

Diversity of the dinoflagellate genus *Alexandrium* along the French coasts, based on morphological and phylogenetic analyses

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INTRODUCTION

In France, the genus *Alexandrium* is mainly known for recurrent toxic blooms of *A. minutum* in some Channel estuaries since 1988 and *A. catenella* in a Mediterranean lagoon since 1998. But studies about its diversity are failing. It has been really approached during the 1990s, using morphological features then molecular analysis more recently. Ribosomal DNA sequences obtained confirm the identification of species and characterize them genetically. They have been used to locate them in a phylogenetic tree including other sequences retrieved from GenBank. And the special case of *A. leei* has been studied, considering its geographical disconnection from other populations.

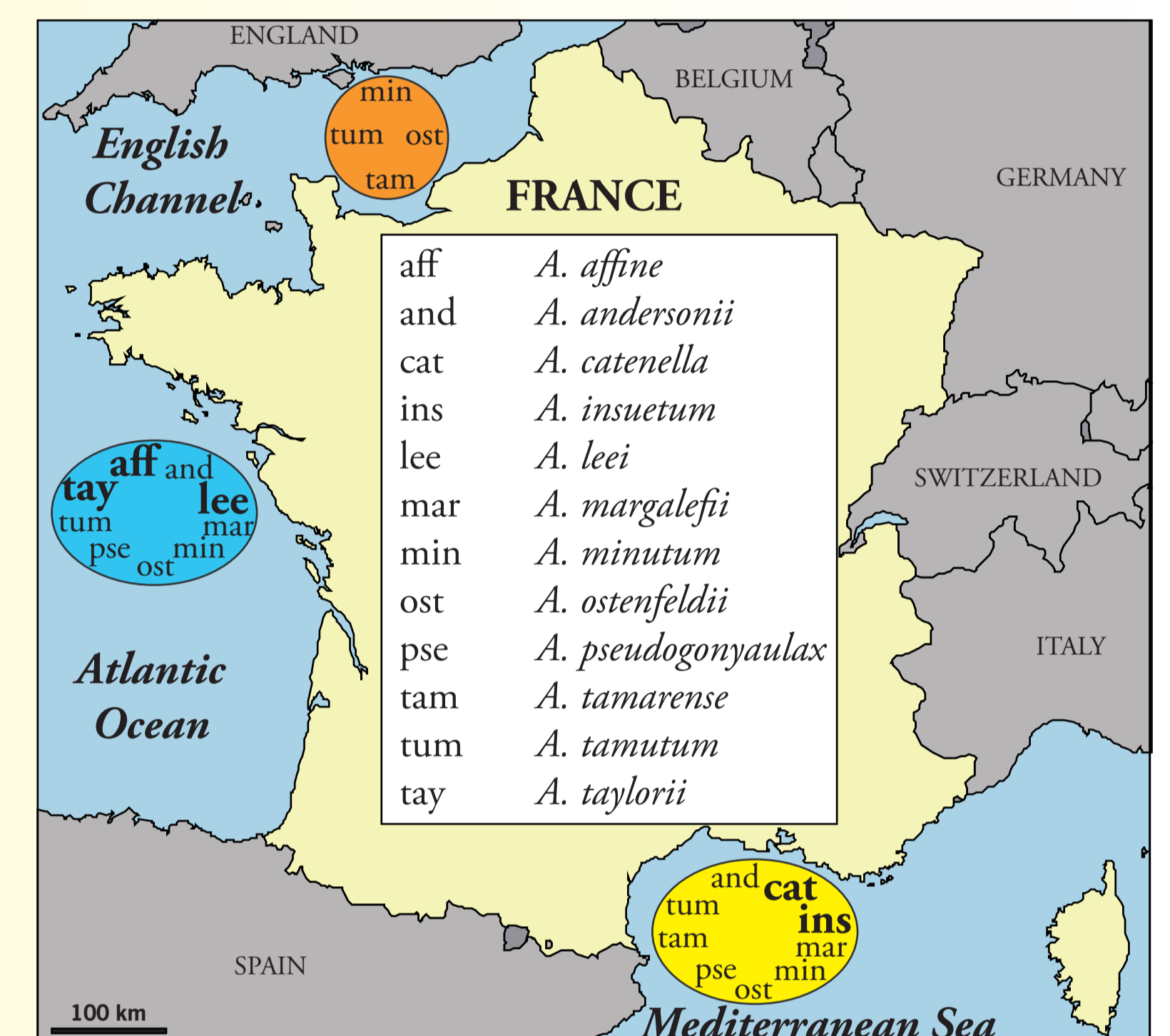
MATERIAL AND METHODS

This study has been achieved thanks to the samples from the national phytoplankton monitoring network (REPHY). Cells were pipetted individually before a morphological identification in light (LM) and scanning electron microscopy (SEM). For amplification of rDNA genes and sequencing, single cells were isolated, rinsed in distilled water and transferred to PCR tubes. Phylogeny was inferred from Bayesian inference (BI), maximum likelihood (ML), maximum parsimony (MP) and neighbour-joining (NJ Kimura2) analyses.

RESULTS

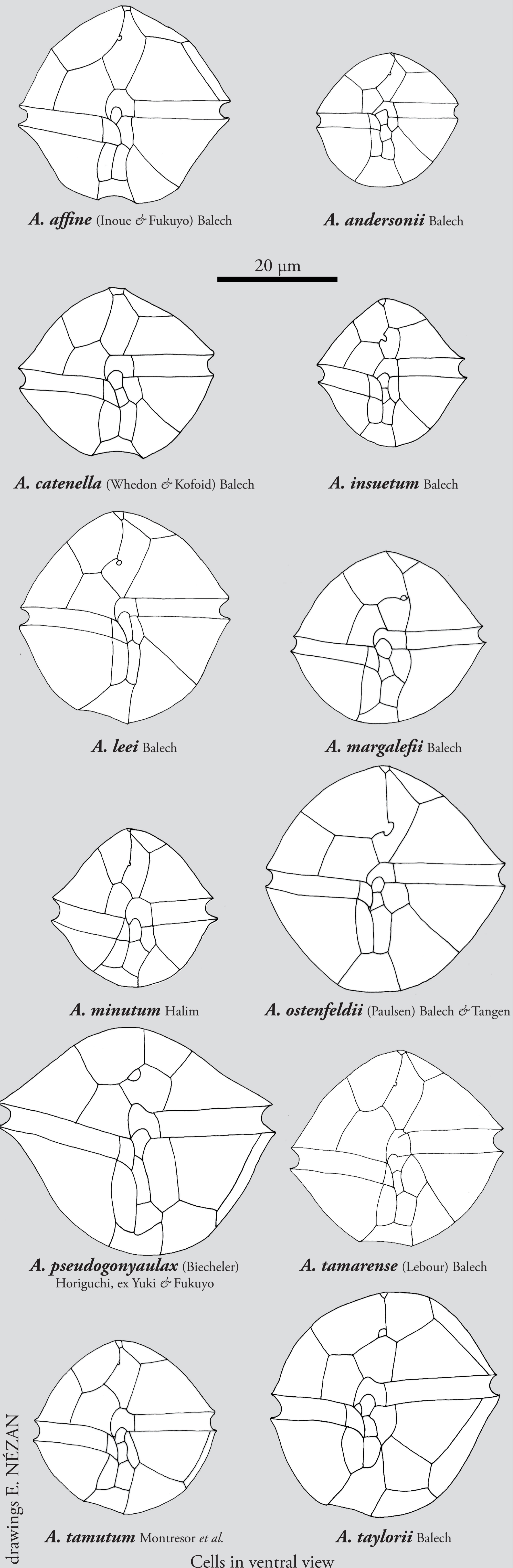
Morphology & ecology

Twelve *Alexandrium* species have been identified. Three of them are present along the coasts of both English Channel, Atlantic Ocean and Mediterranean Sea: *A. minutum* with several morphotypes, *A. ostenfeldii* and *A. tamutum*. As to *A. tamarensis*, it occurs generally in the Mediterranean Sea and English Channel. *A. catenella* and *A. insuetum* are two typically Mediterranean species while *A. affine*, *A. leei* and *A. taylorii* are observed only on the Atlantic coast. A study of the seasonal succession of *Alexandrium* species achieved in the South of Brittany shows an order of appearance from spring to autumn: *A. tamutum*, *A. minutum*, *A. margalefi*, *A. leei*, *A. andersonii*, *A. pseudogonyaulax* and *A. affine*. Concerning *A. ostenfeldii*, we can find it whatever the season and even in winter.



Geographic distribution of *Alexandrium* species

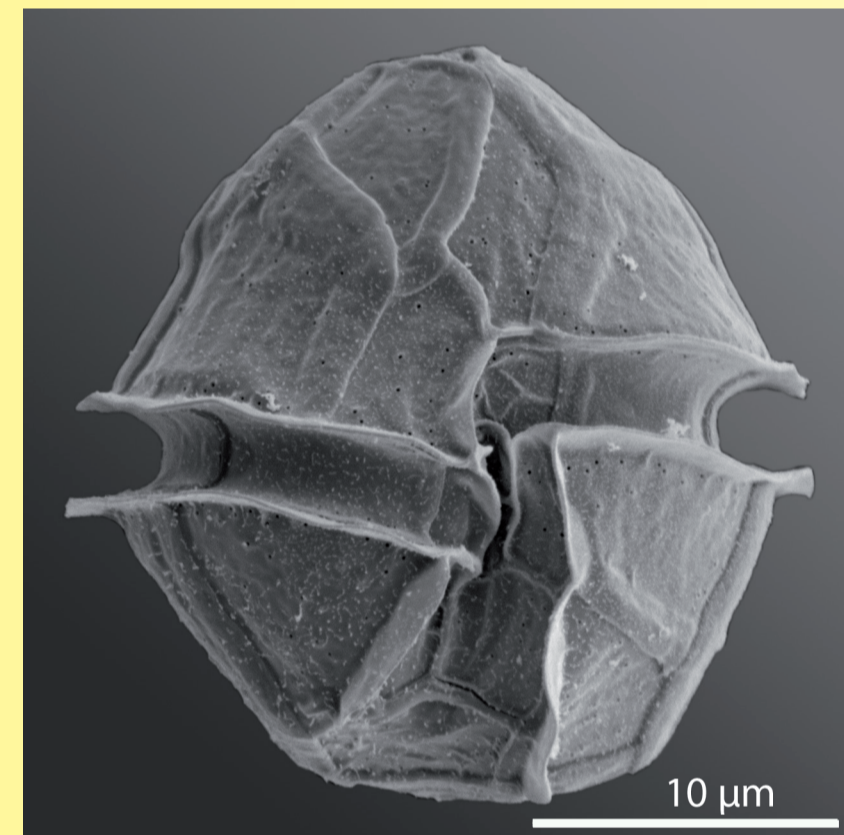
ALEXANDRIUM SPECIES IN FRANCE



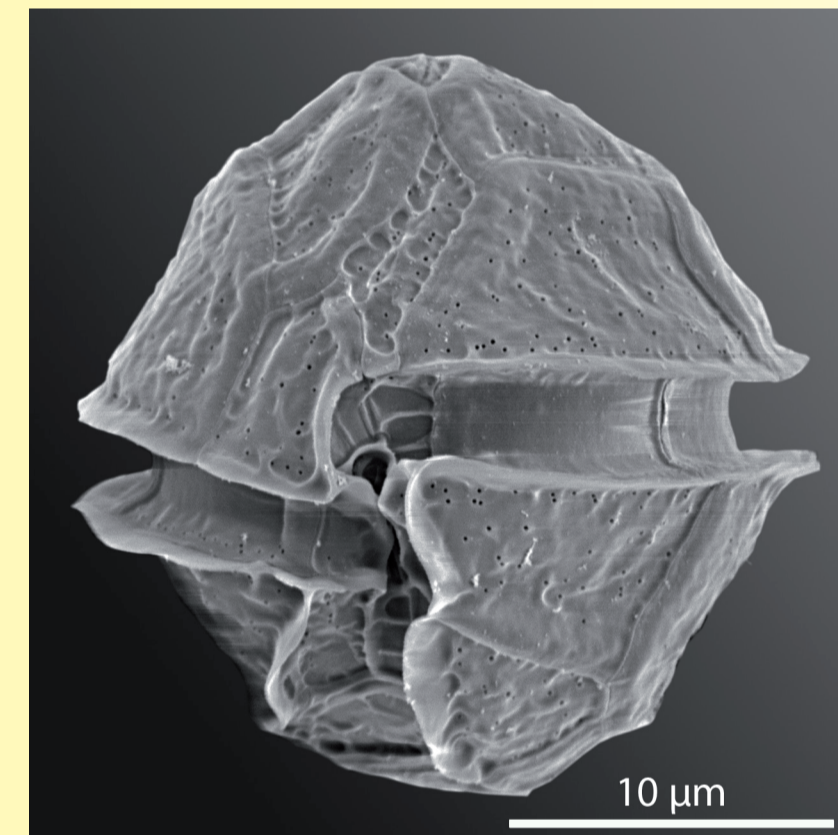
drawings E. NÉZAN

Cells in ventral view

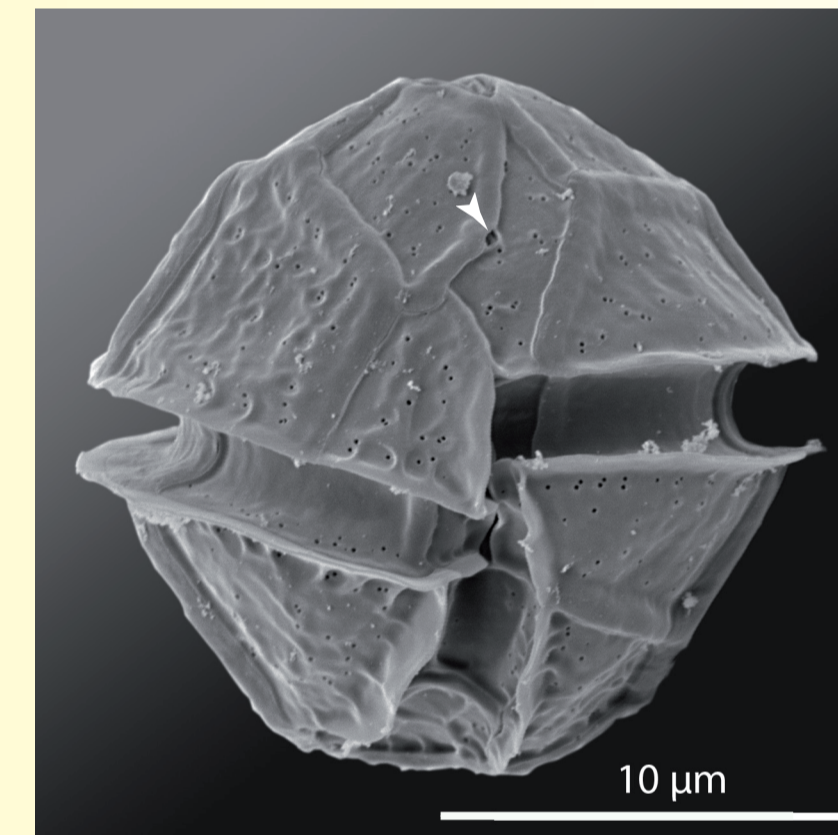
Three morphotypes of *A. minutum*



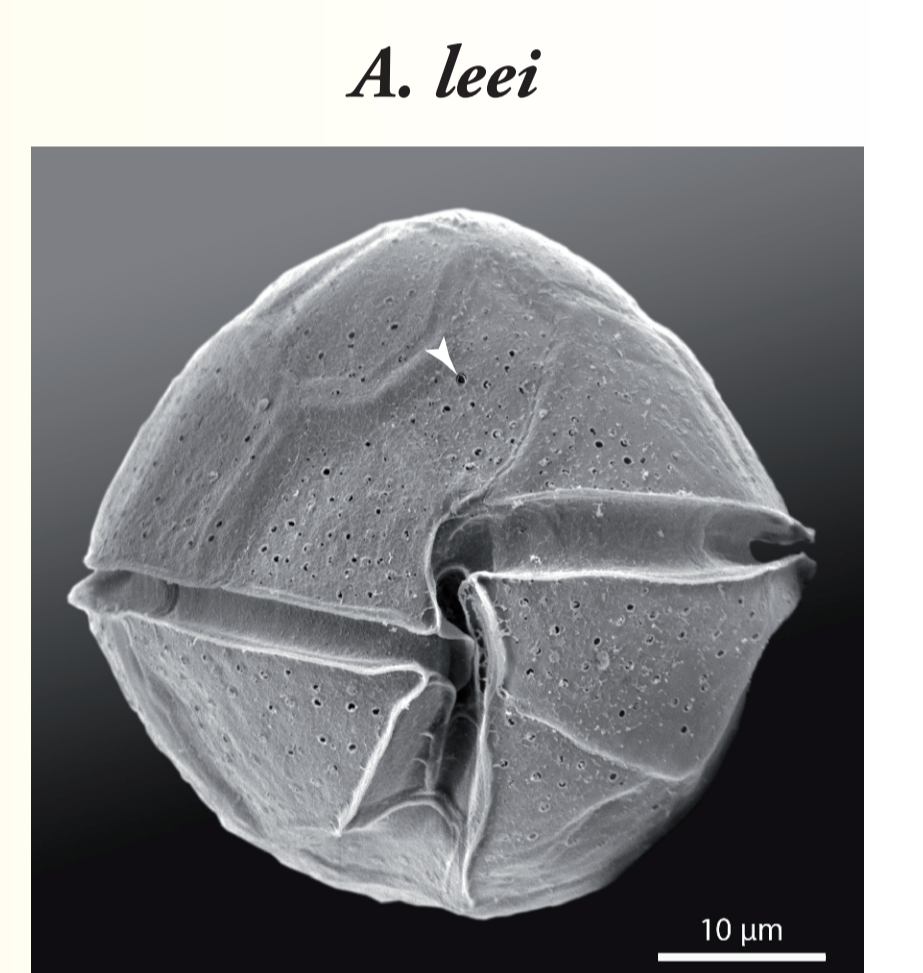
cell with smooth thecal plates and without a ventral pore



cell with thick, ornamented thecal plates and without a ventral pore



cell smooth on the left and ornamented on the right and with a ventral pore



cell with numerous thecal pores and a ventral pore located inside the 1' plate

Molecular analysis

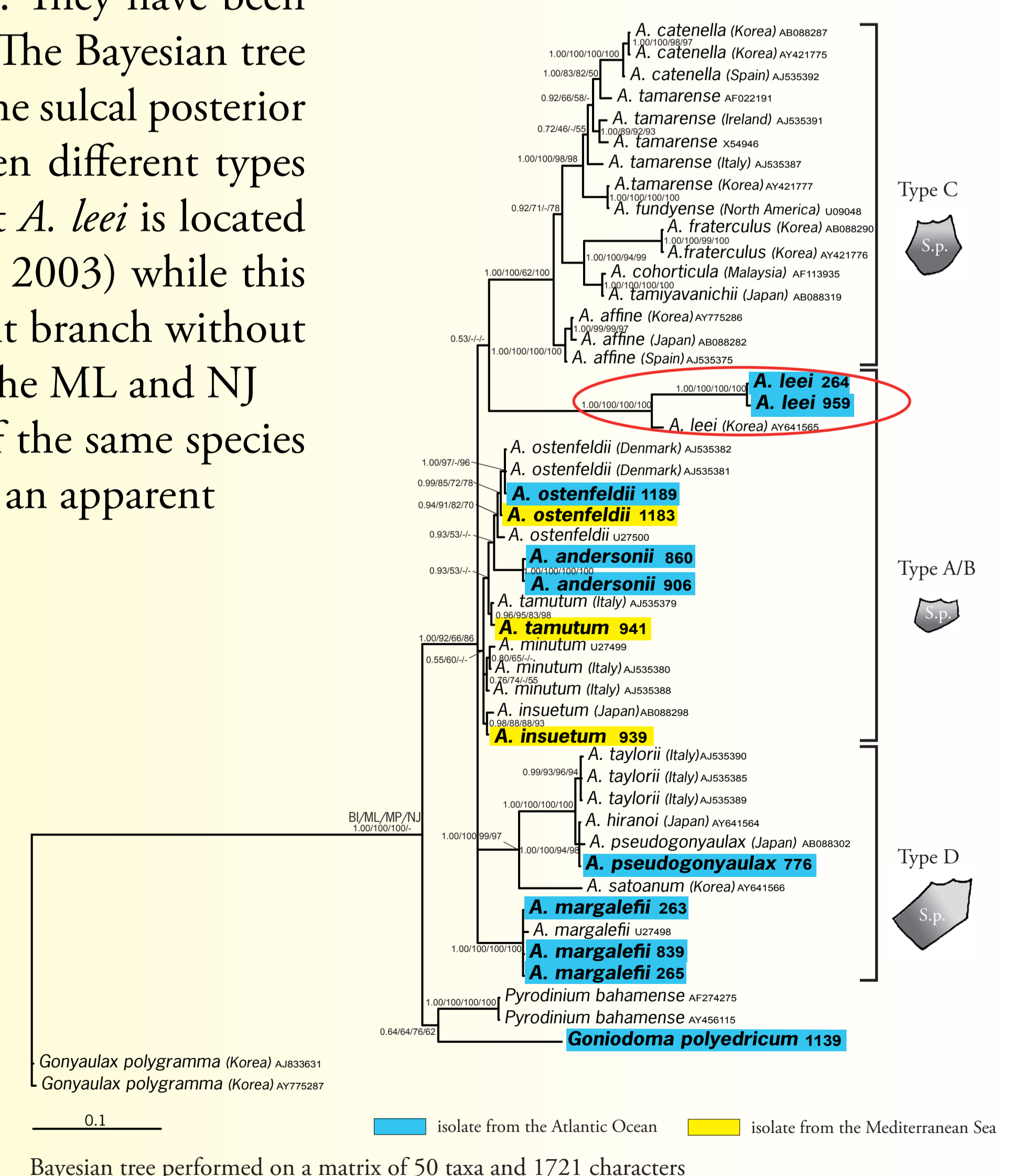
SSU rDNA sequences have been obtained for seven species, including *A. andersonii* of which the sequence was unavailable up to now. They have been used to investigate relationships within the genus *Alexandrium*. The Bayesian tree obtained in this study, in combination with the morphology of the sulcal posterior plate (Sp) are consistent with a previous discrimination between different types (Usup *et al.* 2002, Hansen *et al.* 2003). Moreover, it reveals that *A. leei* is located at the base of a major clade (clade 1 according to Hansen *et al.* 2003) while this species diverges early in the genus and forms a deep independent branch without apparent genetic affiliation to the other *Alexandrium* species in the ML and NJ approaches. It grouped with the Korean strain (JHW0006-2) of the same species but a high genetic divergence (5%) is observed notwithstanding an apparent morphological similarity.

And thanks to a recent phylogenetic analysis of the LSU rDNA gene of the *Alexandrium* species, we confirm that the french *A. leei* strain is distinct not only from the Korean strain but also from the three Malaysian strains (AIMS02, AIMS03, AT2). As a consequence, it does not belong to the Asian clade.

References:

Usup *et al.* 2002. *Harmful Algae* 1: 59-68
Hansen *et al.* 2003. *Harmful Algae* 2: 317-335
Tang *et al.* 2007. *Mar. Biol.* 150: 541-549

PHYLOGENY OF ALEXANDRIUM SPECIES INFERRED FROM SSU rDNA SEQUENCES



Bayesian tree performed on a matrix of 50 taxa and 1721 characters

CONCLUSION

This study has pointed out an unexpected diversity of the *Alexandrium* genus in France, using a combined approach between morphological characters and molecular data. The phylogenetic analysis strengthens the hypothesis of a geographic origin in the intra-specific variation of *A. leei* (Tang *et al.* 2007). However, the available sequences are too few and for a better estimate of the biogeography of this species, phylogeny must be extended to several other countries from different continents and specially from Europe to see if the European clade is a reality.