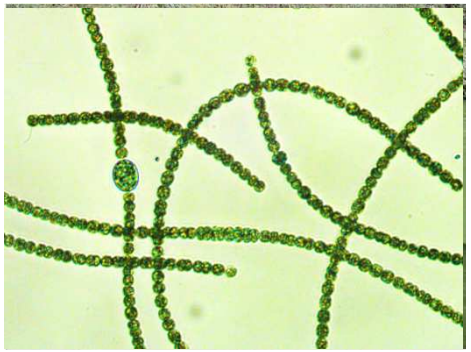
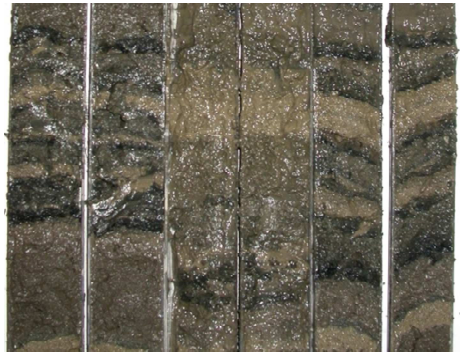
ANATOXIN-A IN SEVERAL FRESHWATER LAKES IN FRANCE: OCCURRENCE AND PHYLOGENY OF BIOSYNTHESIS GENES





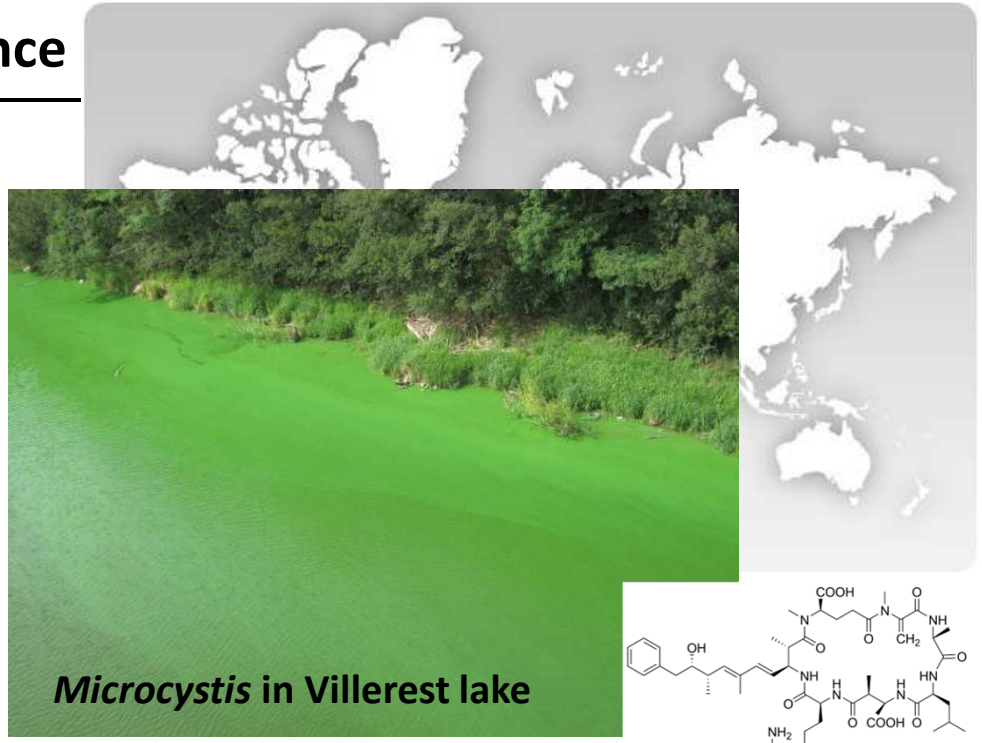
Marion SABART, Benjamin LEGRAND, Jérôme LESOBRE, Pierre SABATIER, Gaël PARIS, Jonathan COLOMBET, Anne-Hélène LEJEUNE and Delphine LATOUR

10th International Conference on Toxic Cyanobacteria, Wuhan, China

Cyanobacteria and cyanotoxins in France

Many freshwaters experiencing heavy cyanobacterial blooms

***Planktothrix rubescens* in Gour de Tazenat**



***Microcystis* in Villerest lake**

The microcystins, the only cyanotoxins monitored by the Health Agency in France

The bloom-forming species are known to produce other cyanotoxins especially neurotoxins

Are there others cyanotoxins in freshwater lakes in France?

What about anatoxin-a?

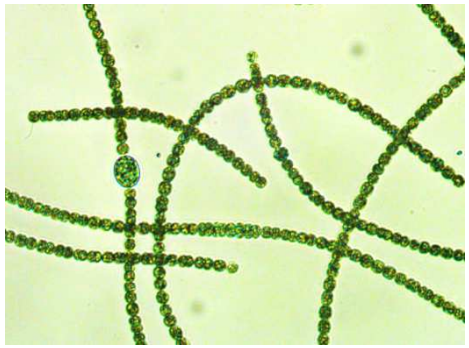


Photo: A. Lamarque

Many lakes with potentially anatoxin-a producing species (*e.g Dolichospermum, Aphanizomenon*)

Photo: J.F. Humbert

Photo: I. Echenique

Dog poisonings in French rivers (Gugger *et al.* 2005, Cadel-Six *et al.* 2009)

ELSEVIER

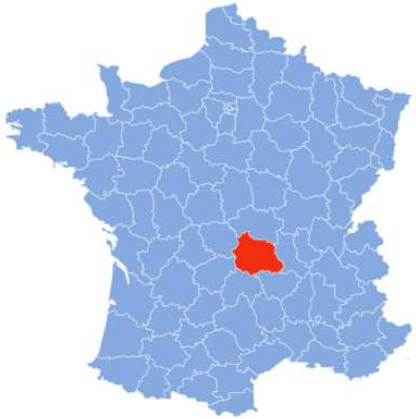
Toxicon 45 (2005) 919–928

TOXICON

www.elsevier.com/locate/toxicon

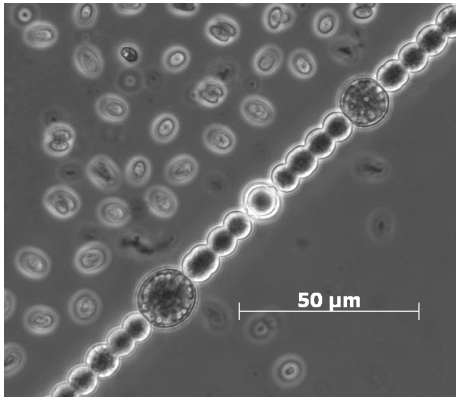
First report in a river in France of the benthic cyanobacterium *Phormidium favosum* producing anatoxin-a associated with dog neurotoxicosis

Muriel Gugger[a,*], Séverine Lenoir[a,b], Céline Berger[a], Aurélie Ledreux[a], Jean-Claude Druart[c], Jean-François Humbert[c], Catherine Guette[a], Cécile Bernard[a]



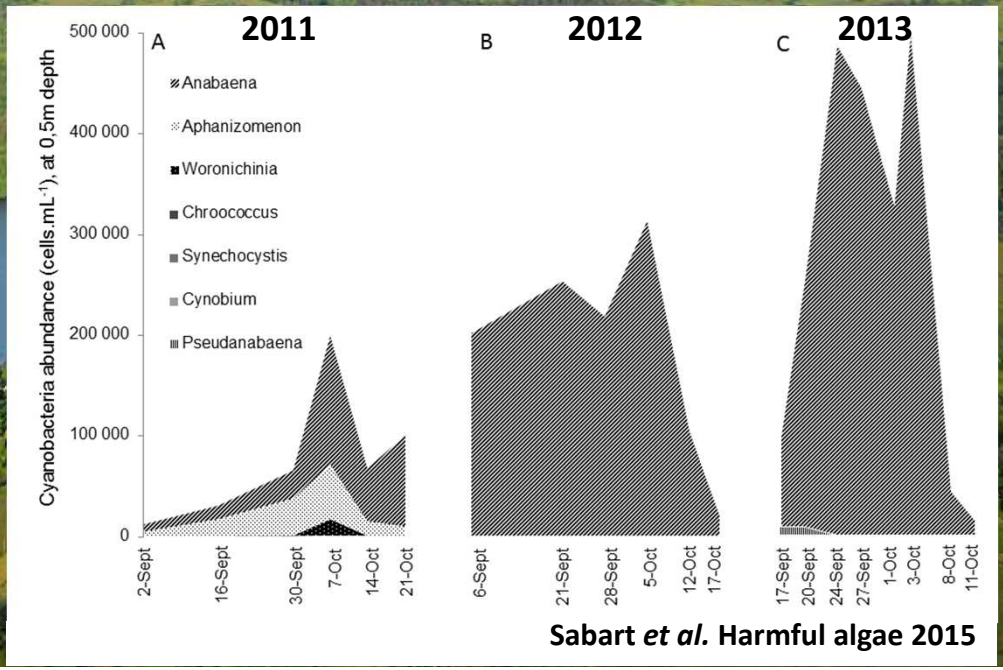
Recent reports in freshwater lakes in Europe (*e.g* Rantala-Ylinen *et al.* 2011, Shams *et al.* 2015) but none in France

A three-year survey in Lake Aydat



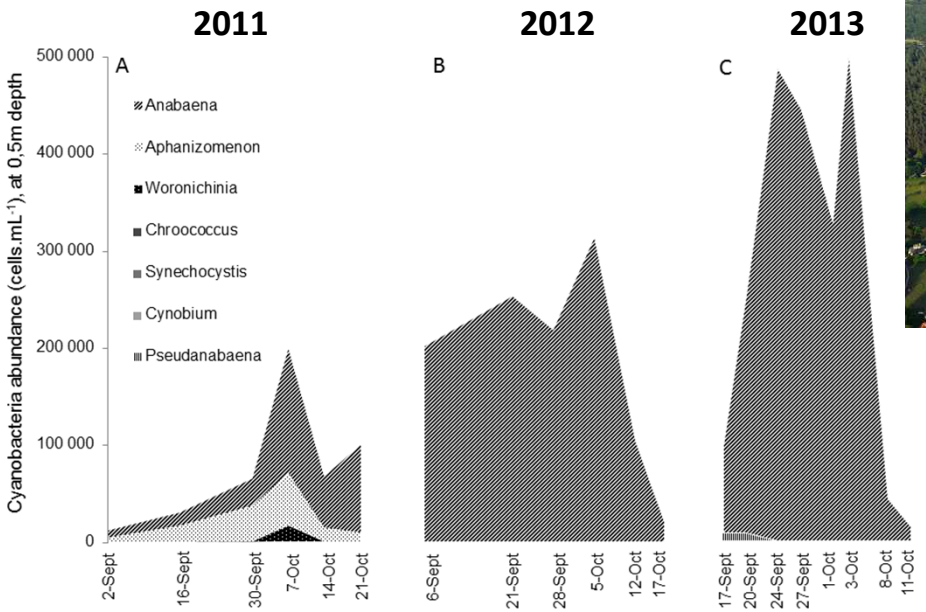
- A natural lake aged of 8000 years
- Hypereutrophic state
- *Dolichospermum (aka Anabaena) macrospora* blooms every year in Autumn

Sampling in the water column in September-October

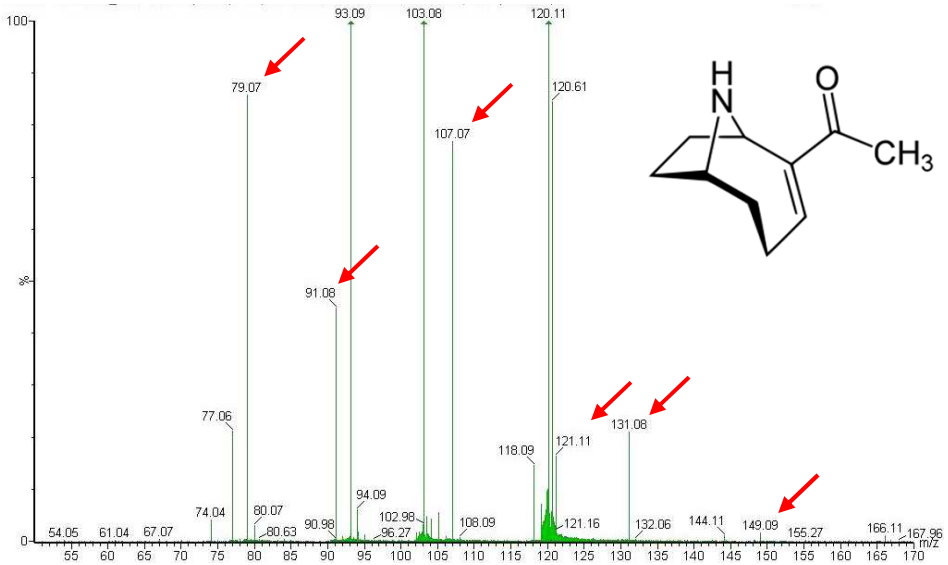


Sabart *et al.* Harmful algae 2015

A three-year survey in Lake Aydat



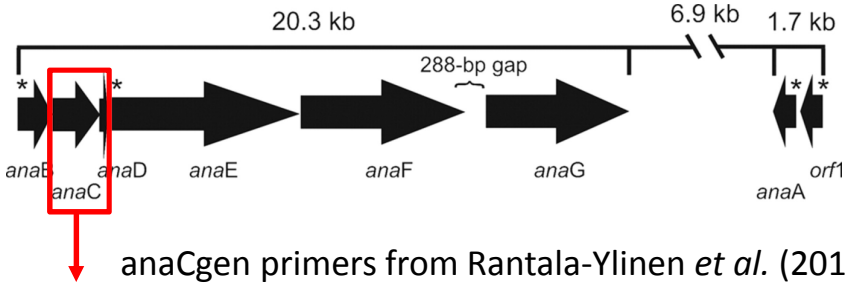
Detection of anatoxin-a by LC-MS-MS analyses



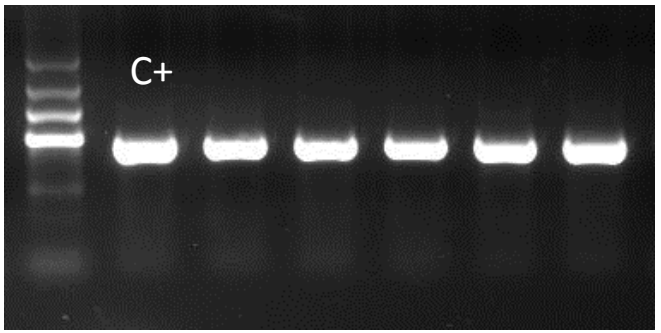
Mass spectra of the 166 m/z ion

Detection of *anaC* gene by PCR amplification

Anatoxin-a biosynthetic gene (*ana*) cluster of *Anabaena sp.* strain 37 (Rantala-Ylinen *et al.* 2011)



anaCgen primers from Rantala-Ylinen *et al.* (2011)



Sabart *et al.* Harmful algae 2015

Occurrence of *ana* genes at a larger scale

Several lakes investigated both in pelagic and benthic compartments

Sampling of the water column

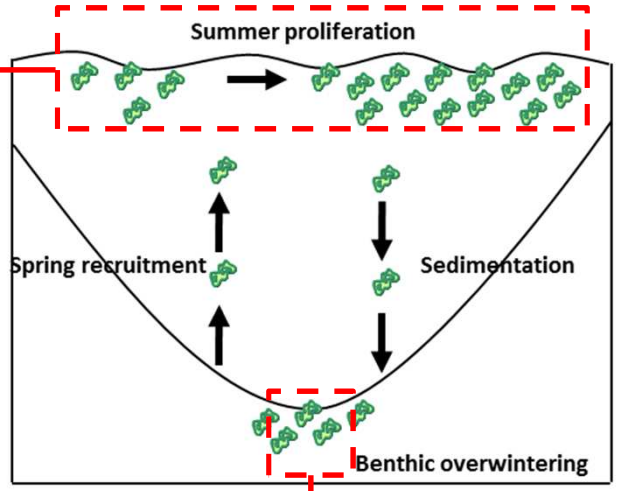
0-2 cm

2-4 cm

4-6 cm

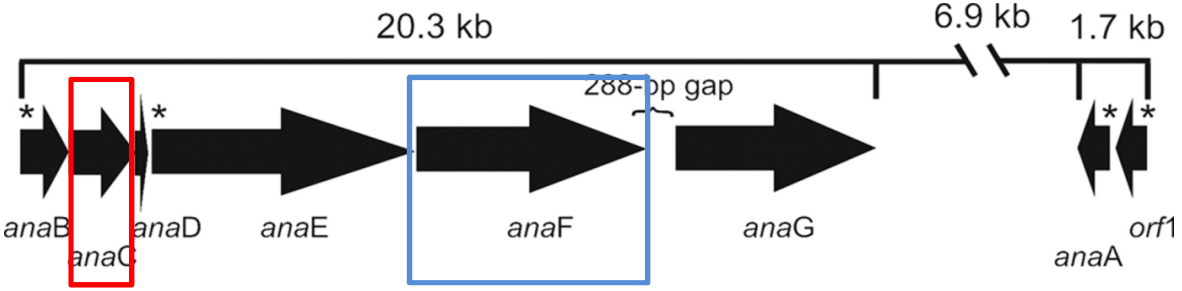
6-8 cm

8-10 cm



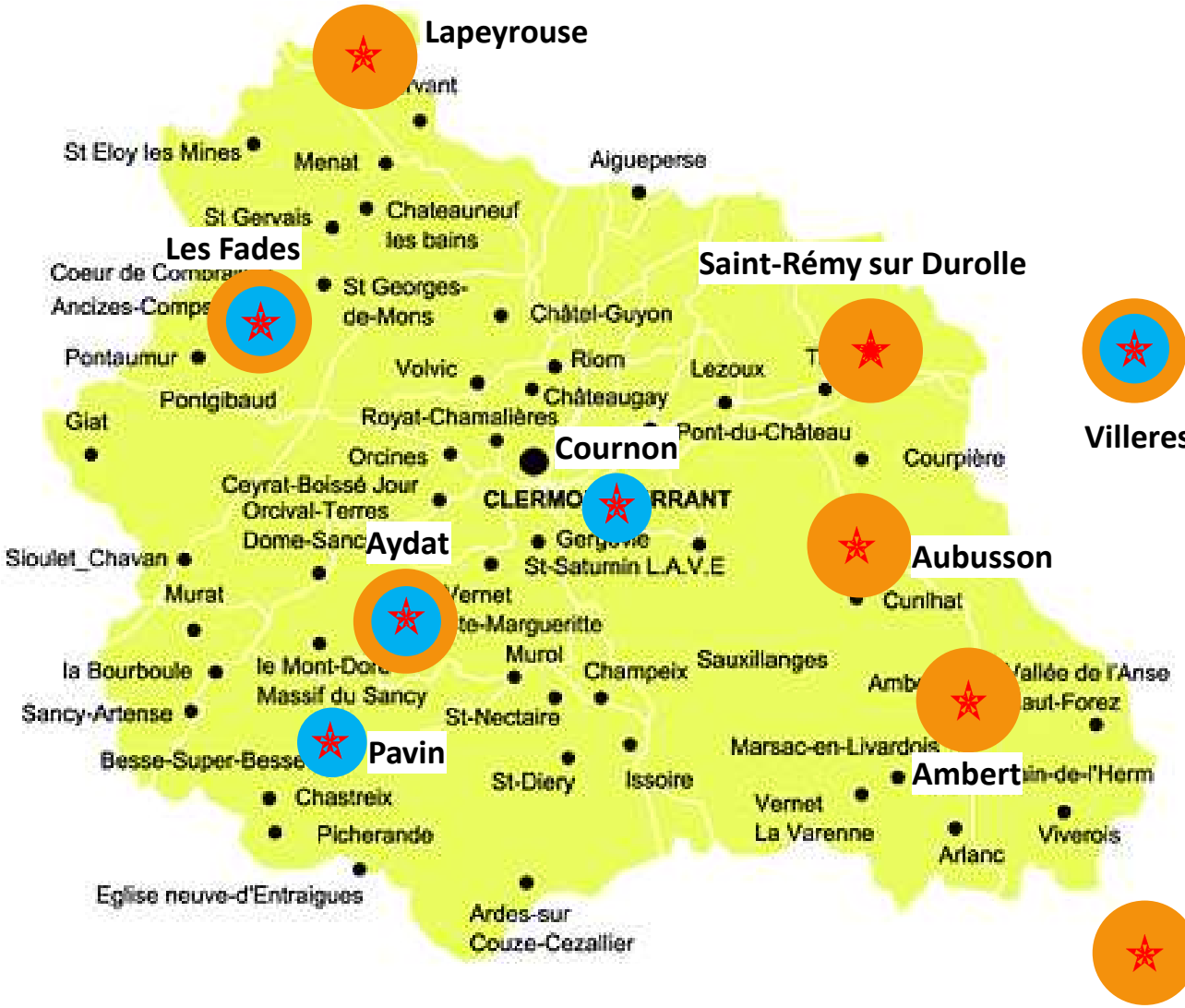
Surviving as vegetative cells or akinetes (Nostocales)

Sediment coring



Two target genes: *anaC* and *anaF*

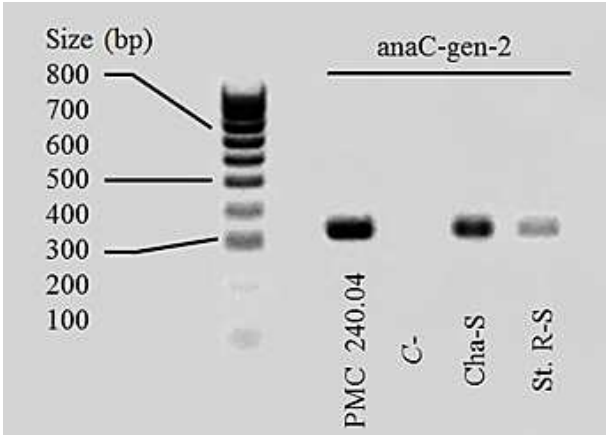
Occurrence of *ana* genes at a larger scale



***anaC* or *anaF* genes**

in the water column

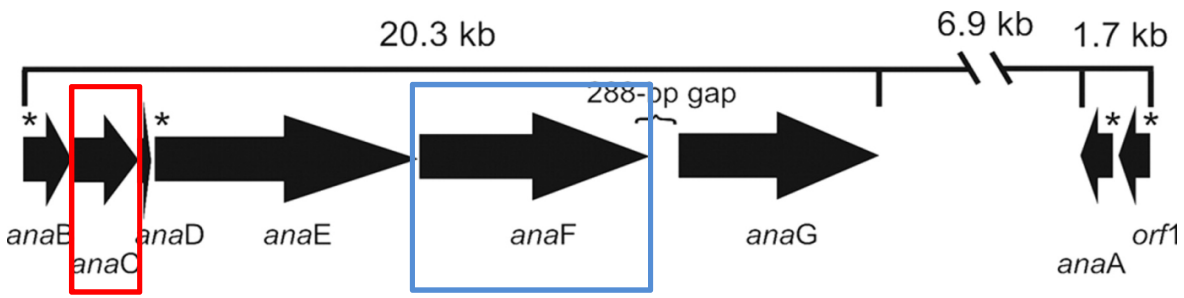
in the sediment



Widespread occurrence of *ana* genes among the studied lakes

Legrand *et al.*, Harmful algae 2016; Sabart *et al.* in preparation

A phylogenetic study of anatoxin-a genes at a larger scale



***anaC* gene**
366 bp

***anaF* gene**
461 bp

PCR amplification

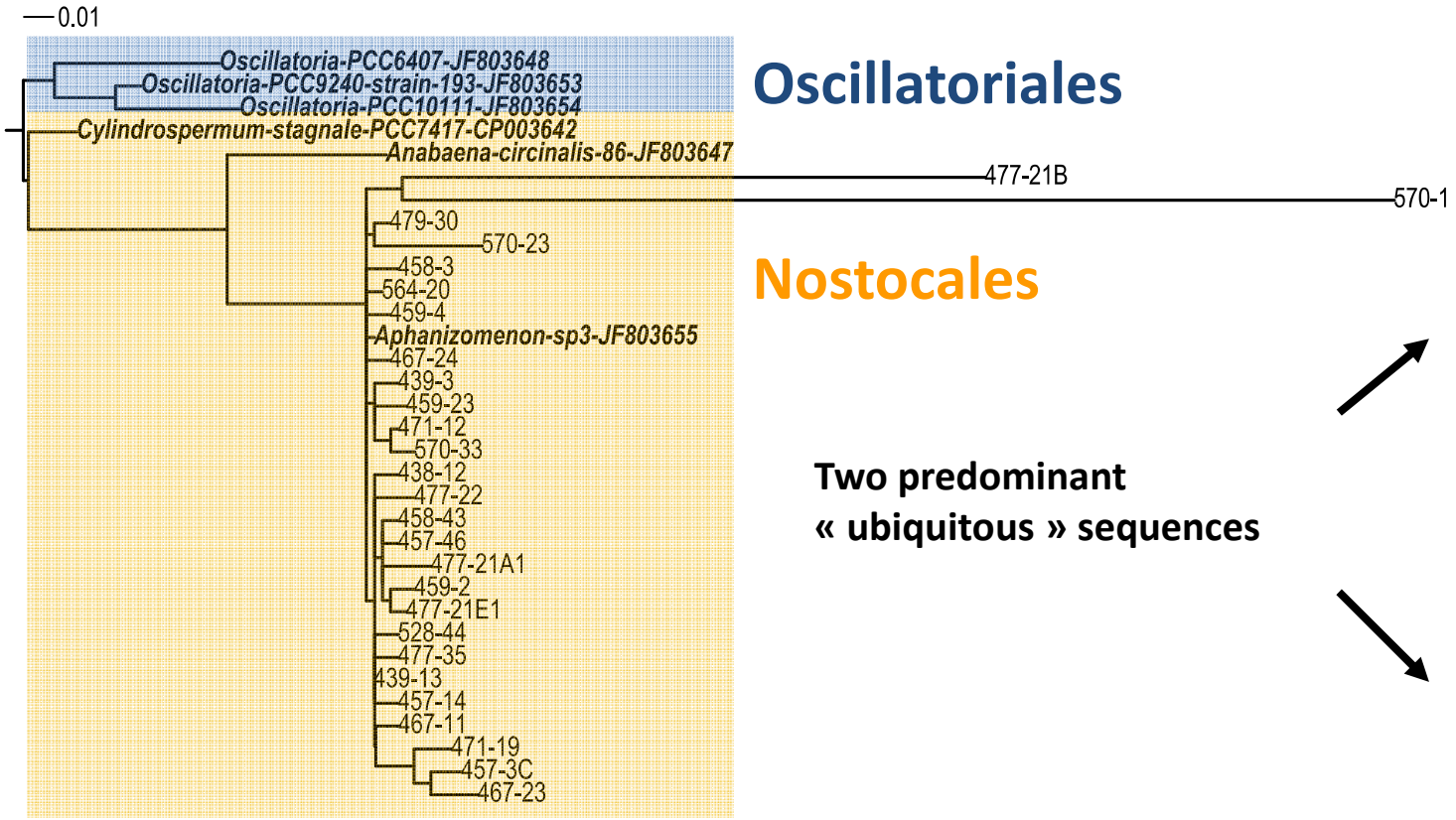
306	**136**	**clones sequenced**	**Cloning and sequencing**
136	**29**	**different sequences**	
134	**27**	**sequences unknown in Genbank**	

Aphanizomenon sp. 3 AnaC (anaC) gene, partial cds	671	671	100%	0.0	99%	JF803655.1
Anabaena circinalis 86 AnaC (anaC) gene, partial cds	483	483	100%	2e-132	90%	JF803647.1
Anabaena sp. 54 AnaC (anaC) gene, partial cds	483	483	100%	2e-132	90%	JF803646.1
Anabaena sp. 37 anatoxin-a synthetase gene cluster, complete sequence	483	483	100%	2e-132	90%	JF803645.1

High number of new sequences underlying the diversity of potentially producing strains

A phylogenetic study of anatoxin-a genes at a larger scale

Maximum likelyhood tree of *anaC* gene (98% identity)

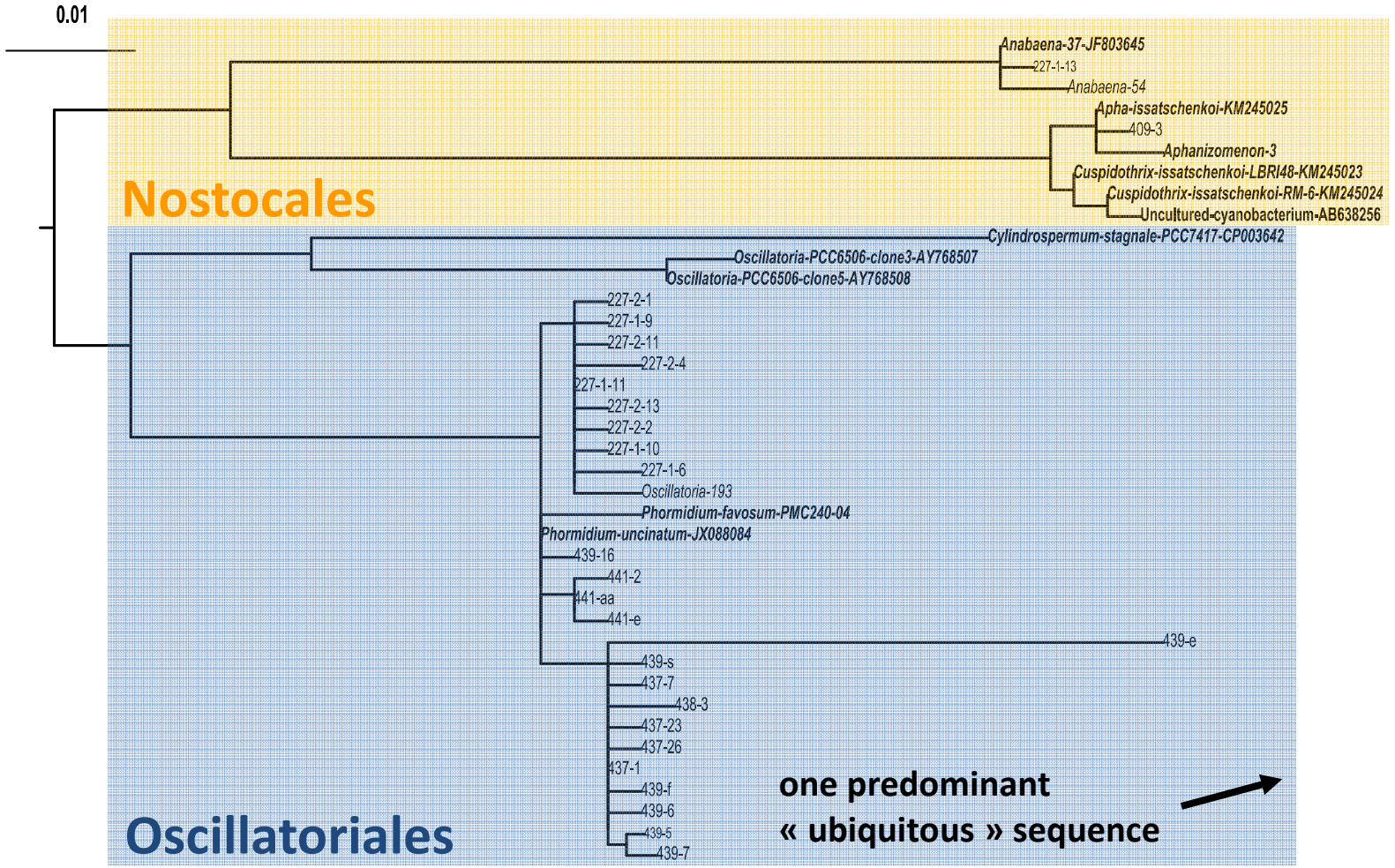


Two predominant « ubiquitous » sequences

⇨ ***anaC* sequences from our study are clustering with Nostocales**

A phylogenetic study of anatoxin-a genes at a larger scale

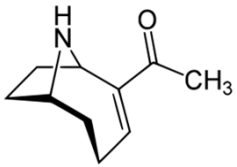
Maximum likelyhood tree of the *anaF* gene (100% identity)



⇨ *anaF* sequences from our study are clustering with Oscillatoriales

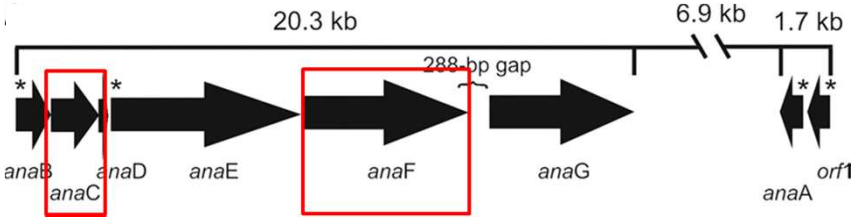
Conclusions

In lake Aydat:



- **First evidence of anatoxin-a in a freshwater lake in France**

At a larger scale:



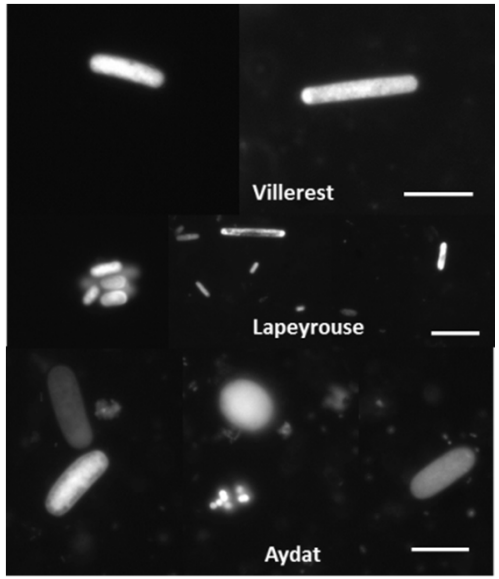
- ***ana* genes widespread among the lakes investigated in Auvergne**
- **Diversity of *ana* sequences**
- **Phylogenetic affiliation of *ana* genes to Nostocales and Oscillatoriales**

Perspectives

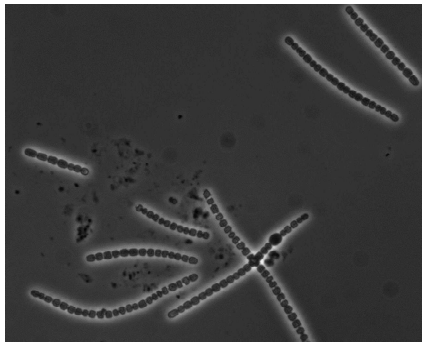
To improve our knowledge of anatoxin-a producers in freshwater lakes:

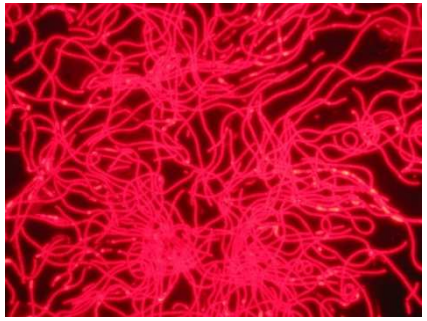
Isolation of anatoxin-a potentially producing strains

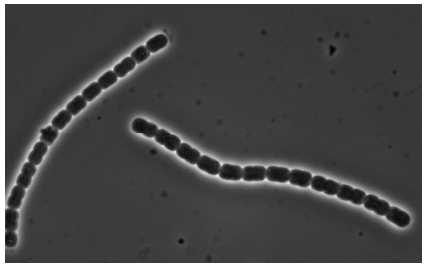
- Akinetes germination experiment
- Screening for the presence of *ana* genes
- Confirmation of anatoxin-a production by LC-MS-MS analyses

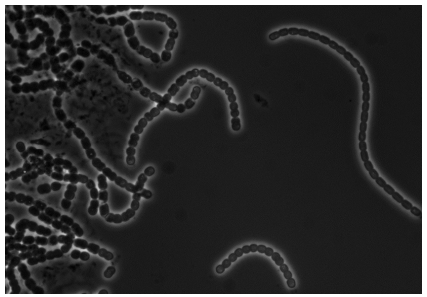


WORK IN PROGRESS…









Acknowledgments

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Thank you for your attention