Molecular characterization of parasites of the genus *Perkinsus* present in clams from French producing areas

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Introduction

PRODUCTION

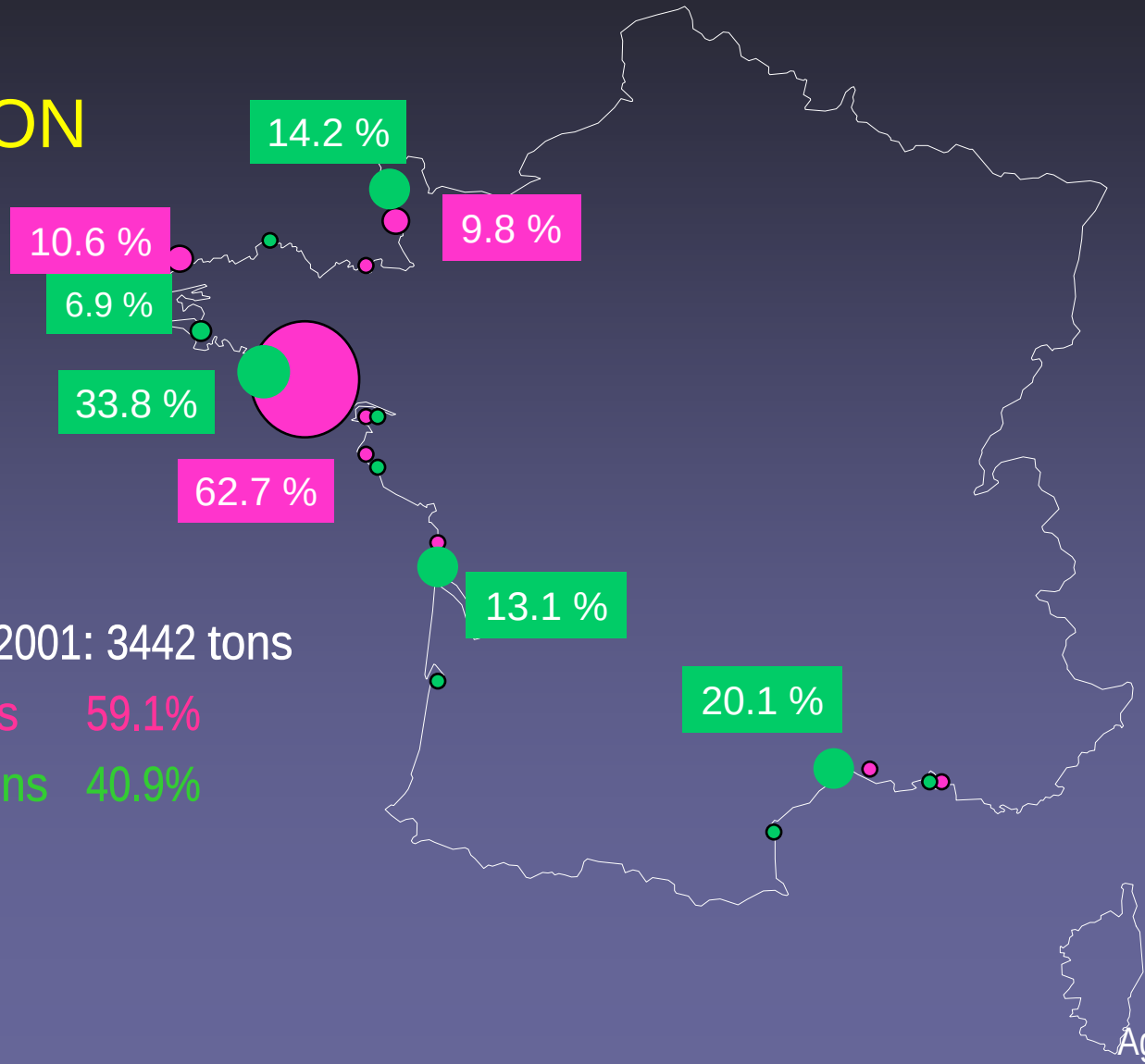
Clams = third most important bivalve production in France

3400 mt produced in 2001 (= 60% from aquaculture and 40% from fisheries)

Two main species : *Ruditapes philippinarum* and *R. decussatus*

Introduction

PRODUCTION



Clam marketing in 2001: 3442 tons

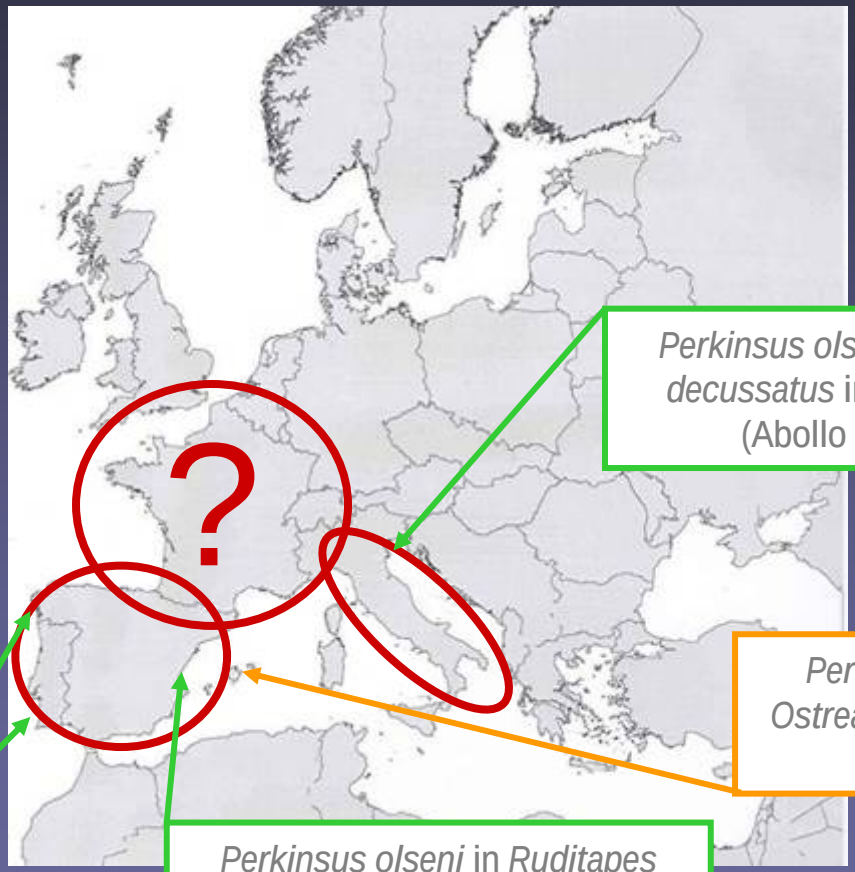
Cultured : 2034 tons 59.1%

Harvested : 1408 tons 40.9%

Agreste, 2005

Introduction

PERKINSUS SPP. IN EUROPE



Perkinsus olseni in *Ruditapes decussatus* in N Adriatic Sea (Abollo et al. 2006)

Perkinsus olseni in *Ruditapes decussatus* in Ria de Arousa (Casas et al. 2002)

Perkinsus mediterraneus in *Ostrea edulis* in Balearic Islands (Casas et al. 2004)

Perkinsus olseni in *Ruditapes decussatus* in Algarve (Robledo et al. 2002)

Perkinsus olseni in *Ruditapes decussatus* in Alfacs Bay (Elandaloussi et al. 2009)

Introduction

SURVEILLANCE OF CLAM DISEASES IN FRANCE

PASSIVE SURVEILLANCE :

notification of abnormal mortality

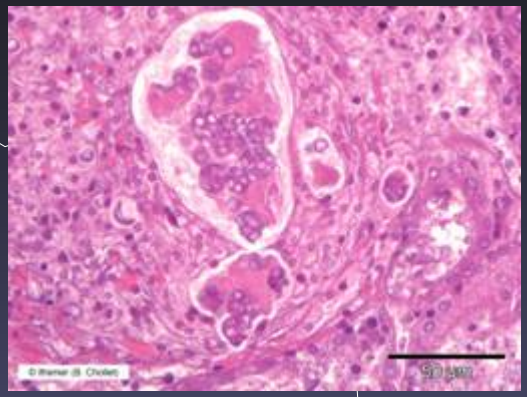
Sampling and investigation by histology, thioglycollate medium culture

ACTIVE SURVEILLANCE:

in 2004-2005 : epidemiological survey to establish the distribution and prevalence of perkinsosis in main producing areas in France

THROUGH THE REPAMO (French network for the surveillance of mollusc diseases)

Introduction



Chausey Island 42%
Est Manche 87%

Aber 2%
Pont Labbé river 31%

Gulf of Morbihan 37%

Marennes-Oléron 12%

Arcachon bay 92%

Thau lagoon 76%

Salses Leucate lagoon 96%

Distribution and prevalence of perkinsosis (2005)

Introduction

EPIDEMIOLOGICAL SURVEY (2004-2005)

Parasites of the genus *Perkinsus* were detected in **all sampling areas** in 2004 and 2005

- **Prevalence variability** : lower in the far West of France (low sea water temperature) and higher in the South of France (clam species ?)
- **Parasite burden variability** between areas and clams from same places (maximum in 2005 : 250 000 par x g^{-1})

Prevalence and parasite burden higher in ***Ruditapes decussatus*** compared to *R. philippinarum*

No associated mortality

Objectives

1. Characterization of parasites of the genus *Perkinsus* detected in clam producing areas in France

2. Studying intra individual and inter individual genetic variability of the parasite

Material and methods

MATERIAL

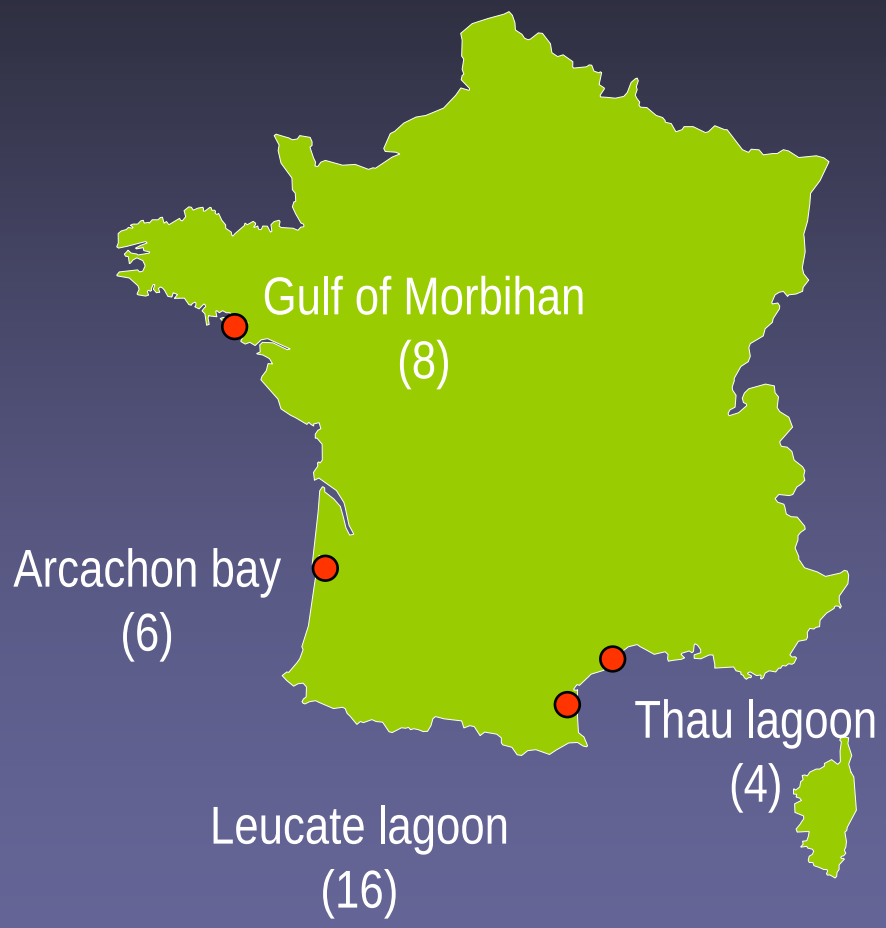
Clams collected in 2005 or 2006

Detected infected by RFTM culture

For which cultures were available

Ethanol fixed tissue from highly infected clams

Cultures = parasites maintained in DMEM:HAM'S F-12 (Invitrogen) according to Gauthier et al. (1995)



Material and methods

Selected parasite cultures or ethanol fixed tissue

↓

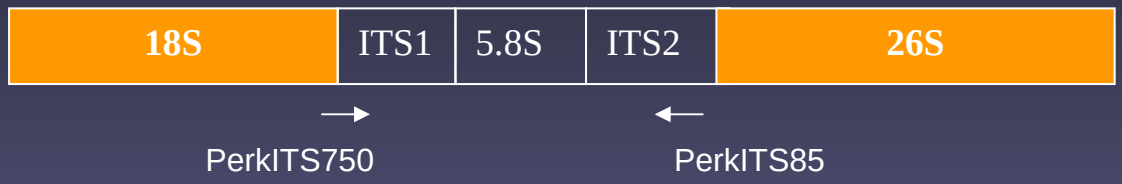
DNA extraction (QIAamp DNA easy kit (QIAGEN)

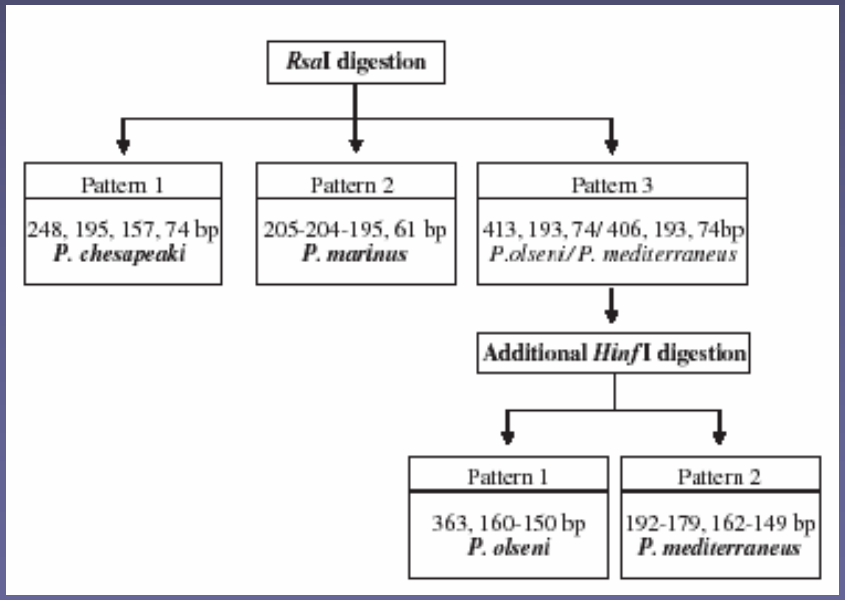
↓

PCR (according to Casas et al. 2002)

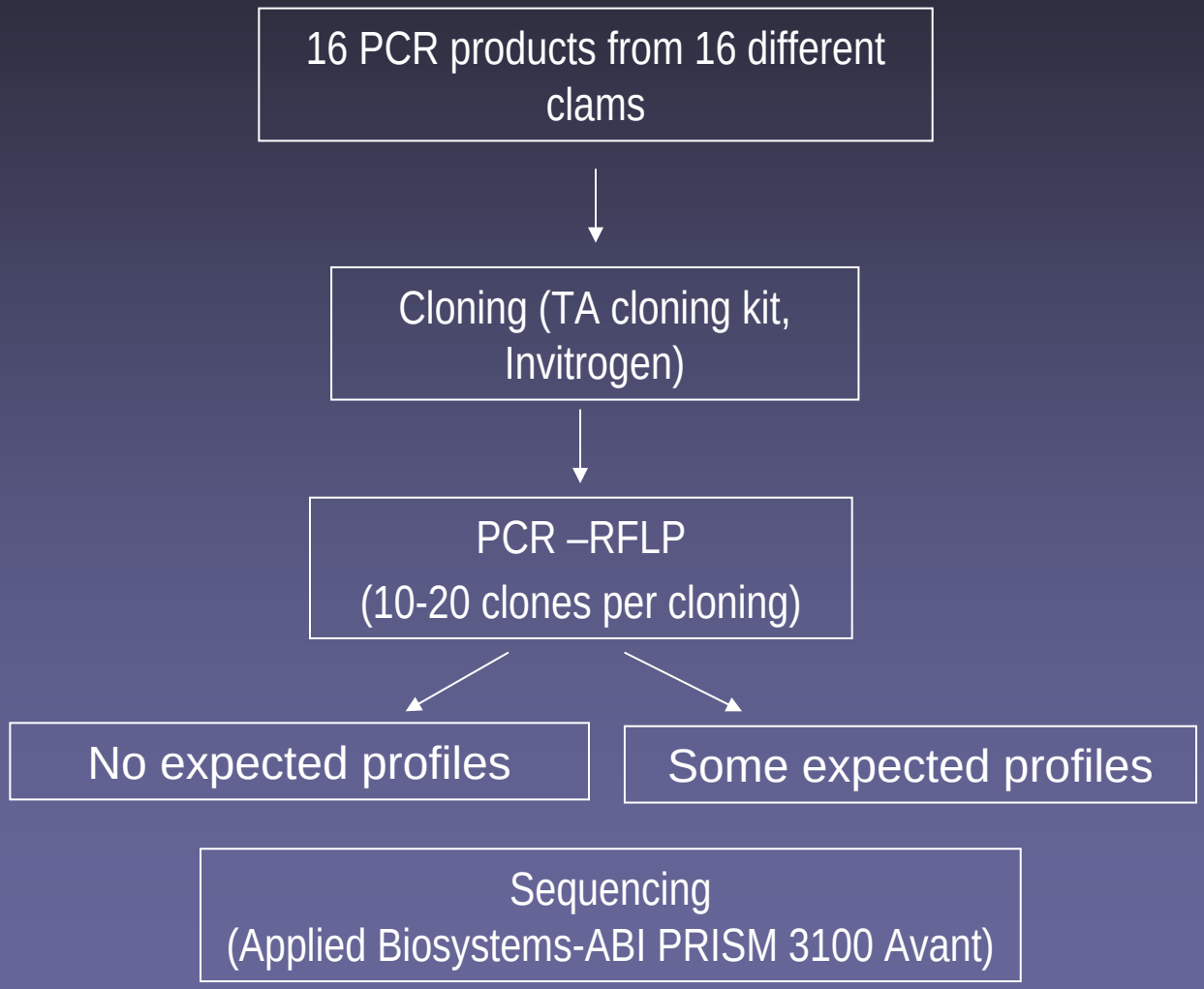
↓

RFLP (according to Abollo et al. 2006)





Material and methods



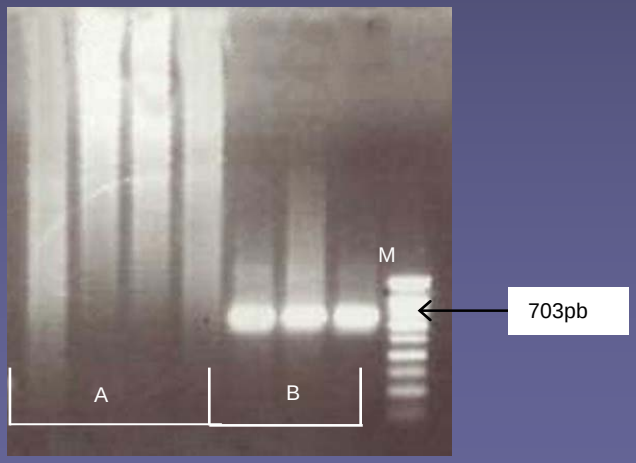
Results

DNA EXTRACTION

ETHANOL FIXED TISSUE VERSUS PARASITE CULTURES :

DNA extracted from ethanol fixed tissue (A) : smears

DNA extracted from parasite cultures (B) : expected size



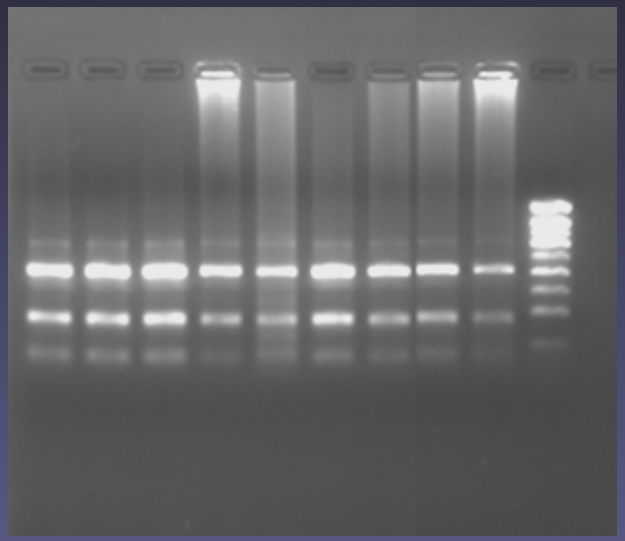
Results

Direct PCR-RFLP

Rsa I DIGESTION

Only profiles " *P. olsenil P. mediterraneus* "

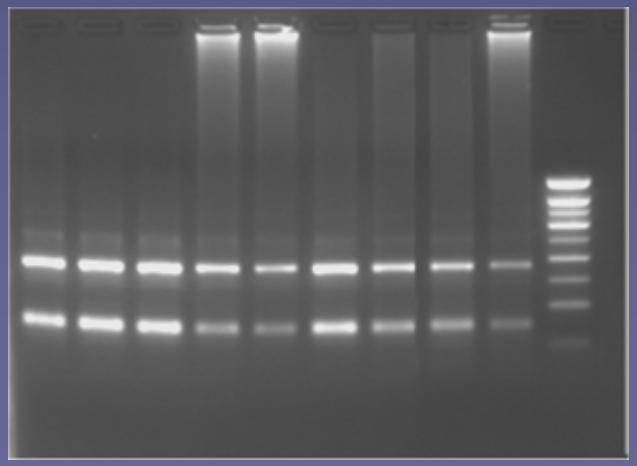
413pb
193pb
74pb



Hinf I DIGESTION

Only profiles "*P. olseni*"

363pb
160pb

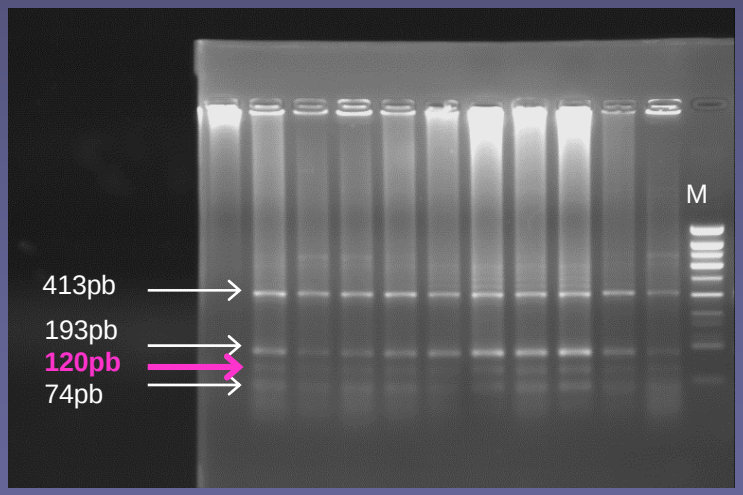


Results

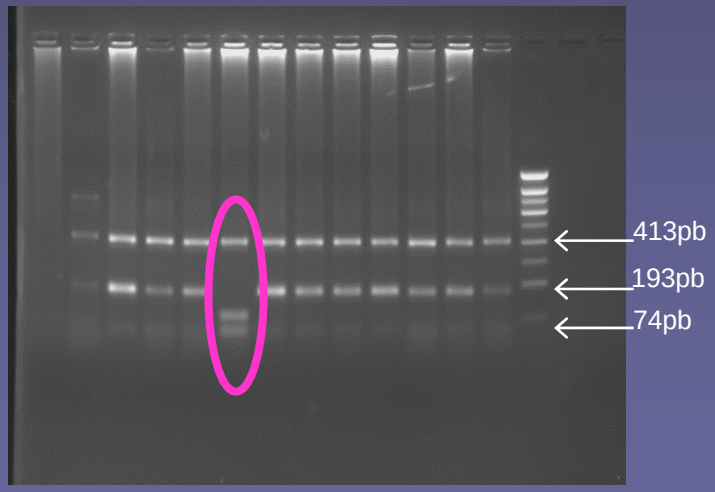
PCR-RFLP after cloning

Most of tested clones showed *Perkinsus olseni* like restriction profiles

Two exceptions :



Rsa I digestion (Clam from Leucates)



Rsa I digestion (Clam from Arcachon)

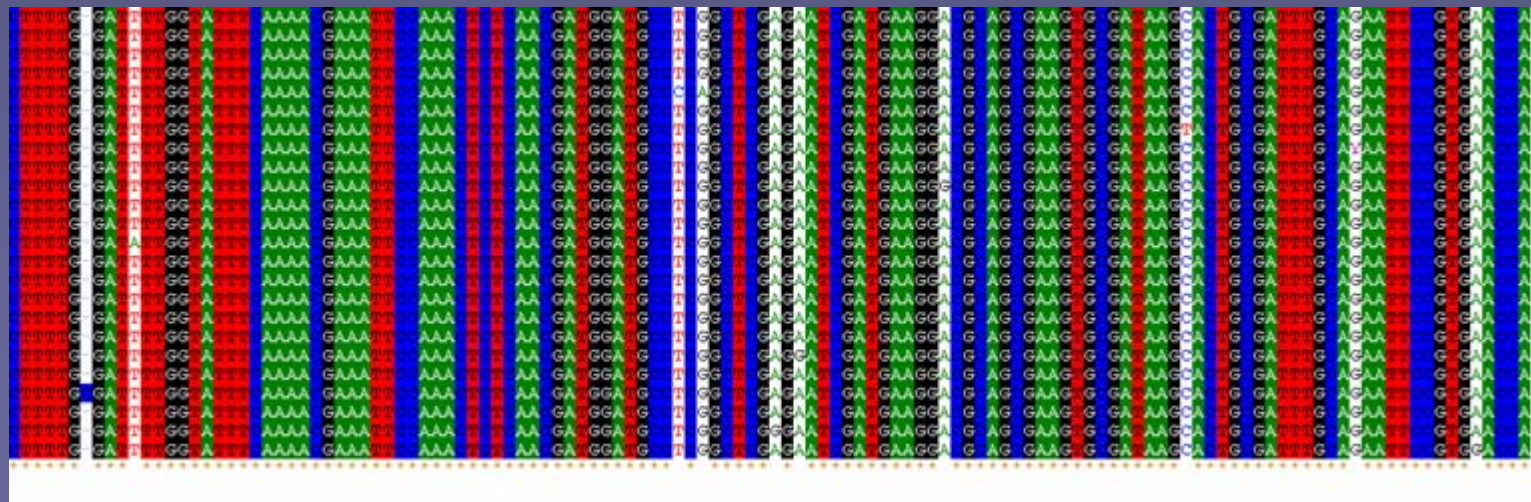
Results

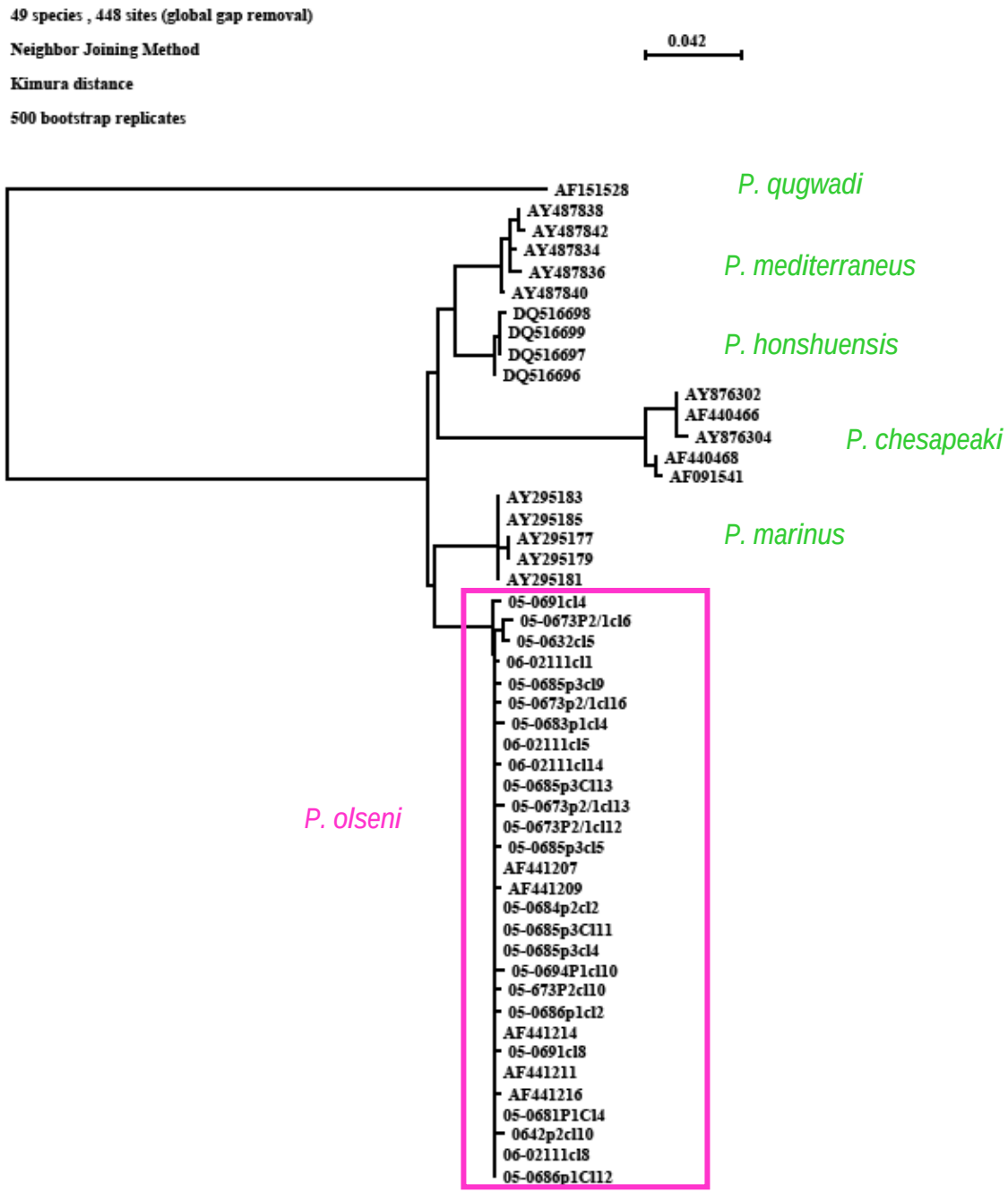
SEQUENCES

All the obtained sequences display between 99 and 100% of identity with *Perkinsus olseni* (even those which did not yield expected restriction profiles)

Random variability : between 1 and 3 punctual nucleotide modification (mainly substitutions) affecting one clone

No correlation with individual or geographic origin




49 species , 448 sites (global gap removal)
Neighbor Joining Method
Kimura distance
500 bootstrap replicates
0.042
AF151528
AY487838
AY487842
AY487834
AY487836
AY487840
DQ516698
DQ516699
DQ516697
DQ516696
AY876302
AF440466
AY876304
AF440468
AF091541
AY295183
AY295185
AY295177
AY295179
AY295181
05-0691cl4
05-0673P2/1cl6
05-0632cl5
06-02111cl1
05-0685p3cl9
05-0673p2/1cl16
05-0683p1cl4
06-02111cl5
06-02111cl14
05-0685p3Cl13
05-0673p2/1cl13
05-0673P2/1cl12
05-0685p3cl5
AF441207
AF441209
05-0684p2cl2
05-0685p3Cl11
05-0685p3cl4
05-0694P1cl10
05-673P2cl10
05-0686p1cl2
AF441214
05-0691cl8
AF441211
AF441216
05-0681P1Cl4
0642p2cl10
06-02111cl8
05-0686p1Cl12
P. qugwadi
P. mediterraneus
P. honshuensis
P. chesapeaki
P. marinus
P. olseni

Conclusions

Parasite culture appeared more appropriate than tissue for our study which has limited the number of samples analysed

Perkinsus olseni is present in at least four different French marine areas : Morbihan gulf; Arcachon bay; Leucate and Thau lagoons.

Genetic variability between geographic sites, between clams from a same location and inside clam is very low.

Perspectives

This work should be completed by more data on other geographic places and on other genes (including LSU and actin genes).

Impact of *Perkinsus olseni* on clam production is still questioned and despite its detection in all producing areas in France, no mortality was reported in 2004 and 2005

Perkinsus olseni is not presently notifiable in Europe which means that clam transfers are not regulated… = risk?

Thanks for your attention