

Detection and molecular identification of *Pseudo-nitzschia* species in natural samples from the French coasts

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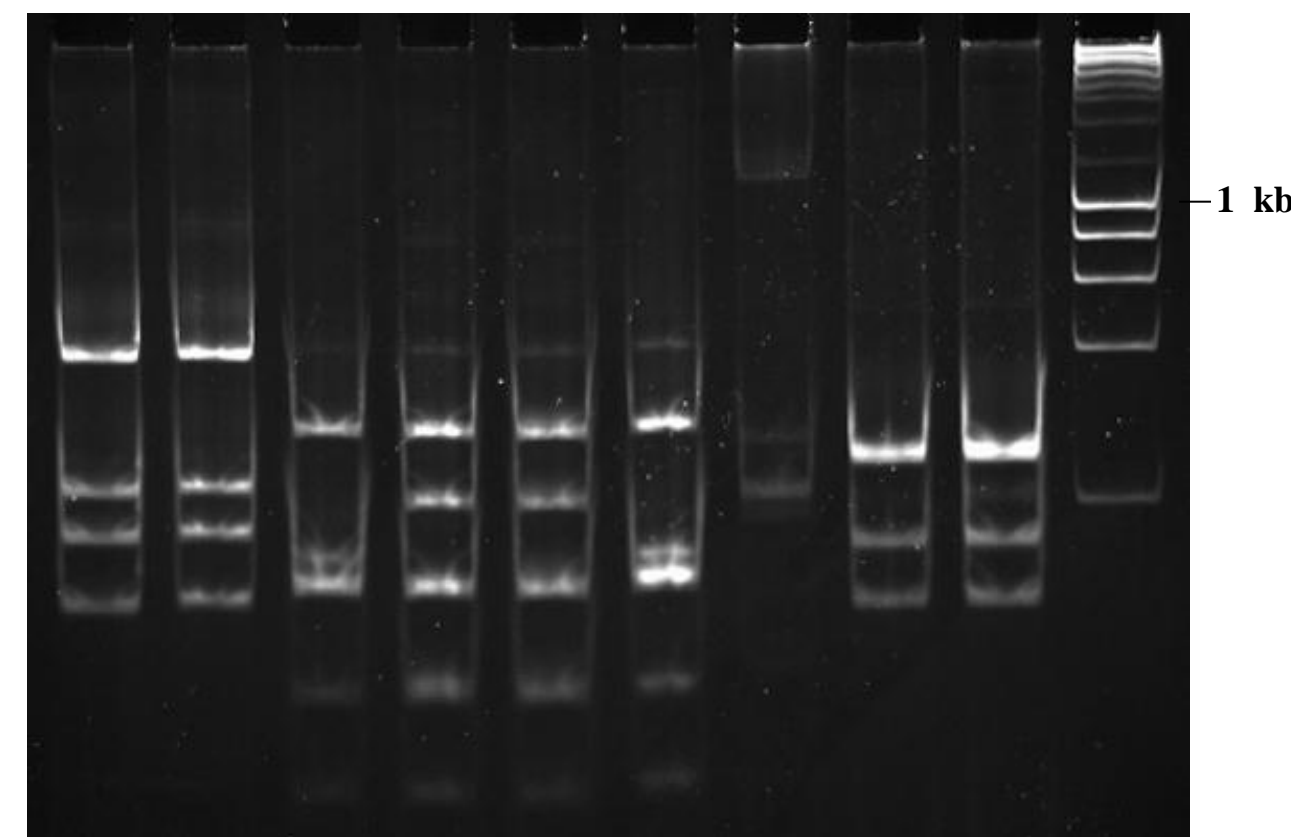
Introduction

Domoic acid (DA) was first detected in France in mussel flesh in 1998, outbreak that was linked to the presence of *Pseudo-nitzschia pseudodelicatissima*. Ever since, the phytoplankton monitoring program (REPHY, Réseau de surveillance du Phytoplancton et des Phycotoxines) also monitors DA content in shellfish flesh. Amnesic shellfish poisoning (ASP) has been recurrently detected, and was over the sanitary threshold in the years 2000 and 2004 in Brittany and in 2002 in the Mediterranean coast (Amzil, 2001; Amzil pers. comm.). Toxicity events are usually dominated by several *Pseudo-nitzschia* species, although different strains might be or not be toxic (Bates *et al.*, 1998). This is why an inventory of the species present in the French coasts and their molecular characterisation needed to be carried out. A methodology to get *Pseudo-nitzschia* DNA directly from natural samples fixed with lugol is presented. The DNA sequences obtained in this study will be used to produce specific molecular markers of *Pseudo-nitzschia* species.

Natural water sample conserved with lugol



RFLP fragments after restriction enzyme digestion with Alu I



Methods

From lugoled natural water samples:

Decantation of 50-100 ml of sample

Pseudo-nitzschia chains pick up

MDA reaction

ITS amplification by PCR

Cloning reaction
Plasmid extraction
Insert amplification by PCR

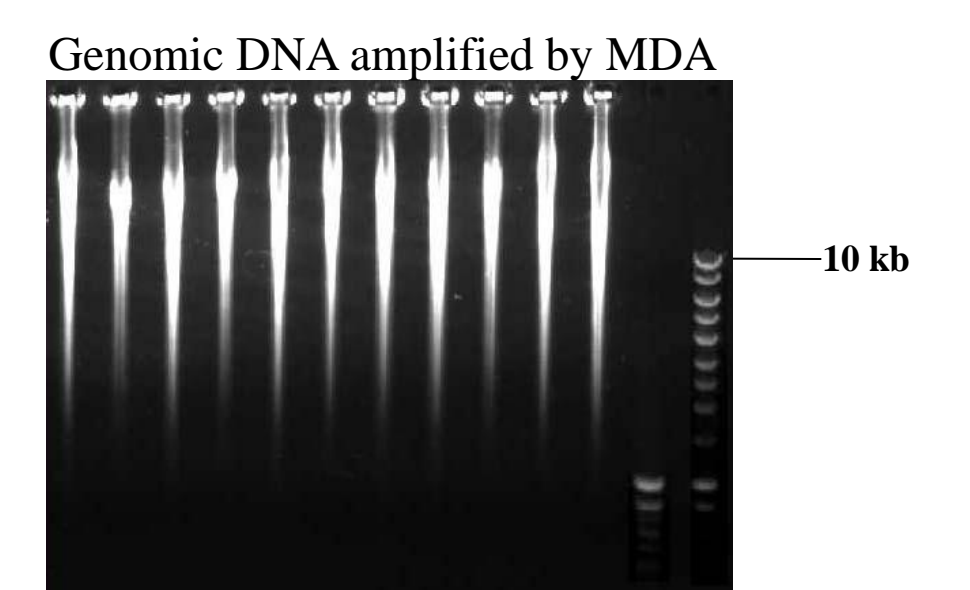
Restriction enzyme digestion
Clone selection

Amplified DNA purification

Sequencing

Multiple Displacement Amplification (MDA)

The MDA reaction copies repeatedly DNA using random primers and a polymerase that amplifies DNA in a 30 °C isothermal reaction (Lasken, 2007).



Clone selection by restriction enzyme digestion

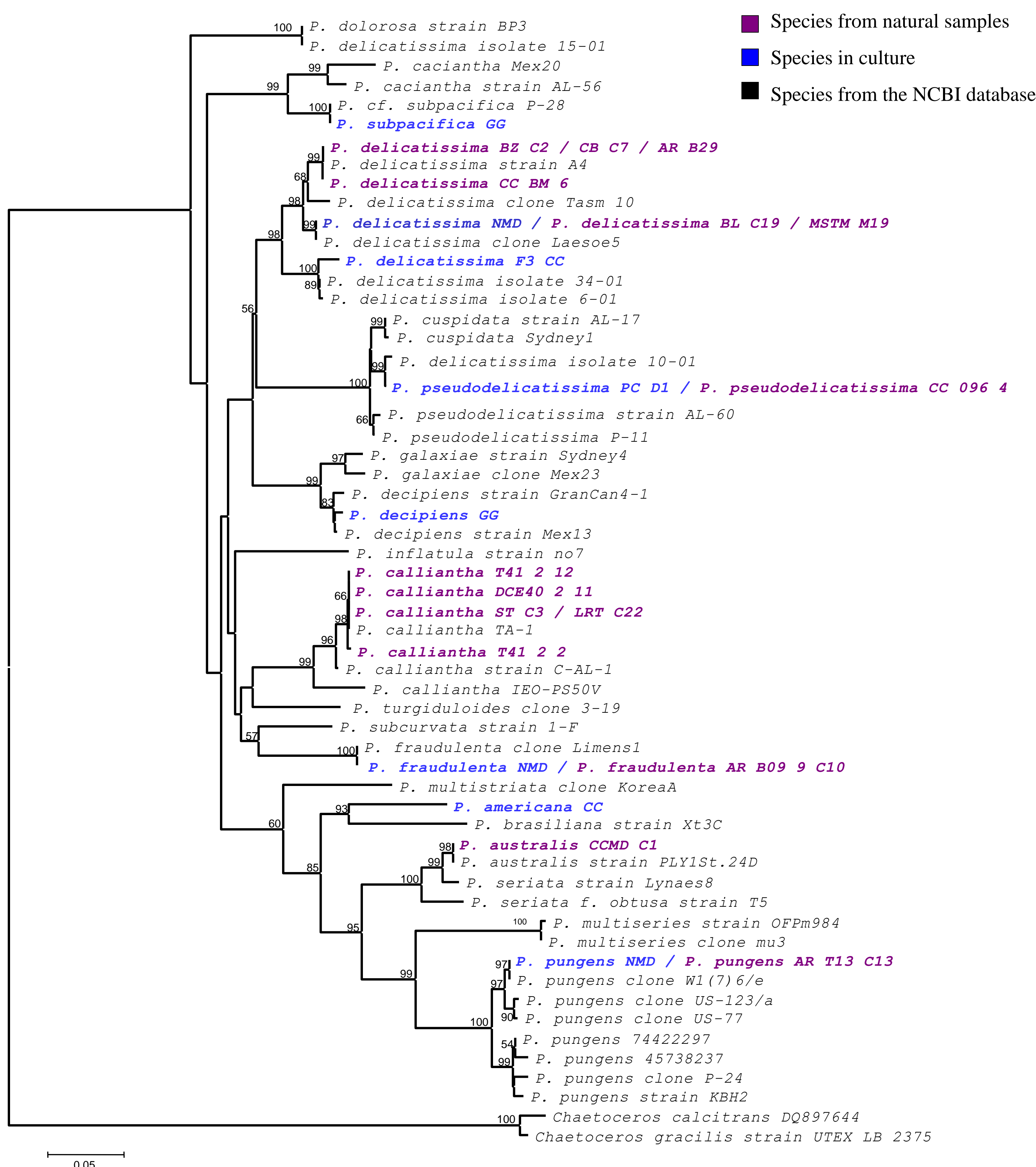
Amplified ITS fragments were digested with restriction enzymes (Alu I, Hha I, Mse I, Rsa I) to select different clones by RFLP.

DNA sequences

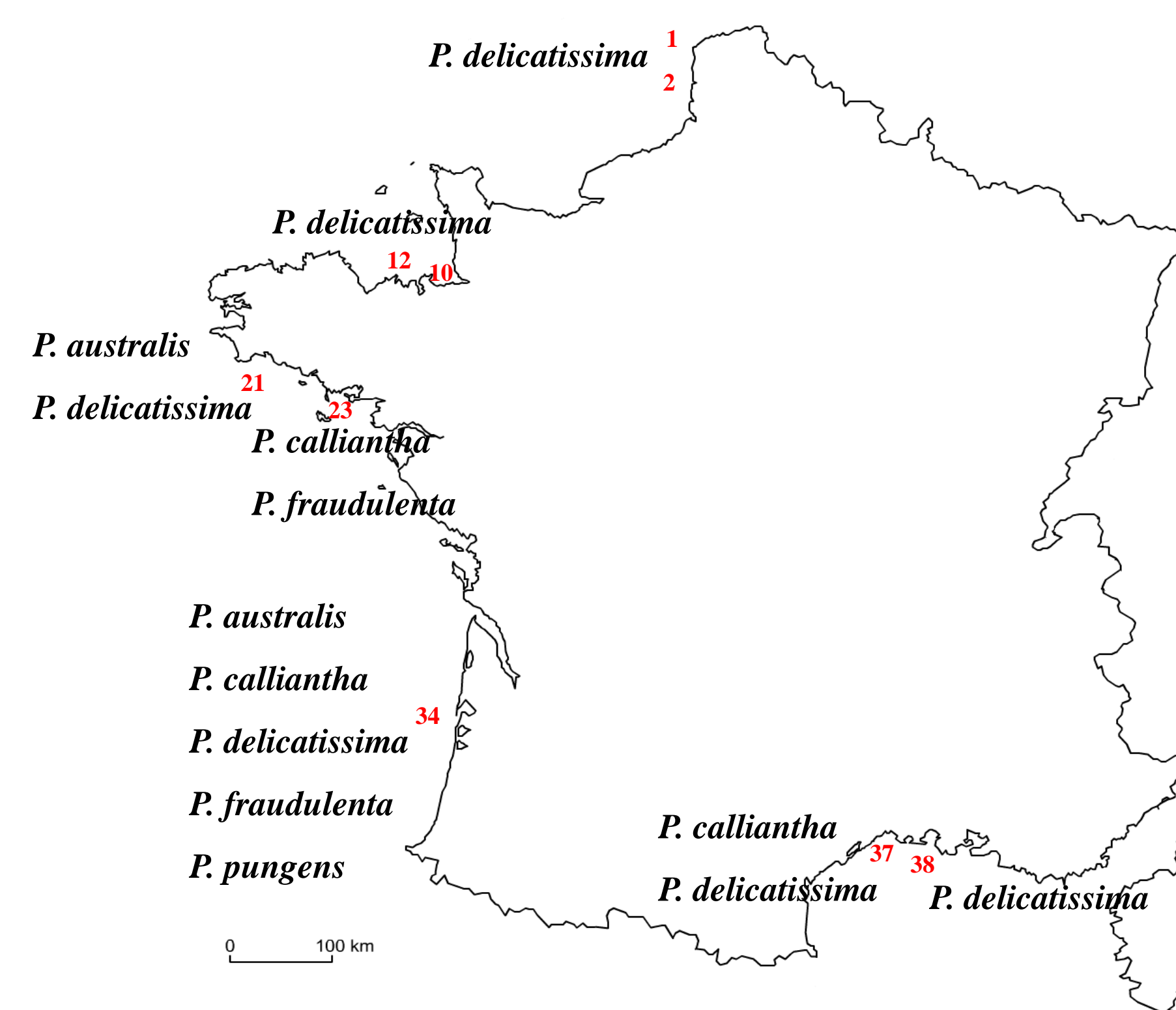
Sequence analysis was carried out in Bioedit and sequences were input into the NCBI database for BLASTn. Sequences were also aligned using CLUSTALW and a phylogenetic tree was inferred using NJ in Mega 4 with all sequences found in the samples and in the database.

Results

Phylogenetic tree of *Pseudo-nitzschia* species



Distribution of *Pseudo-nitzschia* species along the French coasts



List of sites REPHY:

- 1 Dunkerque
- 2 Boulogne
- 10 Mont Saint Michel
- 12 Saint Cast
- 21 Concarneau
- 23 Lorient
- 34 Arcachon
- 37 Bouzigues
- 38 Sète Mer

Conclusions

We have developed a methodology that allows the amplification of *Pseudo-nitzschia* genomic DNA from natural samples. Thus, making possible the study of the biodiversity in the sample and taxonomic characterisation and identification of species by specific DNA fragments amplification and sequencing. This work allowed mapping of *Pseudo-nitzschia* species along the French coasts and increased the DNA sequences available in the database. Future work will focus on the search of specific molecular markers for each species as a tool of detection and identification.

References

- Amzil, Z., Fresnel, J., Le Gal, D. and Billard, C. (2001). Domoic acid accumulation in French shellfish in relation to toxic species of *Pseudo-nitzschia multiseriata* and *P. pseudodelicatissima*. *Toxicon*. 39: 1245-1251.
- Bates, S. S., Garrison, D. L. and Horner, R. A. (1998). Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. In: Anderson, D. M., Cembella, A. D. and Hallegraeff, G. M.; (eds.). *Physiological ecology of harmful algal blooms*. Springer-Verlag, Heidelberg, pp. 267-292.
- Lasken, R.S. (2007). Single-cell genomic sequencing using Multiple Displacement Amplification. *Current Opinion in Microbiology*. 10: 510-516.