoodles.com