

Workshop

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Technology and Metallurgy in
Water Biomonitoring

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Workshop, Belgrade, October 2023

BIOLAWEB presentation



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the European Union

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Diatom metabarcoding for biomonitoring and for basic ecology

F. Rimet

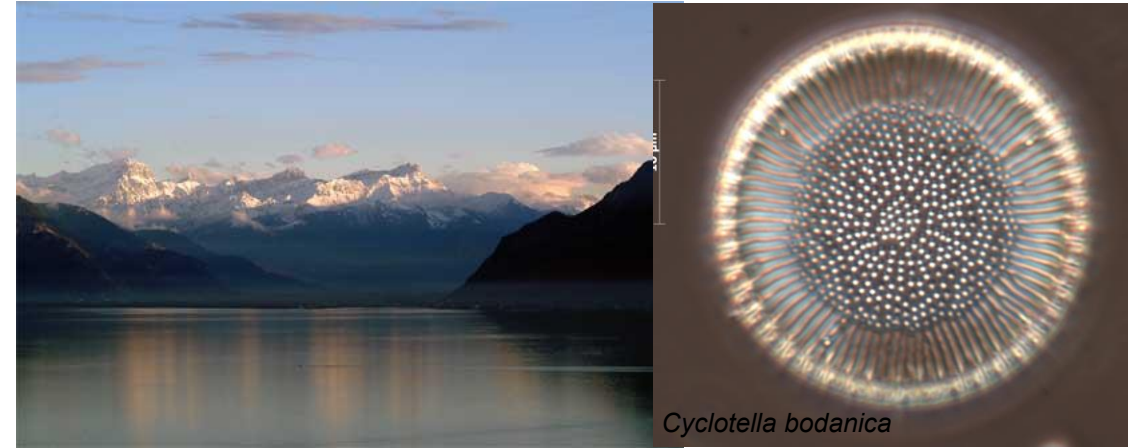
UMR Carrtel, INRAE, France

The INRAE logo is located in the bottom left corner. It consists of the letters "INRAE" in a bold, teal, sans-serif font, with a stylized leaf-like shape integrated into the letter "E".

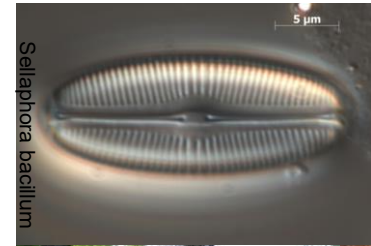
INRAE

Diatoms: diversity and importance in the biosphere

- Microalgae: $3 > 500 \mu\text{m}$, photosynthetic
- 100 000 species (Mann & Vanormelingen 2013)
- 10% of the total biomass in oceans (500 gt C / 6 000 gt C Leblanc et al. 2012, Bar-On et al. 2017)
- Colonize all habitats: lakes, rivers, oceans, soils, wet walls, caves ...



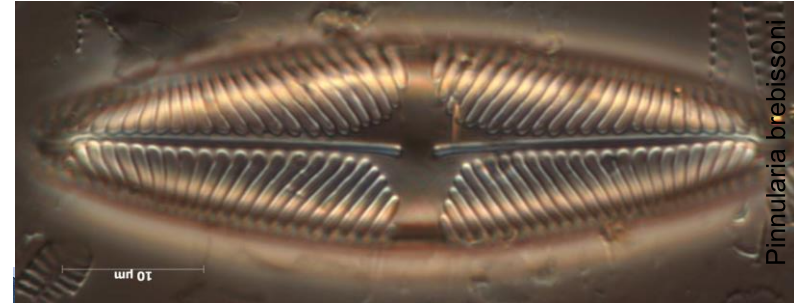
Cyclotella bodanica



Seliaphora bacillum



Luticola ventricosa



Pinnularia brebissoni



Licmophora flabellata
Dr. Arlene Wechezak

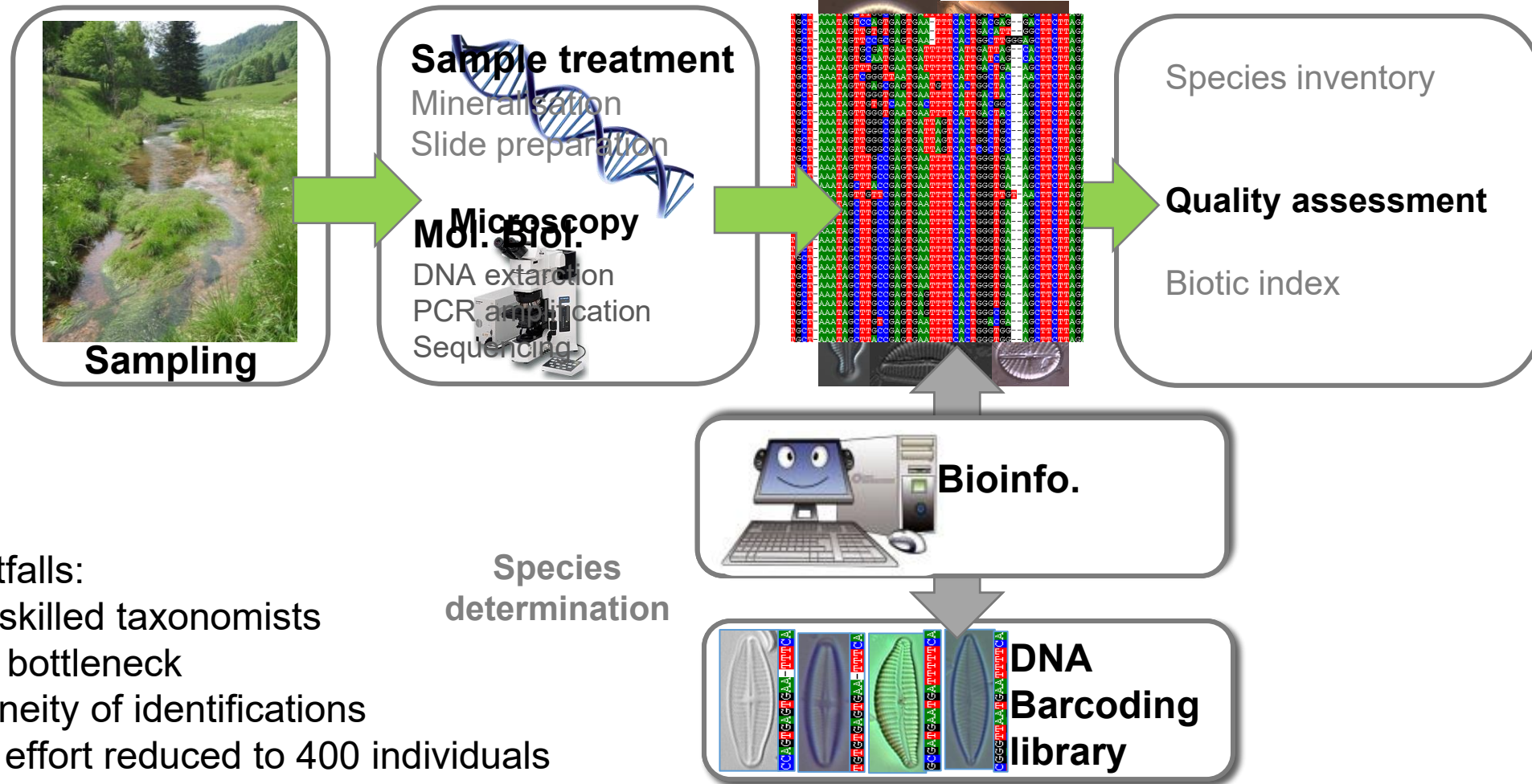


Cymbella erhenbergii



Diatom are used as regulatory biomonitoring tool

(European Water Framework directive)



But some pitfalls:

- Need for skilled taxonomists
- Analyses bottleneck
- Heterogeneity of identifications
- Counting effort reduced to 400 individuals

What is DNA metabarcoding?

2003: DNA barcoding (Hebert et al. 2003)

Standardized approach to identify organisms

Barcode: Short DNA fragment

Characteristic of a taxon

Easy to sequence (universal primers)

Barcoding gap

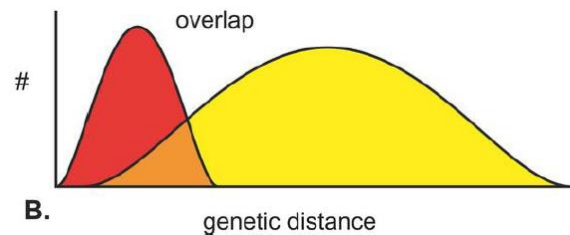
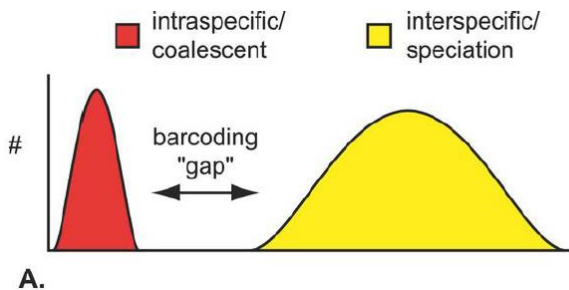
Diatom barcoding pioneers:

Evans et al. 2007 (cox, rbcl, 18s, ITS)

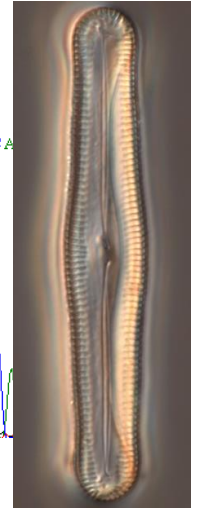
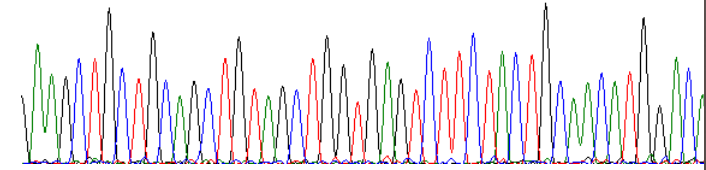
Hamsher et al. 2011 (cox, rbcl)

>Sellaphora pupula complex (D. Mann)

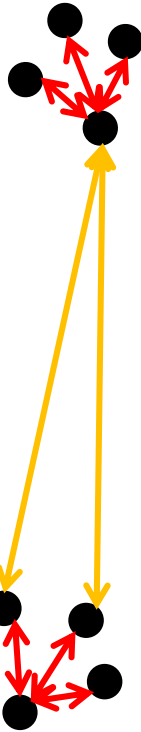
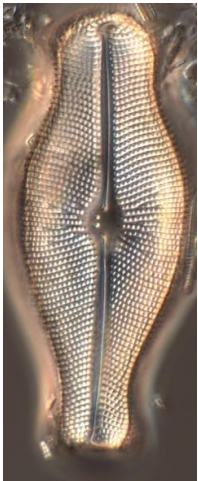
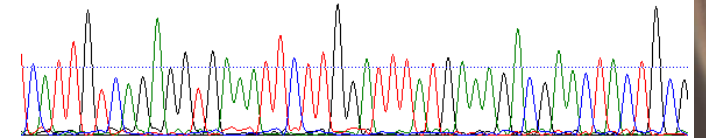
Moniz & Kaczmarska 2009 (5.8S, ITS)



160 170 180 190 200
} AAGC TGCTGCAGC TGTAGCTGGTGGAGTCTTTCTACTGCAACA TGGACA



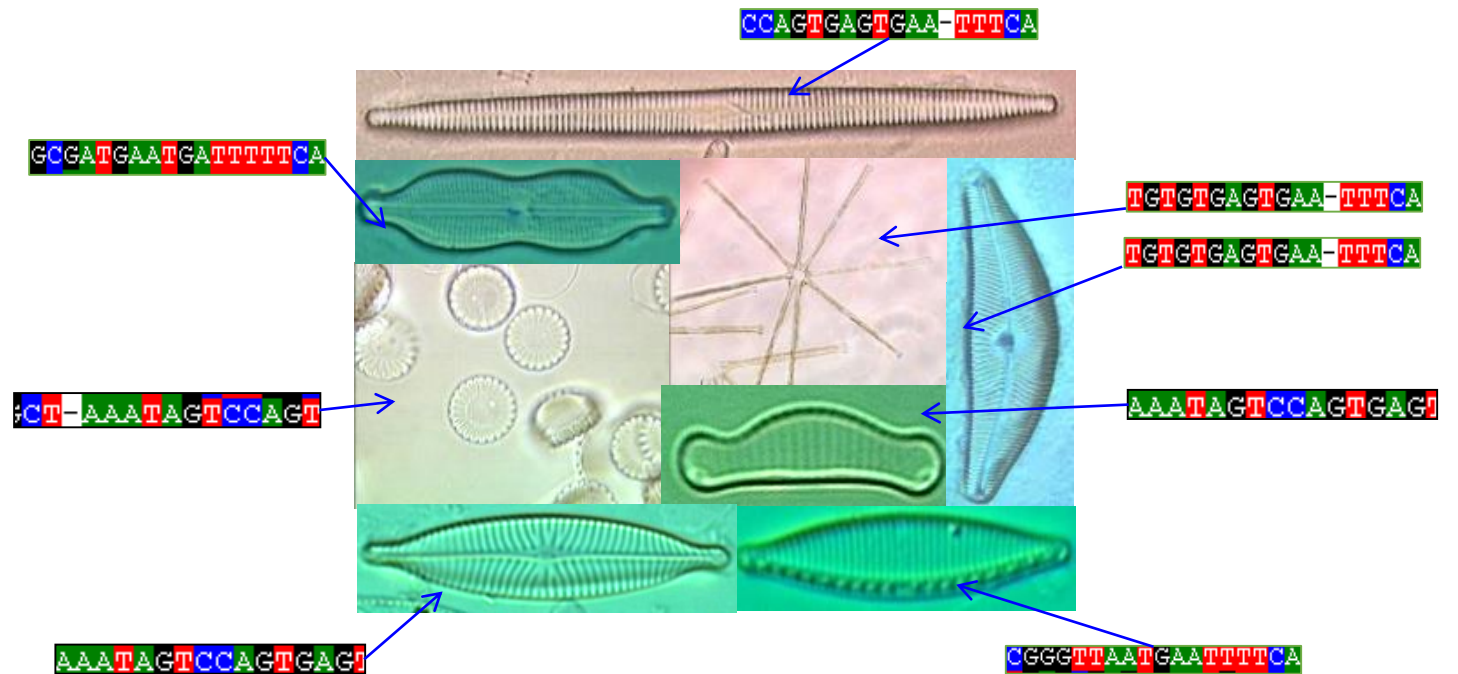
0 300 310 320 330
} CATTGTCAGAGGTGAAATTC TTGGATTTATGAAAGACGAACTACTGCG



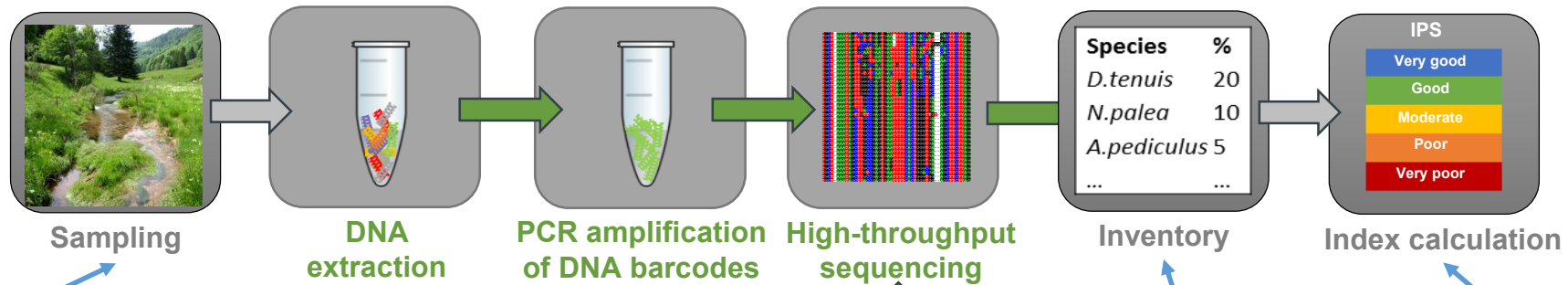
What is DNA metabarcoding?

2012: metabarcoding (Taberlet et al. 2012)

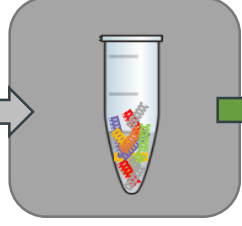
Concept expanded to study natural samples
Identify organisms in communities
Use of High Throughput Sequencing
Sequencing of environmental DNA



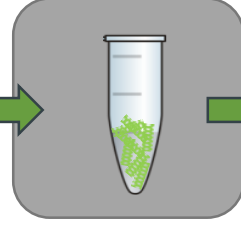
Methodological developments



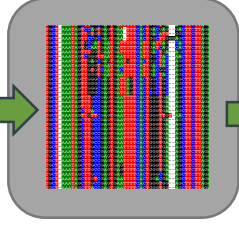
Sampling



DNA extraction



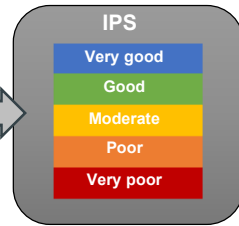
PCR amplification of DNA barcodes



High-throughput sequencing

Species	%
<i>D.tenuis</i>	20
<i>N.palea</i>	10
<i>A.pediculus</i>	5
...	...

Inventory



Index calculation



Sampling (*CEN TR 17245 2018*)
Preservation (*Baricevic et al. 2022*)

Choice of extraction kit (*Vasselon et al. 2017a*)

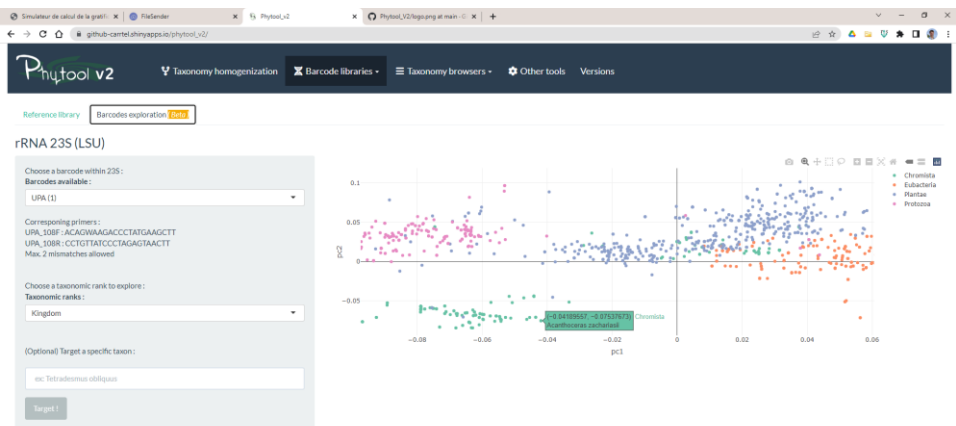
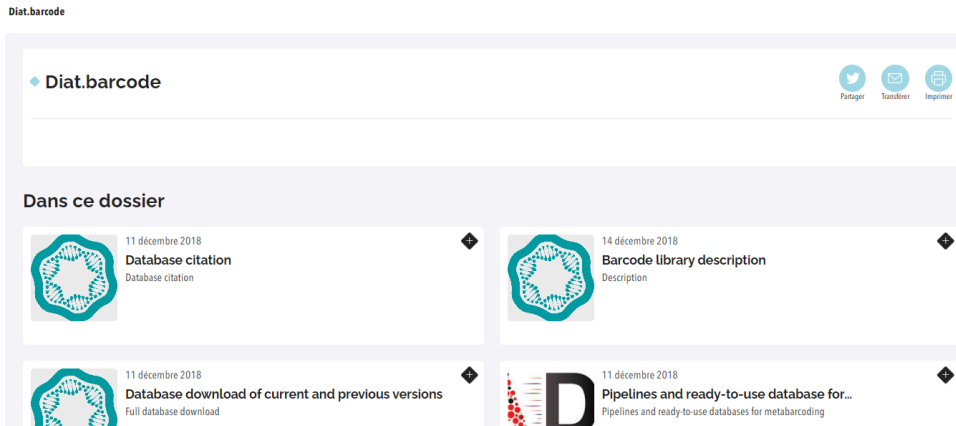
Barcode choice (*Kermarrec et al. 2014*)
rbcL primers (*Vasselon et al. 2017b*)

Reference Barcode library (*Zimmermann et al. 2014, Rimet et al. 2016, CEN TR 17244 2018*)

Pipeline comparisons (*Bailet et al. 2020, Rivera et al. 2020*)

Correction factor (*Vasselon et al. 2018*)
Taxonomie-Free index (*Tapolczai et al. 2020*)

Protocols and tools are open access



River biofilms sampling for both downstream DNA analysis and microscopic counts V.1

DOI
dx.doi.org/10.17504/protocols.io.ben6jdhe

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⁴University of Applied Sciences Southern Switzerland, SUPSI, Cannobio, Switzerland;
⁵Austrian Agency for Health and Food Safety Ltd.(AGES), Austria

VERSION 1
JUN 11, 2020

SHARE

WORKS FOR ME 1

EcoALpsWater

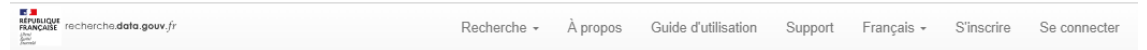
COMMENTS 0

Create an editable copy for use in your research

GOT IT

COPY / FORK

MORE ↓



Data INRAE

(Institut national de recherche pour l'agriculture, l'alimentation et l'environnement.)

Recherche Data Gov > Data INRAE >

Diat.barcode, an open-access barcode library for diatoms

Version 15.1



Rimet, Frederic; Chonova, Teofana; Gassiole, Gilles; Gusev, Evgeny; Kahlert, Maria; Keck, François; Kelly, Martyn; Kochoska, Hristina; Kulikovskiy, Maxim; Levkov, Zlatko; Maltsev, Yevhen; Mann, David; Pfannkuchen, Martin; Trobajo, Rosa; Vasselon, Valentin; Vidakovic, Danijela; Wetzel, Carlos; Zimmermann, Jonas; Bouchez, Agnès. 2018, "Diat.barcode, an open-access barcode library for diatoms", <https://doi.org/10.15454/TOMBYZ>, Recherche Data Gov, V15, UNF:6:82rxzGfFpV91mu78sPuXlg== [fileUNF]

Citer le jeu de données -

Pour en apprendre davantage sur le sujet, consulter le document Data Citation Standards [en].

Modalités d'accès au jeu de données

Contact Partager

Statistiques d'utilisation sur les jeux de données

6 644 consultations

2 980 téléchargements

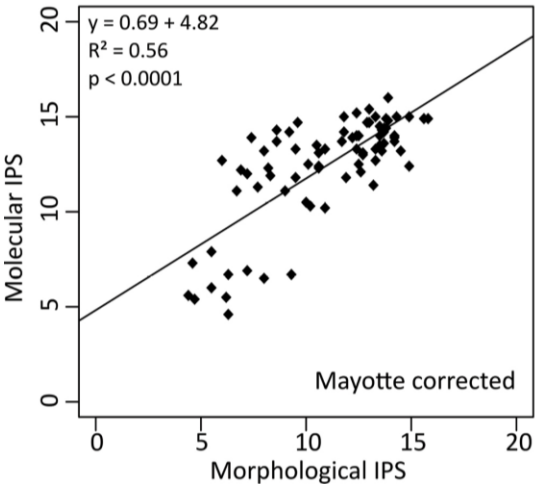
0 citation

Description

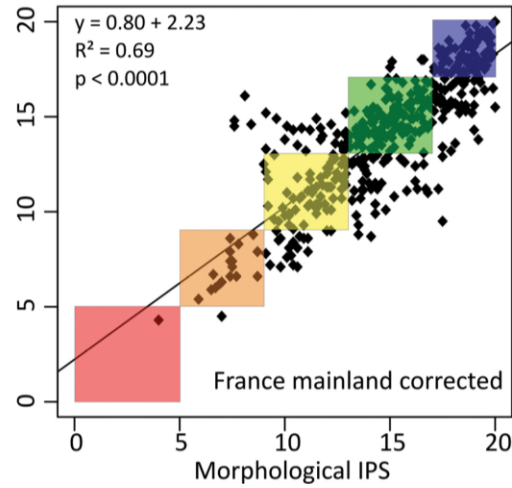
Diatoms (Bacillariophyta) are ubiquitous microalgae which produce a siliceous exoskeleton and which make a major contribution to the productivity of oceans and freshwaters. They display a huge diversity, which makes them excellent ecological indicators of aquatic ecosystems, and can also be used to reconstruct paleoenvironments. Usually, diatoms are identified using characteristics of their

Application to monitoring networks

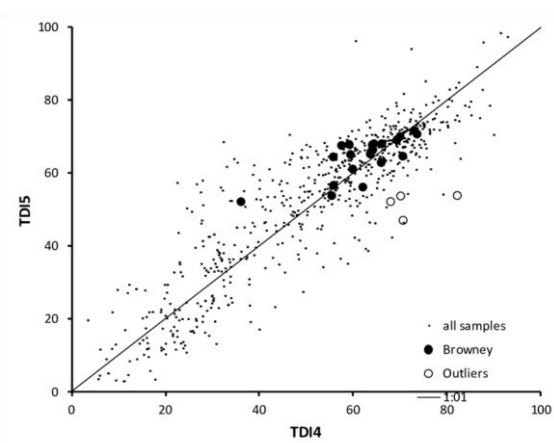
Mayotte



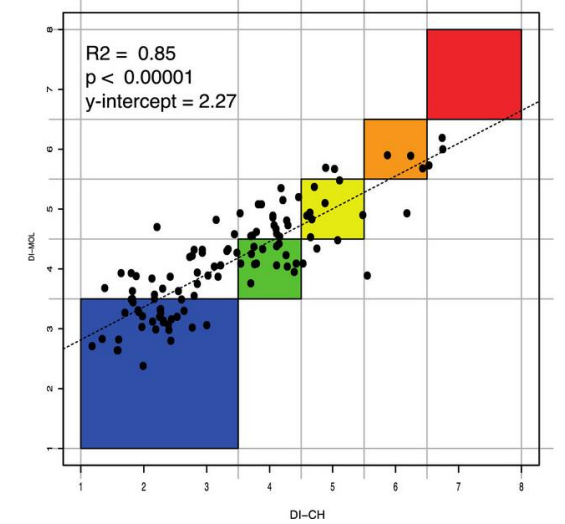
France



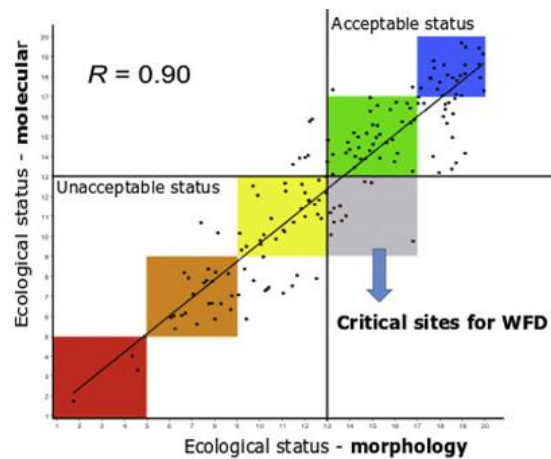
UK



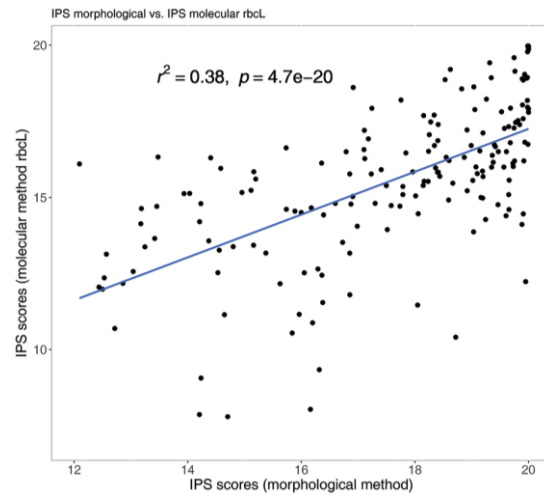
Switzerland



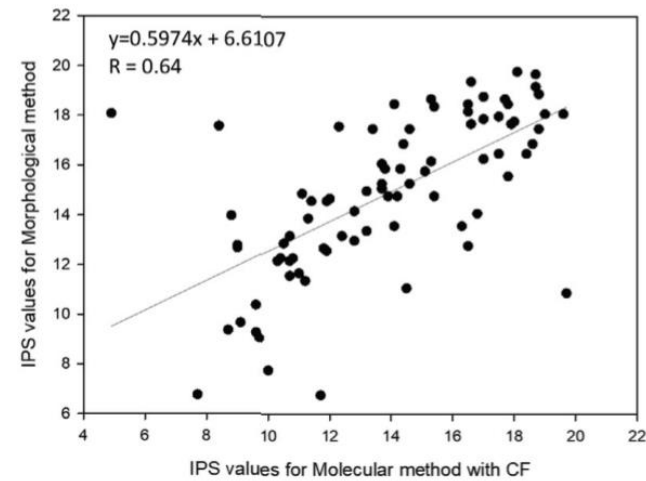
Spain



Sweeden



Portugal



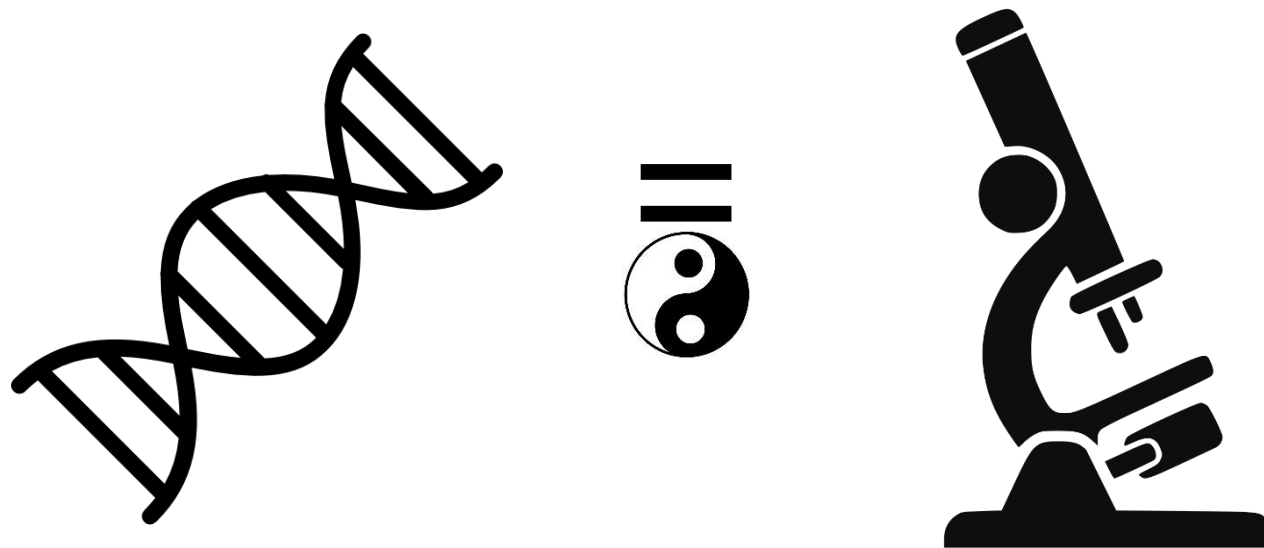
Not used as regulatory tool, except in UK

Application to monitoring networks

Basic ecology

In this framework (biomonitoring)
DNA metabarcoding is used to replace (to mimic) microscope identifications

Can diatom metabarcoding bring additional and complementary information to morphological data?





Schedule

4 topics

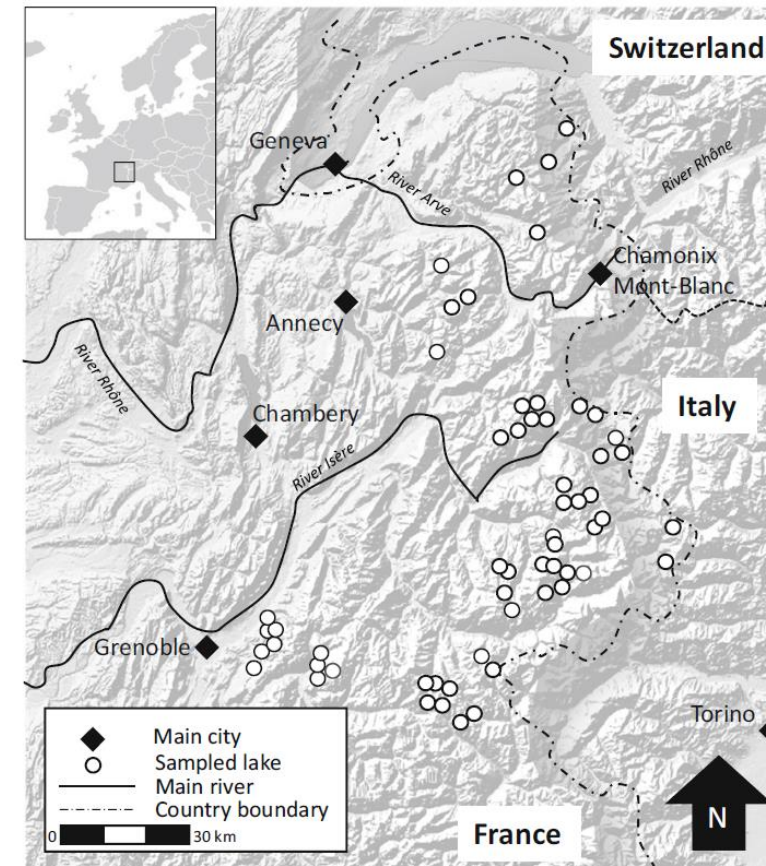
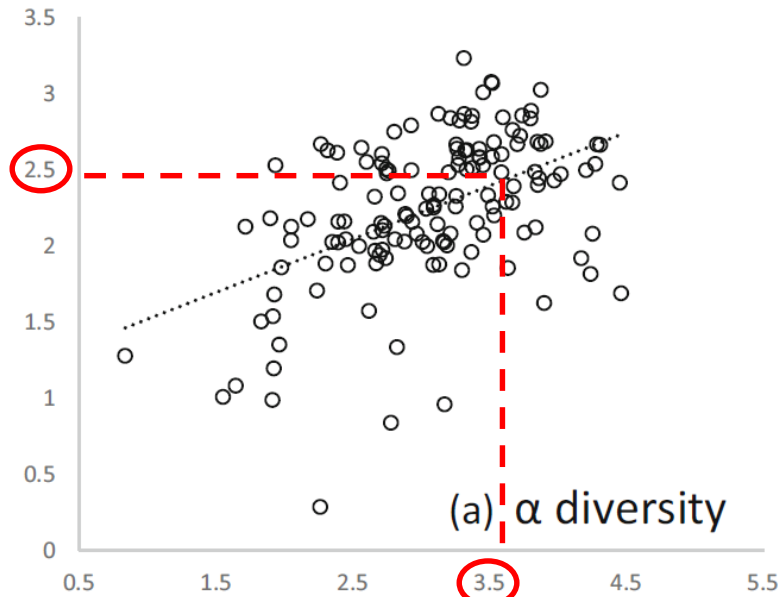
- Biodiversity
- Autecology
- Biogeography
- Community assembly



Biodiversity assessment

When compared to microscopy, DNA metabarcoding measure a higher diversity

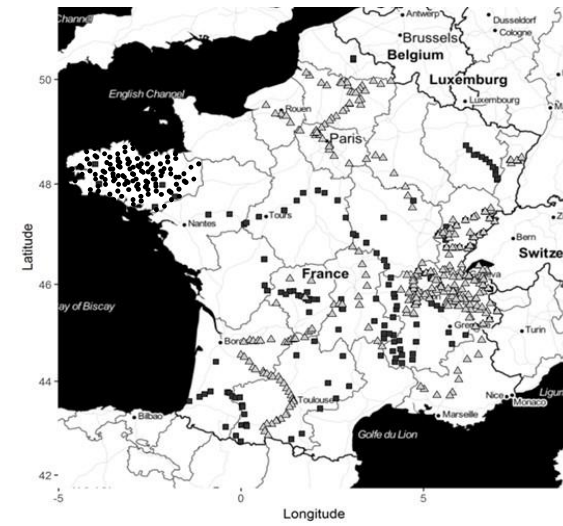
- Why?
- deeper sampling effort (400 individuals vs several 10 000 of reads)
 - > rare species
 - finer taxonomical resolution with rbcl
 - > sub-species level



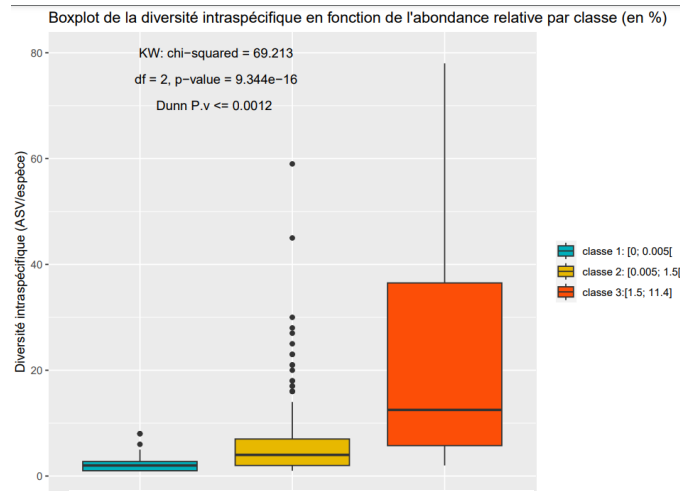
Biodiversity assesement

Can we characterise this intraspecific diversity?

- > 658 sampling sites in rivers of France
- > campains 2016, 2017, 2019

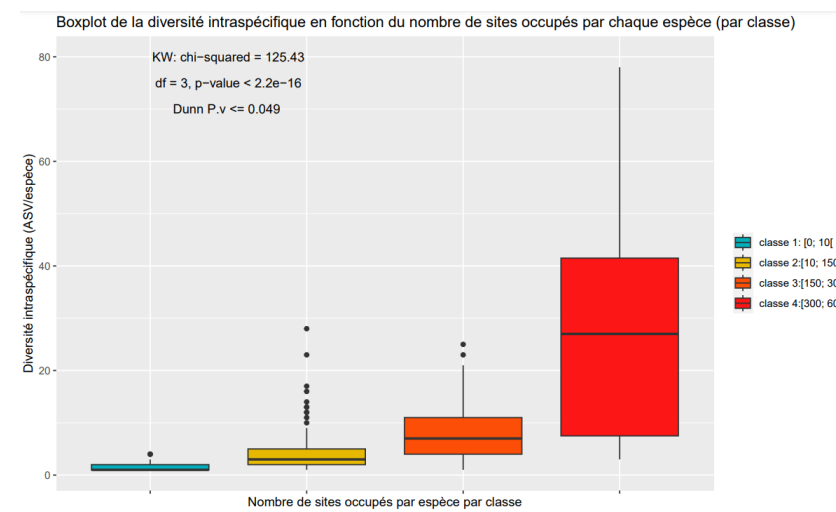


Intrasp.
genotypic
diversity



Species abundance

Intrasp.
genotypic
diversity



Number of sites occupied

Species with the highest genotypic diversity:

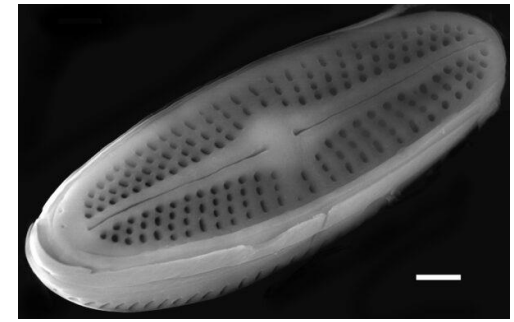
- big population size
- wide niche breadth

Autecology

Being able to measure cryptic diversity -> New insights for diatom species ecology

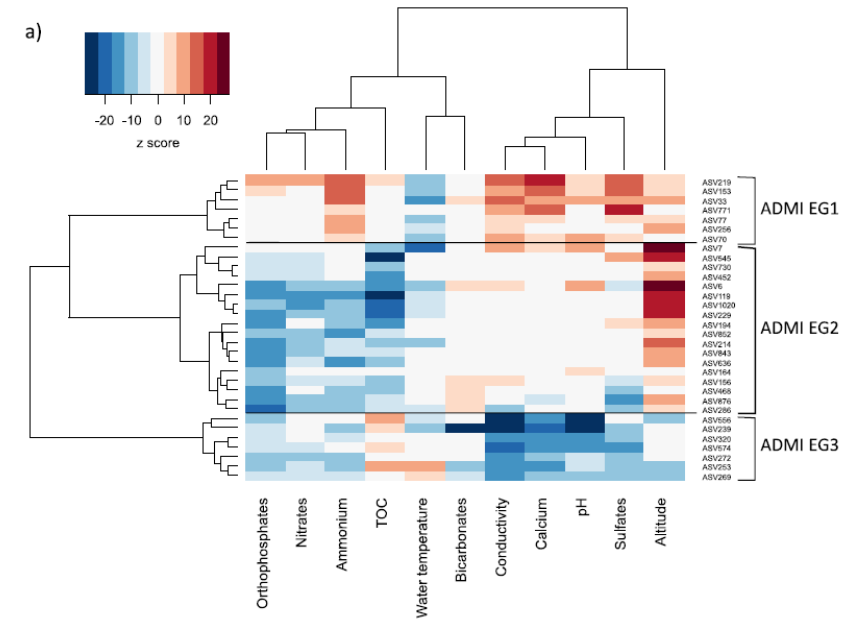
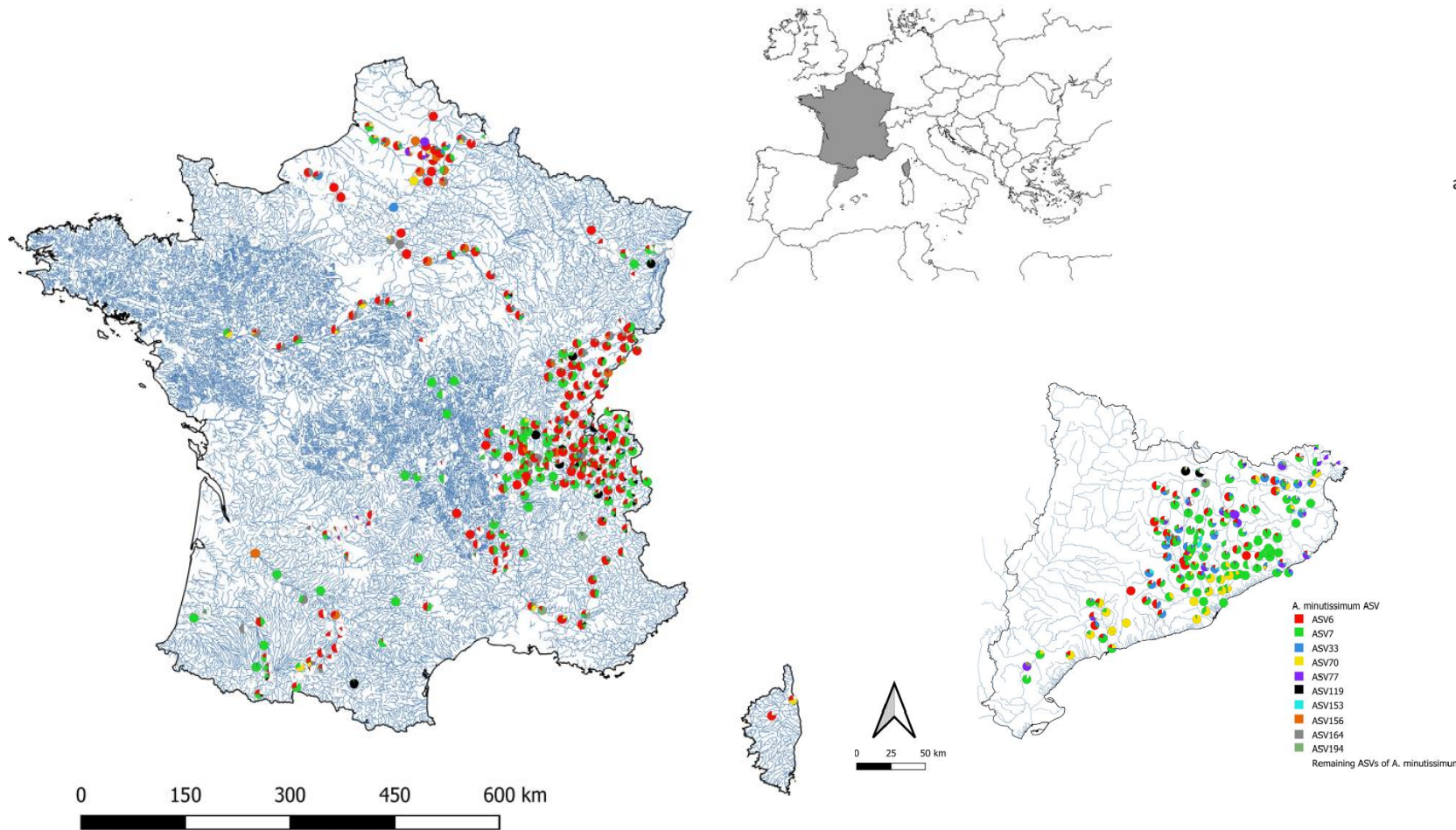
Study on rivers in France and Catalonia

- Genetic variants within species show different patterns of distribution.
- Some genetic variants within species differ in their ecological preferences



Achnantheidium minutissimum

https://diatoms.org/species/achnantheidium_minutissimum/images#image-93141

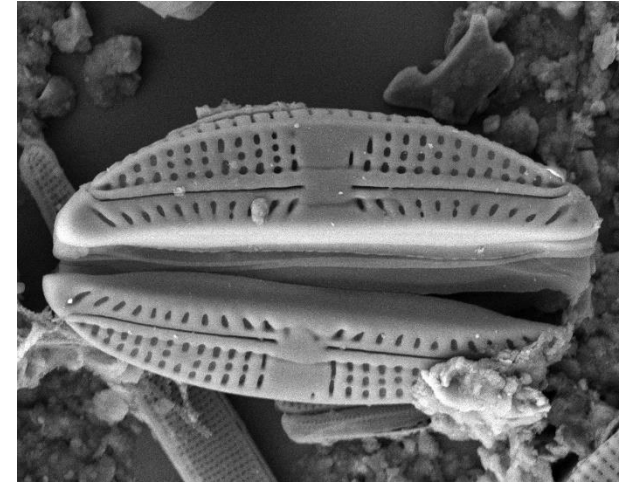


Subspecies/genetic variants have different requirement for conductivity, calcium

Autecology

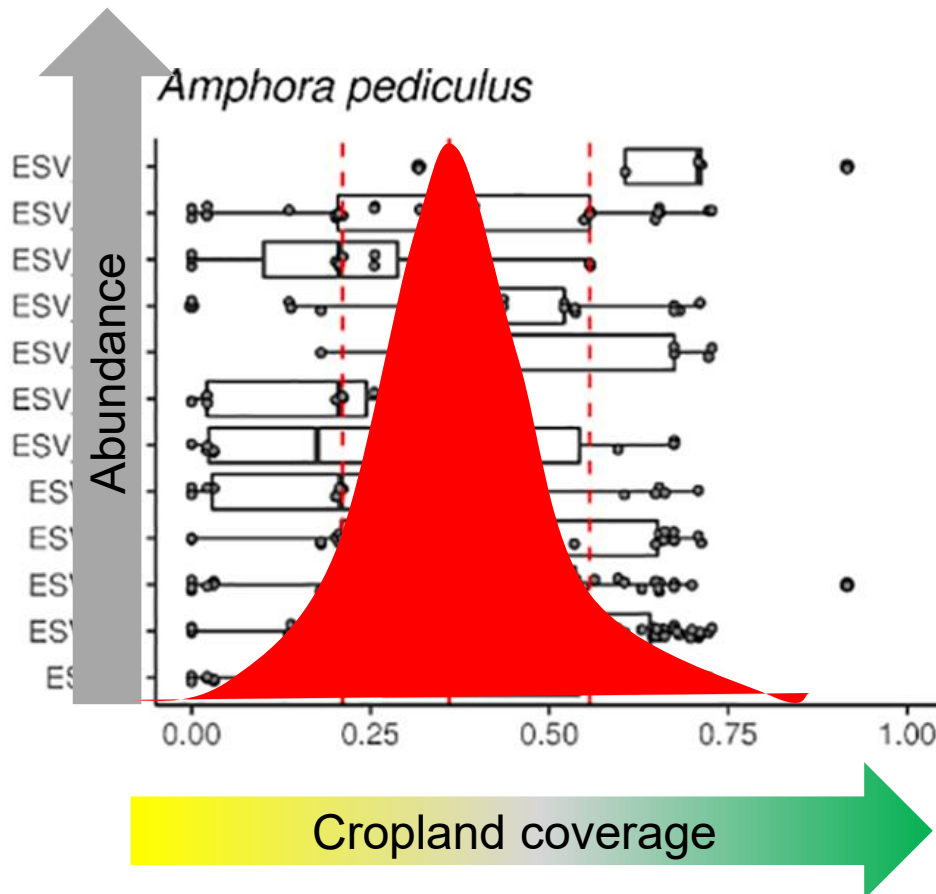
Development of an index to assess agriculture land-use (Hungary)

- Development of a genotype index enable to better assess land-use
- But species assignation is necessary to have an ecological interpretation



Amphora pediculus

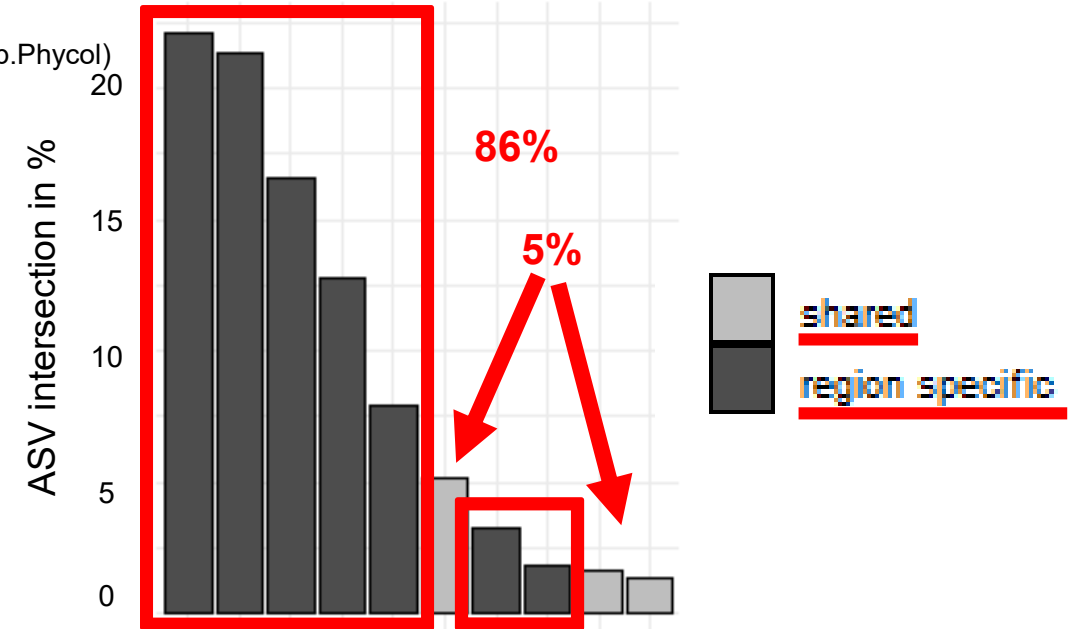
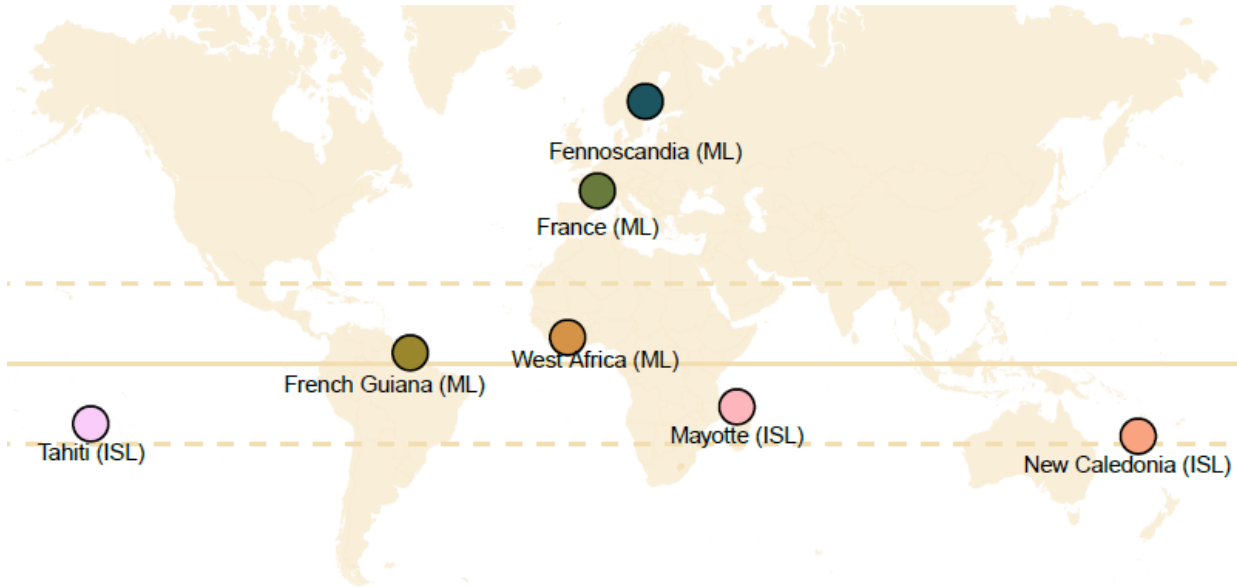
<https://diatoms.org/images/18280>



Biogeography

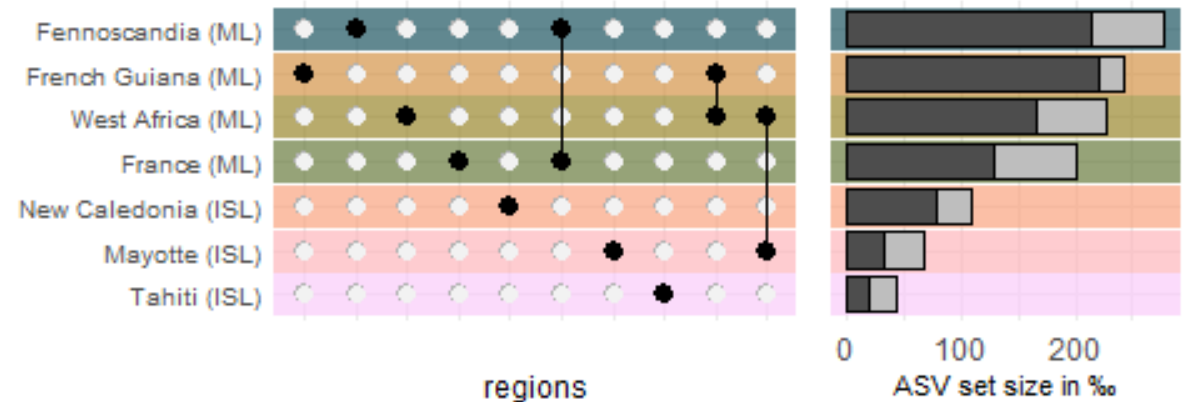
Interest of DNA metabarcoding for large biogeographical studies:

- Taxonomic precision
- Robustness of sequencing vs microscope identification (Kahlert 2009 J.App.Phycol)



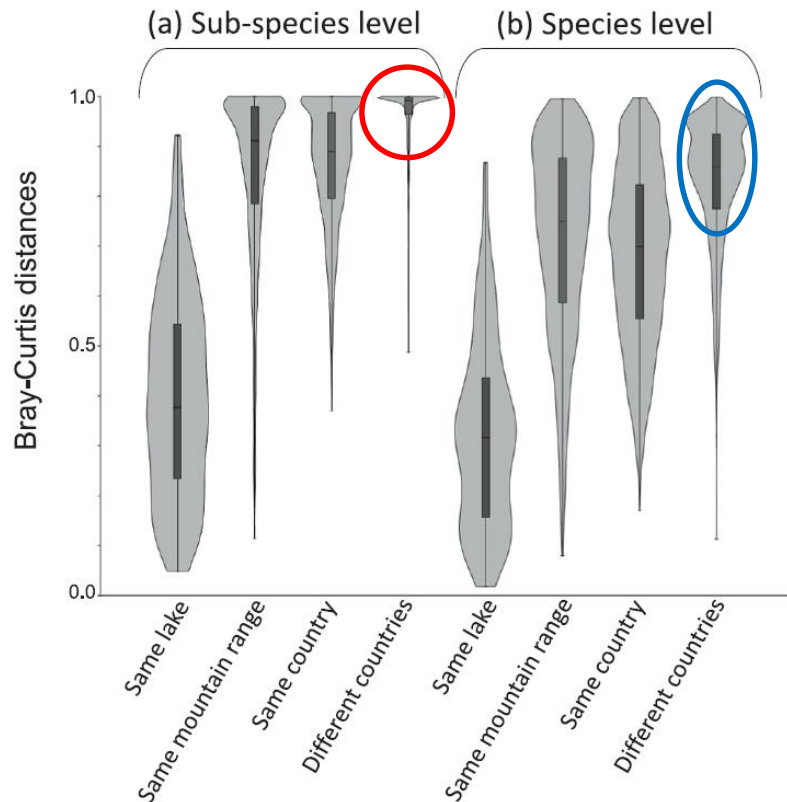
Very strong geographical structure, strong limitations to dispersal, high endemism (≠Finlay et al. 2002)

But what happen if we compare similar ecosystems? Do we still have endemism?



Biogeography

Comparison of similar ecosystems: high altitude lakes in alpine climate

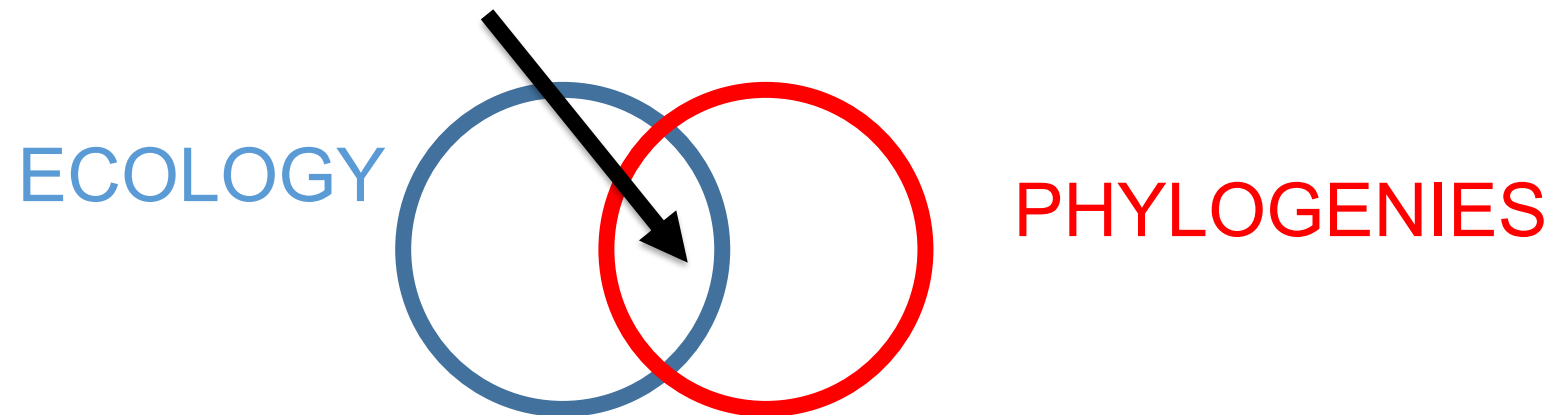


- Only 1,5% of genotype shared
- 15% of species shared
- At least 1 species is shared between samples
- Where in 40% of the case 0 genotypes are shared

There is still a very high genotypic endemism. For species level, “endemic” species of France or Georgia are in fact not characteristic of lakes but of neighbor habitats (soil, sediments, peat-bogs...)

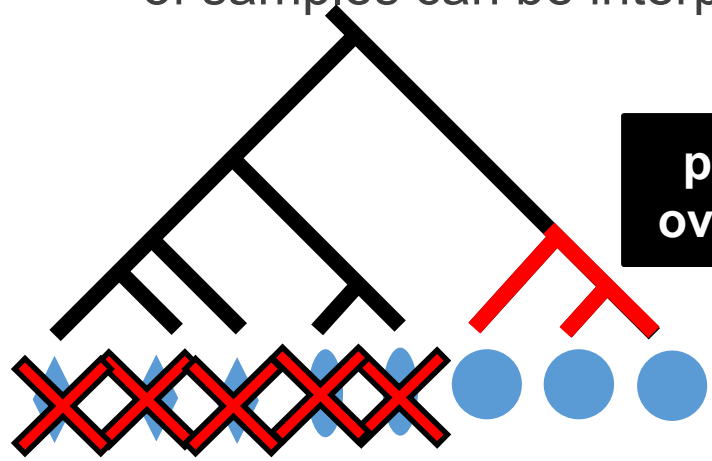
Community assembly

- There are 2 main predictors in community assembly:
 - **Environmental filtering**: Ecologically similar species should coexist in ecologically similar environments.
 - **Limiting similarity**: Ecologically dissimilar species should coexist because too similar species competing for the same resources cannot stably coexist.
- **Including phylogeny into ecological** thinking is an opportunity for biologists because:
 - Species distributions are shaped by evolutionary and ecological processes
 - These 2 processes are intimately related
 - So, it is important to study them together
- “Ecophylogenetic” Mouquet et al. 2012 (Biological Reviews)



Community assembly

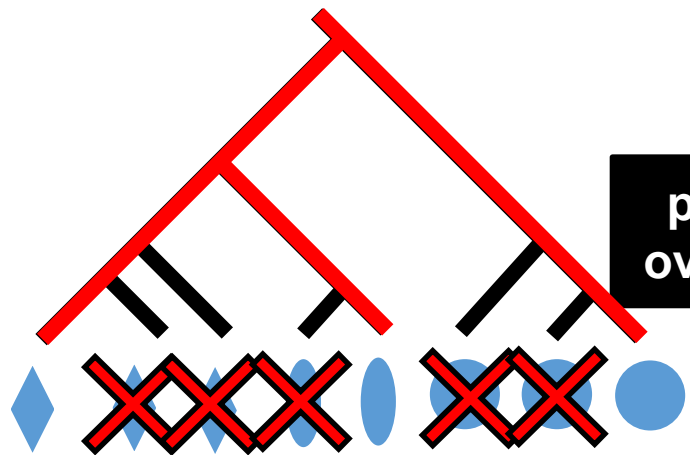
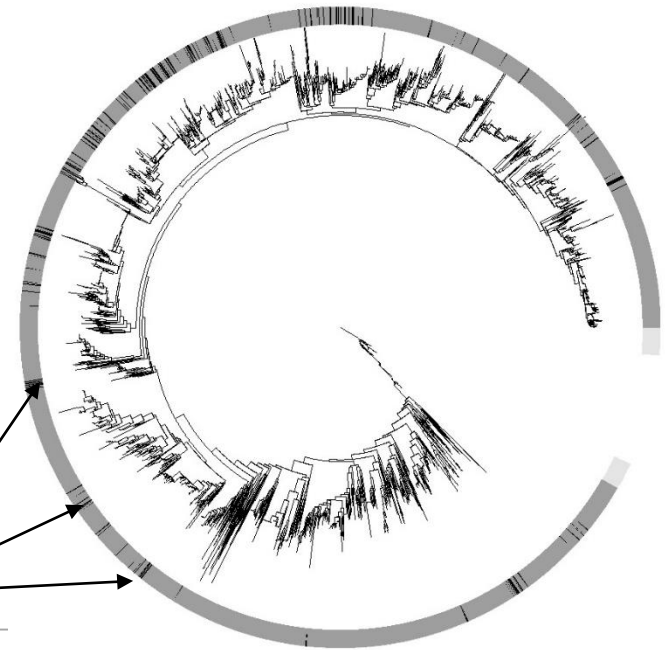
- If niches are conserved in the evolution, then phylogenetic structure of samples can be interpreted in terms of ecological processes



phylogenetic
over-clustering

Environmental
filtering
dominates

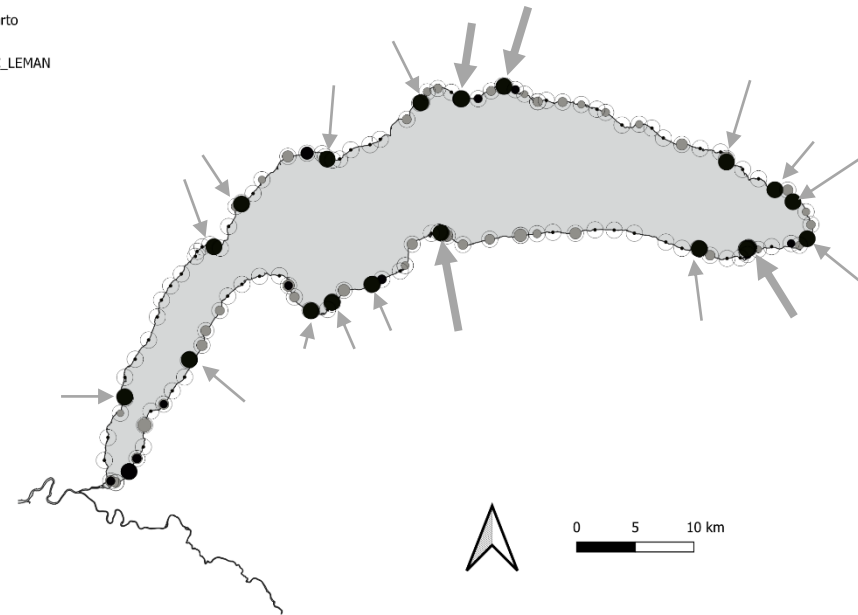
Environmental
sequences



phylogenetic
overdispersion

Competition
dominates

NRI all carto
■ NRI.All
NTI all carto
■ NTI.All
GEO_LAC_LEMAN

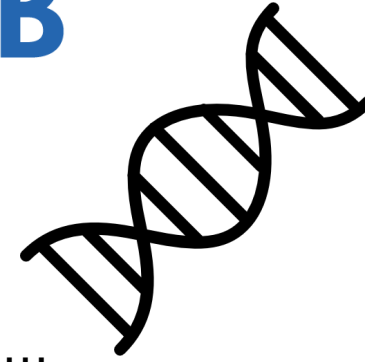


Conclusions

Complementarity

Microscope:

- possibility to see species size, how species move, make colonies...
- long tradition -> good knowledge of species ecology

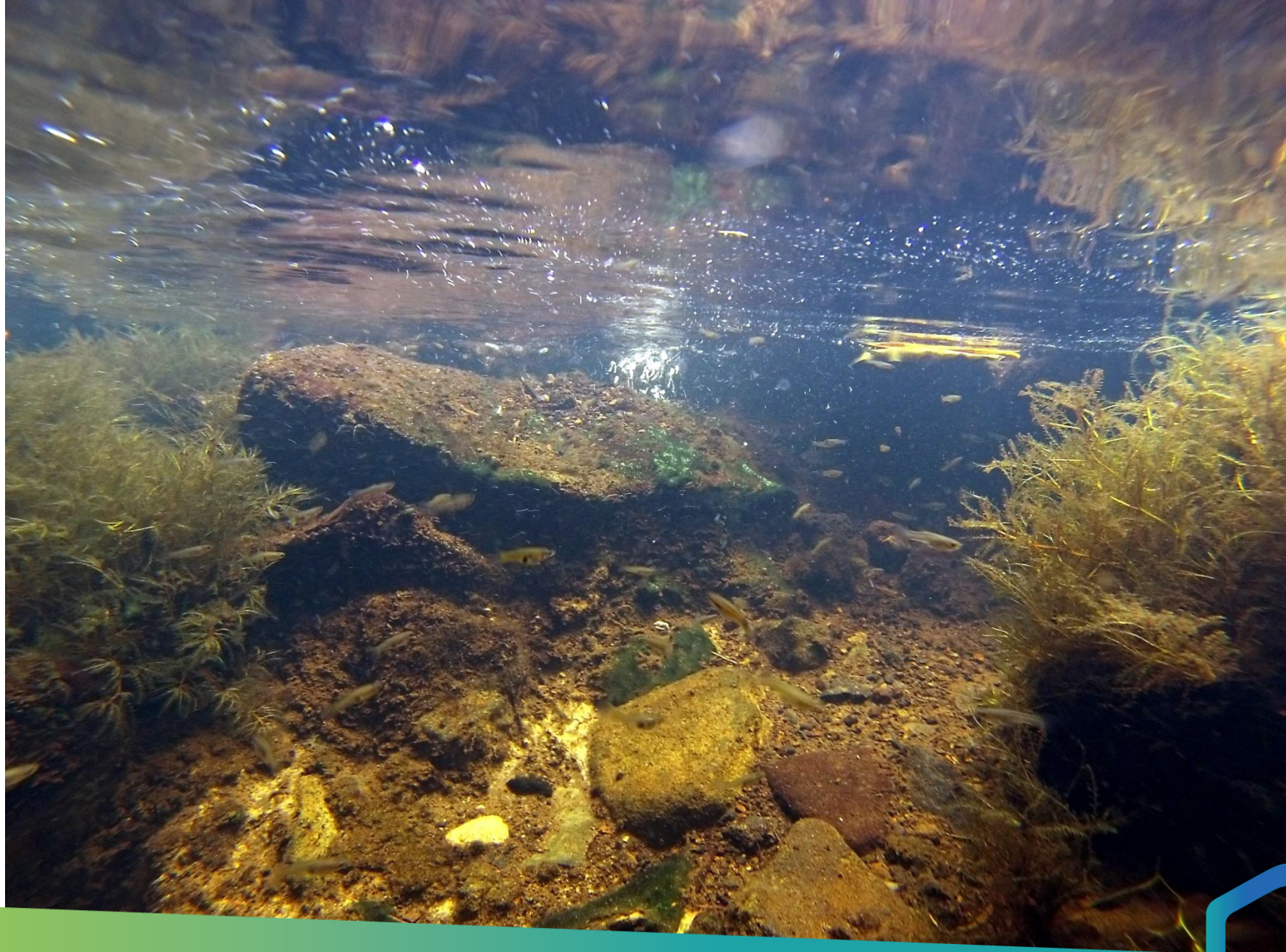


« DNA barcodes lie within the gray area between phylogenetics and population genetics » (Hajibabaei 2007 Trends in Genetics)

DNA metabarcoding :

- easily handle cryptic diversity
 - reveal a high degree of endemism
 - enable to have new insights of diatom species ecology
 - should help in diatom population genetic
- phylogenetic dimension:
 - address community assembly questions (phylogenetic overclustering / overdispersion)
 - evolutionary processes (phylogenetic signal)

Thank you for
your attention



Acknowledgement



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Thank you for your attention!

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