

Exploring cryptic Labyrinthulomycetes (Stramenopiles) diversity in *P. olsenii*-infected clam populations along the French Atlantic Coast

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Introduction

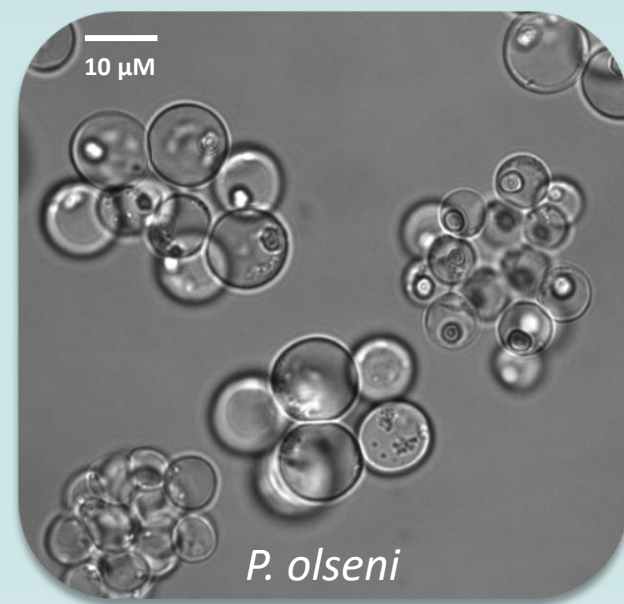


Manila clam (*Ruditapes philippinarum*)

- Imported to Europe in 1972 for aquaculture purposes
- Found along the French Atlantic coast

Host to several phyla of micro-eukaryotic organisms, including *Perkinsus olsenii* (Alveolata):

- Exotic unicellular parasite introduced in Europe in 1972
- OIE-listed, causing clam mortalities in Portugal and Spain



What is the diversity of micro-eukaryotic organisms associated with *P. olsenii* infected manila clams?

We will use a culture-dependant approach to explore this diversity

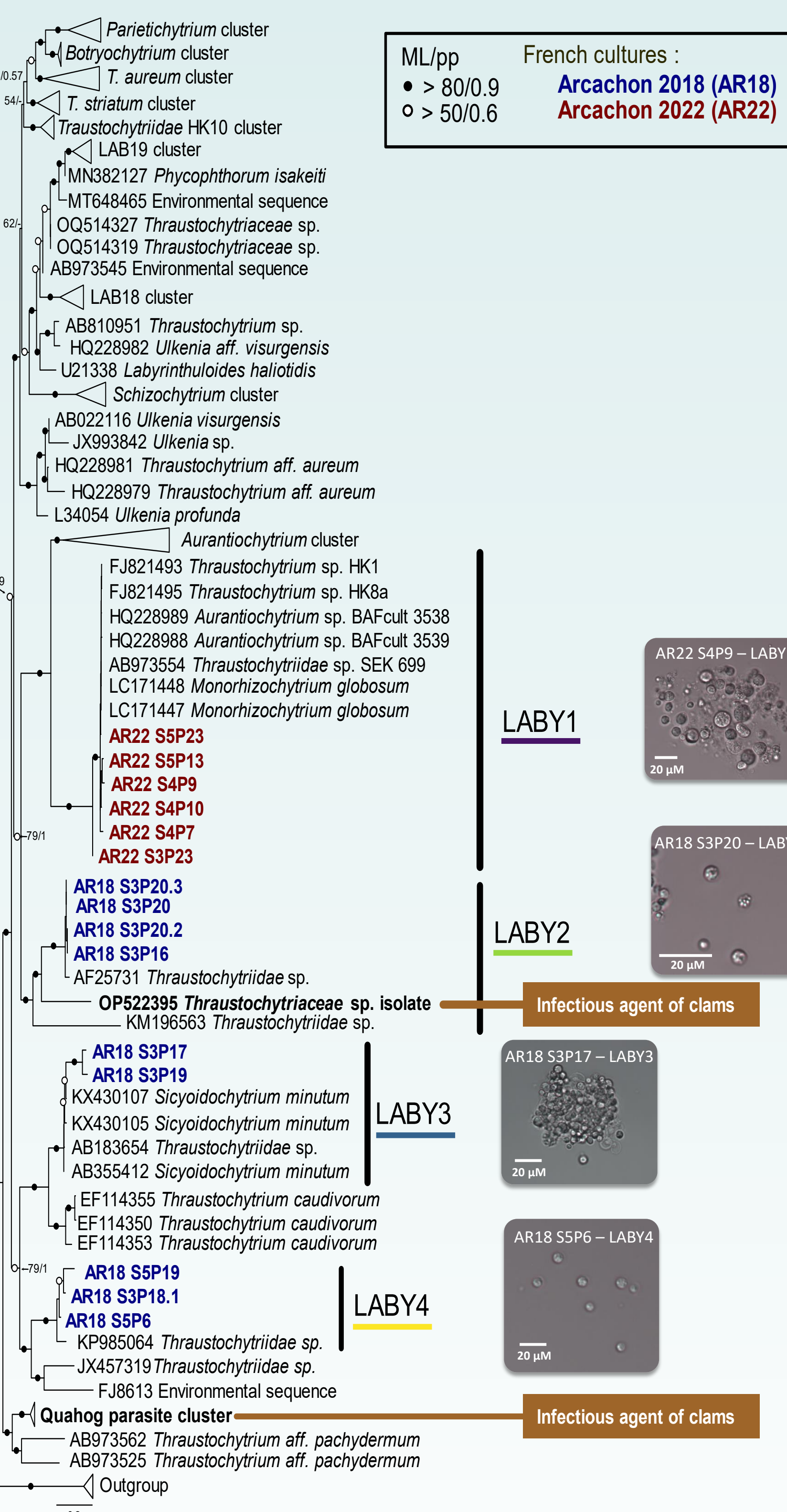
Results

1. Culture and DNA extraction using *Perkinsus* broth media

7 and 6 cultured strains belonging to the Labyrinthulomycetes were obtained following a sampling campaign in Arcachon Bay respectively in 2018 and 2022.

Labyrinthulomycetes (Stramenopile) are protists, mostly saprophytes but some are parasitic (QPX, MCPX). Their diversity is greater than currently described.

2. Genetic characterization of associated eukaryotic culture



3. Exploring the presence of underdescribed clusters of Labyrinthulomycetes along latitudinal French coastline

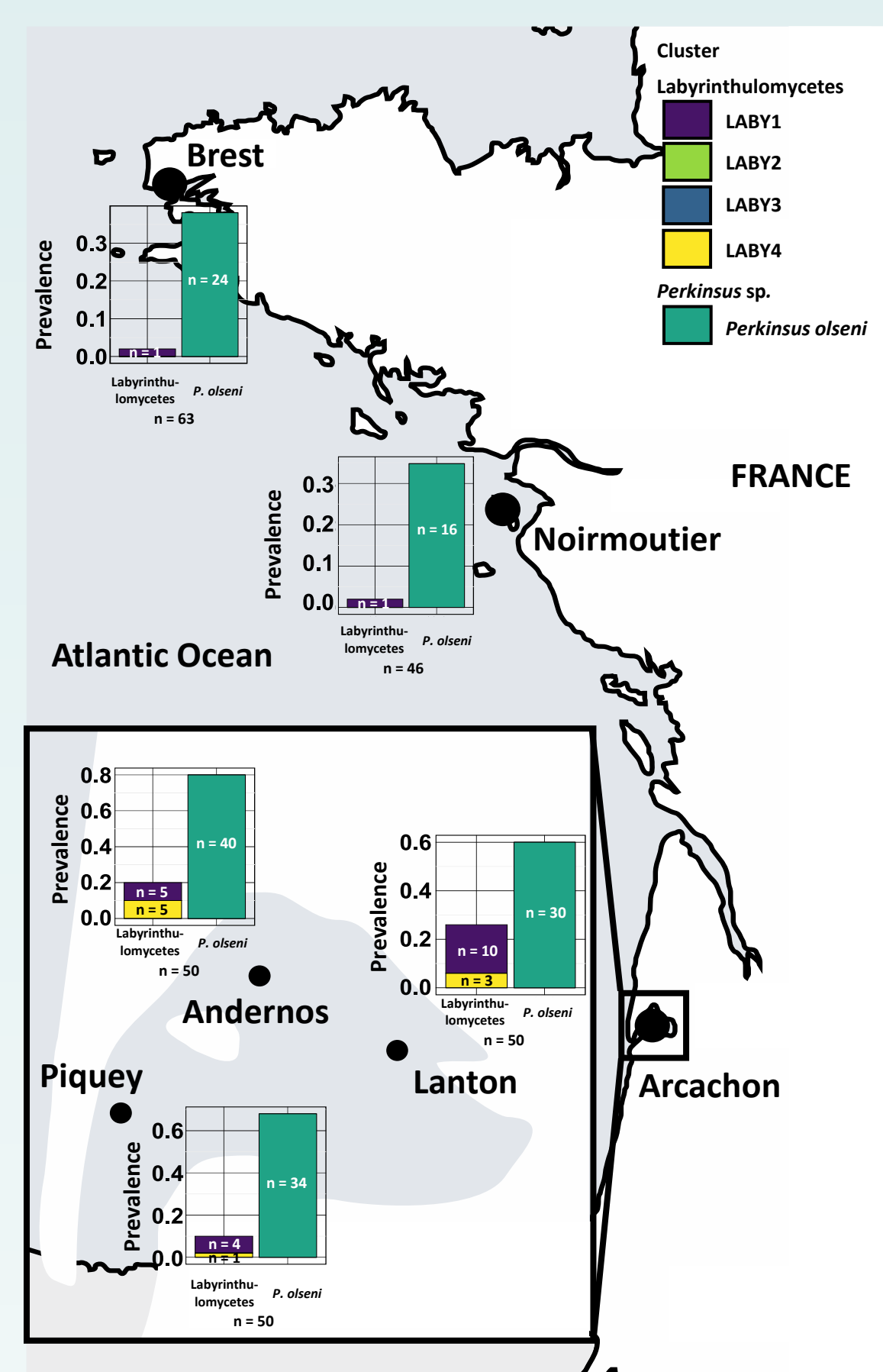


Fig 2: Map of the French Atlantic coast with the prevalence of Labyrinthulomycetes and *P. olsenii* in 3 sites: Brest, Noirmoutier and Arcachon

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Methodology

1. Sampling where *P. olsenii* is recurrent

50 clams harvested per site in the Arcachon Bay, 63 in Brest and 46 in Noirmoutier (France)

| Site | <i>P. olsenii</i> prevalence |
|---------------------|------------------------------|
| Brest | 38% |
| Noirmoutier | 35% |
| Lanton (Arcachon) | 60% |
| Piquey (Arcachon) | 68% |
| Andernos (Arcachon) | 80% |

Table 1: Prevalence of *P. olsenii* in 3 sampling sites in Arcachon Bay in 2022, in Brest and Noirmoutier in 2023

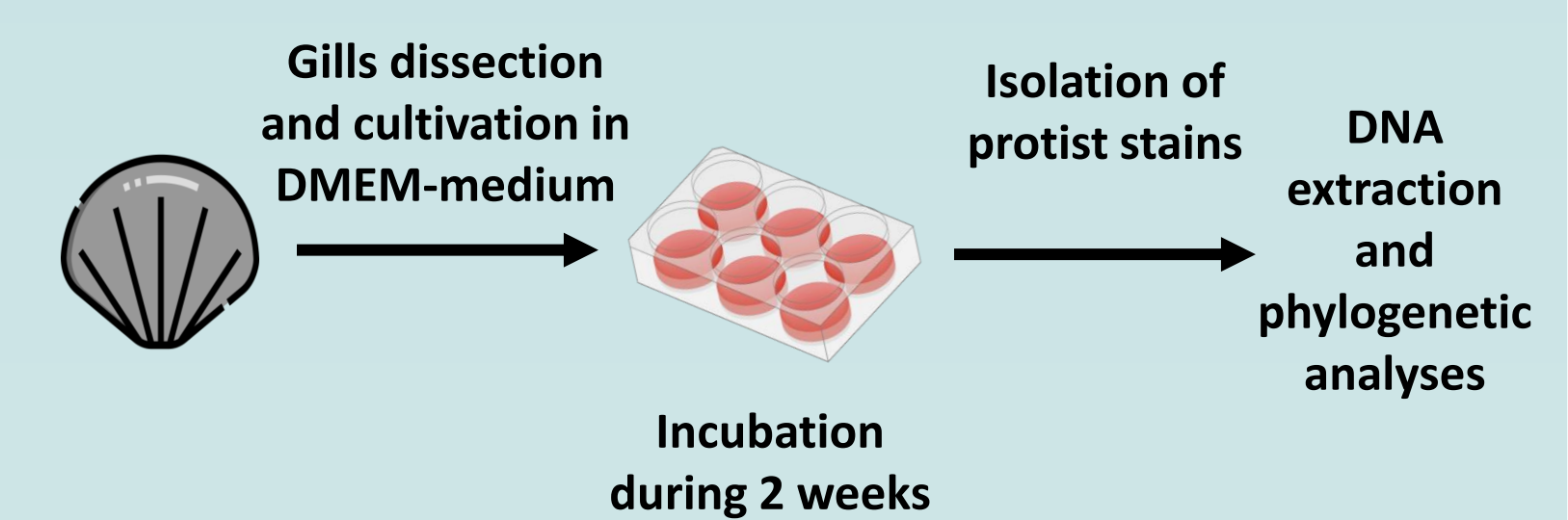
3. Genetic characterization of associated eukaryotic culture

Targeting the 18SrDNA with generalist eukaryotic primers

Construction of a consensus tree using:

- Bayesian methods (Mr Bayes)
- Maximum Likelihood methods (IQtree)

2. Culture and DNA extraction using *Perkinsus* broth media



4. Exploring the presence of underdescribed clusters of Labyrinthulomycetes along latitudinal French coastline

Search for Labyrinthulomycetes in a total of 259 clam samples from 3 sites (Brest, Noirmoutier and Arcachon) using specific primers

| Targeted cluster | Primers | Sequence (5'→3') |
|------------------|---------|----------------------|
| LABY1 group | LABY1_F | GGGCGCATTATTAGATT |
| | LABY1_R | GCACCGCCGATCGCTA |
| LABY2 group | LABY2_F | TCGGAGTGAAGCAATCCTGC |
| | LABY2_R | CCACCAACTAAGAACGGCCA |
| LABY3 group | LABY3_F | TTGTTTGTCTGGGGCGCC |
| | LABY3_R | GCCGCGGGGTCAATTAA |
| LABY4 group | LABY4_F | AAGCCGAATCTGGAAACG |
| | LABY4_R | TGCATCTGTGCCAAACCA |

Table 2: Specific primers designed and used in this study.

Conclusion and Perspectives

- Presence of unexpected Labyrinthulomycetes communities associated to *P. olsenii* infected manila clams
- The majority of Labyrinthulomycetes species found along the French Atlantic coast belongs to LABY1 group
- No detection of genetic signature belonging to LABY3 and LABY2 groups
- The prevalence and diversity of Labyrinthulomycetes is more important in Arcachon Bay, where *P. olsenii* is more prevalent

- Design qPCR primers for Labyrinthulomycetes to have a better sensibility and precision in our results
- Setting up *in vivo* co-infection experiments with LABY strains and *Perkinsus olsenii*
- Labyrinthulomycetes infection may be confused with *P. olsenii* infection and therefore not be detected