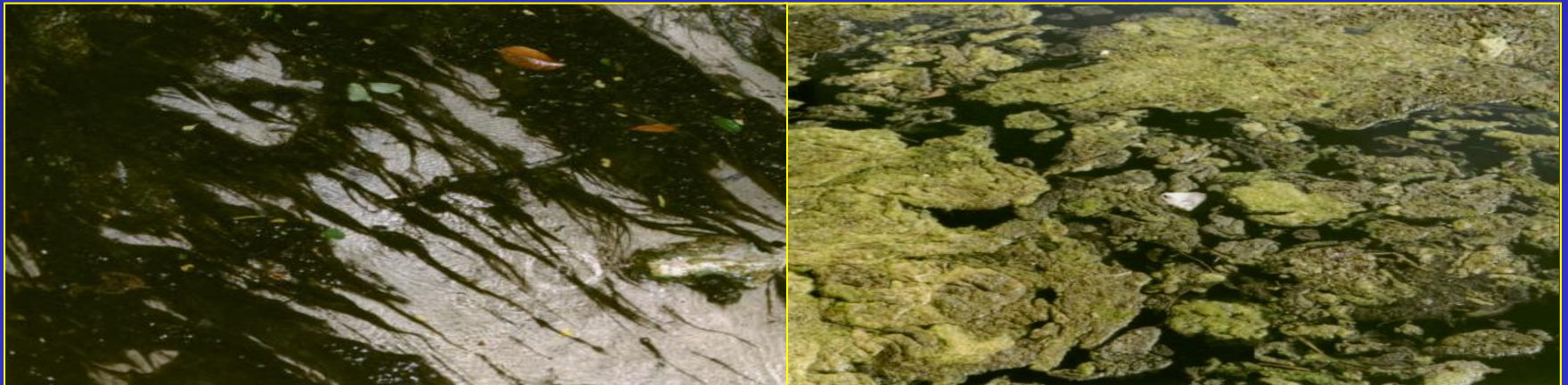


Lyngbya wollei, a Toxic
Bloom-forming
Cyanobacterium Present in
Florida Springs

Jennifer Joyner, Hans W. Paerl, Glen
Shaw, Nick Osborn and John Burns

Outline

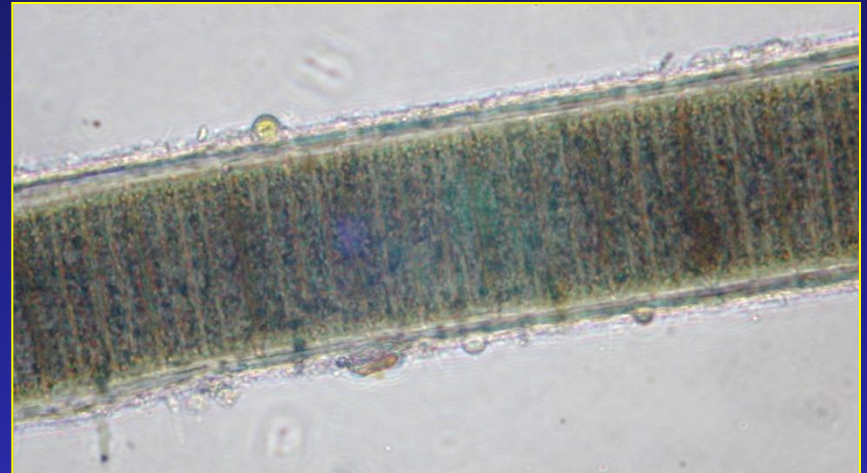
- What is *Lyngbya wollei*?
- What toxins are associated with *Lyngbya*?
- Research Questions
- Necessary research
 - Bioassays *in situ*
 - Molecular characterization of *Lyngbya wollei*
- Conclusions
- Future Research



Lyngbya wollei

- *L. wollei* is a freshwater benthic cyanobacterium
- found in lakes, rivers, springs, and water supply reservoirs throughout southeastern US
- capable of forming thick nuisance blooms
- “nitrogen fixer”
 - can convert N_2 to a biologically available form of N (NH_3)
- proliferates in eutrophying waters
 - a potentially useful bioindicator of eutrophication

Kings Bay, FL



City Lake, NC

Lyngbya

Bioactive Compounds

Dermatotoxic Alkaloids

- lyngbyatoxin-a
- aplysiatoxin
- debromoaplysiatoxin

Neurotoxins

- saxitoxins



Lyngbya

Human Health Aspects

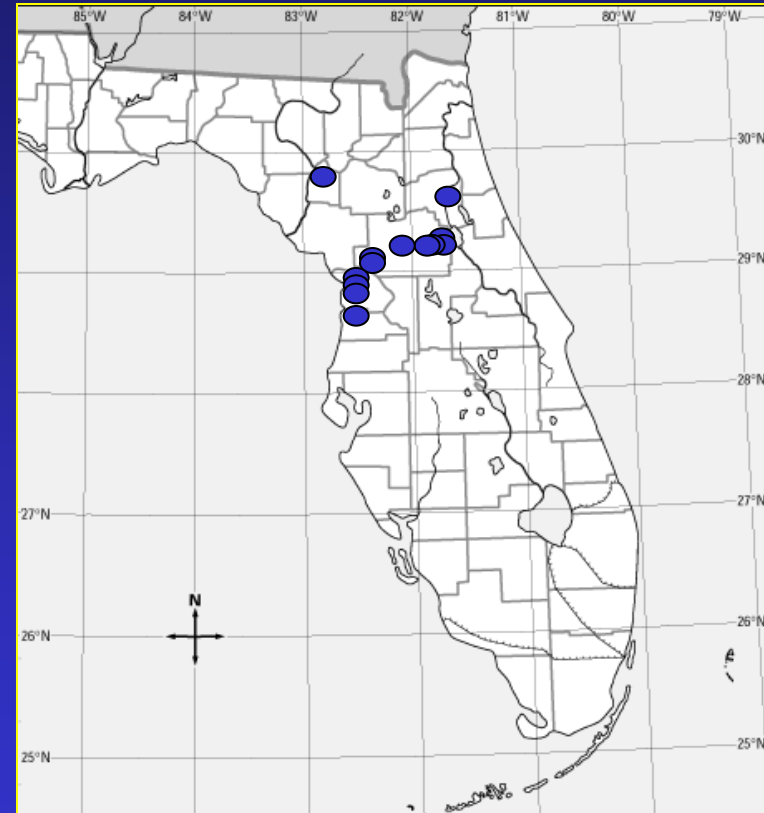
- Cutaneous inflammation with signs of erythema, blisters and desquamation within 12 hrs of exposure
- Severe oral and gastrointestinal inflammation
- Skin tumor promoters and protein kinase C activators



Rash associated with *Lyngbya majuscula* bloom in Australia

Florida Distribution of *Lyngbya*

Salt Spring	-
Silver Glen Spring	+
Juniper Spring Run	-
Juniper Spring	-
Fern Hammock Spring	+
Silver Spring	+
Crystal River/Kings Bay	+
Withlacoochee River	+
Rainbow Spring	+
Homosassa Spring Run	+
Chassahowitzka Spring Run	+
Weeki Wachee Spring Run	+
Ichetucknee Spring	+



Lyngbya

Florida Toxins

✓ **Debromoaplysiatoxin**

✓ **Lyngbyatoxin-a**

Others?

Courtesy: Glen Shaw & Nick Osborn / National Research Centre
for Environmental Toxicology, PO Box 594, Archerfield, Qld.,
4108, Australia.

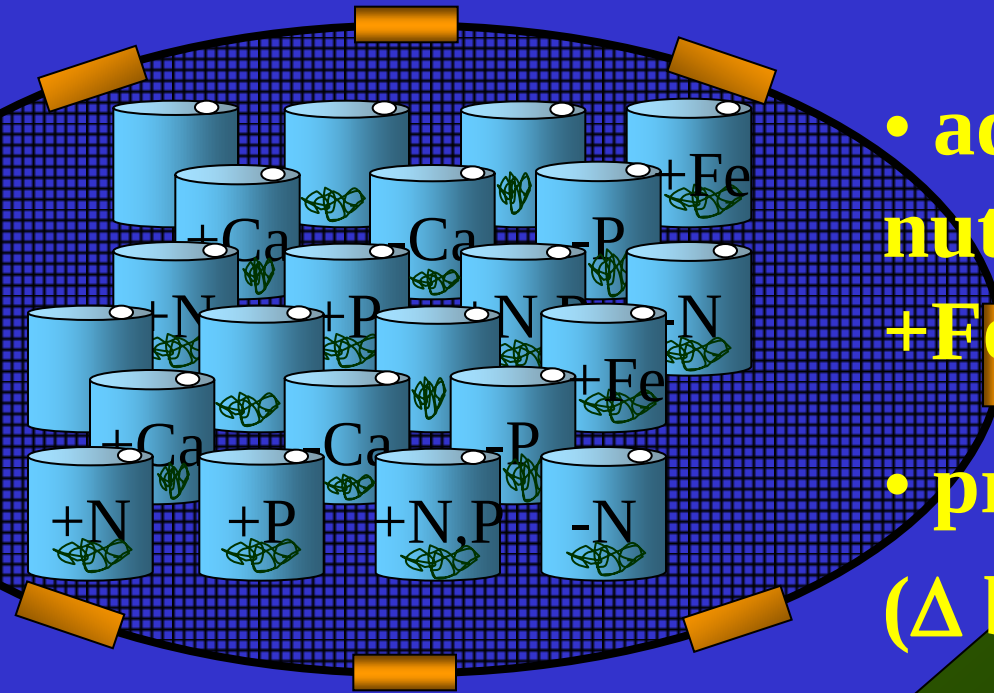
Research Questions

1. Is *Lyngbya wollei* a bioindicator of eutrophication?
2. Which nutrients strongly influence the growth of *Lyngbya wollei*?
3. Is there a way to quickly identify toxic strains of *Lyngbya wollei*?

Bioassays

- bioassays *in situ* indicate which nutrient limits growth in the organism's natural environment
- potential limiting nutrients are
 - Fe, P in *L. majuscula* (marine analog)
 - N? P? Ca^{2+} ? Fe? in *L. wollei*
- important for future management decisions and strategies

L. wollei bioassays *in situ*



- additions and dilutions of nutrients ($\pm$ N, $\pm$ P, $\pm$ NP, $\pm$ Ca, +Fe)
- primary productivity (Δ biomass, Δ chlorophyll *a*)
- acetylene reduction experiments to measure nitrogenase activity

Promoters of *Lyngbya* blooms

- Cowell and Botts (1994) found P limiting; Ca^{2+} promoted growth in *L. wollei* cultures
- Watkinson (2000) found P and Fe limiting in *L. majuscula* (in situ)
- Yin, Carmichael, and Evans (1997) found Ca^{2+} to promote *L. wollei* growth and toxicity in culture



Silver Glen Spring 1/22/02

The Bioassay Plan

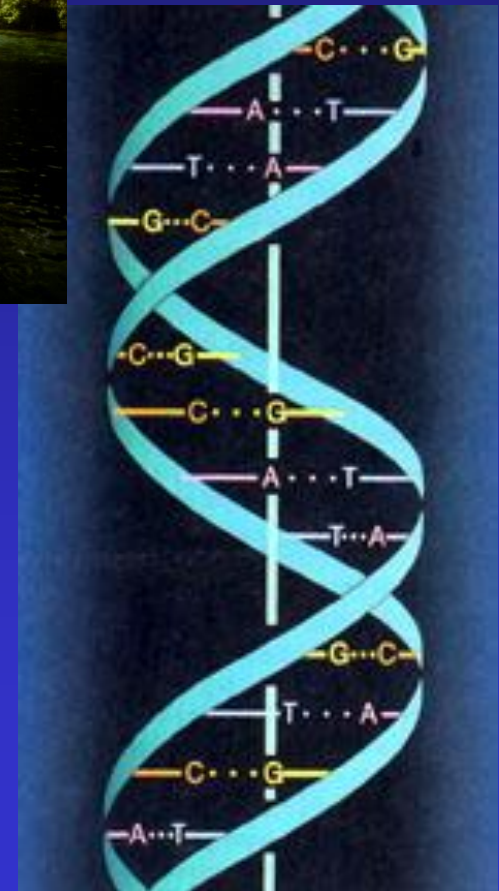
- ◆ perform bioassays in Silver Glen Spring as well as Salt Spring
- ◆ begin by adding N, P, Ca^{2+} , and Fe; dilute P, Ca^{2+}
- ◆ 5 repetitions/nutrient alteration + control group
- ◆ 5 day incubation
- ◆ toxicity analyses of each nutrient treatment

Research Questions

1. Is *Lyngbya wollei* a bioindicator of eutrophication?
2. Which nutrients strongly influence the growth of *Lyngbya wollei*?
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Purpose of Molecular Characterization

- to compare strains of *Lyngbya* to one another through use of the sequence of one or more genes
- *nifH* is a known gene with successful detection in *L. wollei* and other *Lyngbya* species (*L. lagerheimii*, *L. majuscula*, *L. aestuarii*)
- 16S rRNA gene is present in all bacteria



Why not use toxin genes to detect toxic species?

There are no known genes associated with toxins or toxin production in *Lyngbya*.

Molecular characterization of *Lyngbya* species

- ◆ extract DNA from *Lyngbya* blooms
- ◆ compare samples from multiple sites based on *nifH* and 16S gene (conserved genes involved in the nitrogen fixation process and ribosomes respectively)
- ◆ compare sequences to toxin analyses of strains



Correlation between toxin-producing forms of *L. wollei* and *nifH* and/or 16S sequence

- **detection of toxic forms of *Lyngbya wollei* possible within 2 weeks of sample collection**

The Molecular Plan

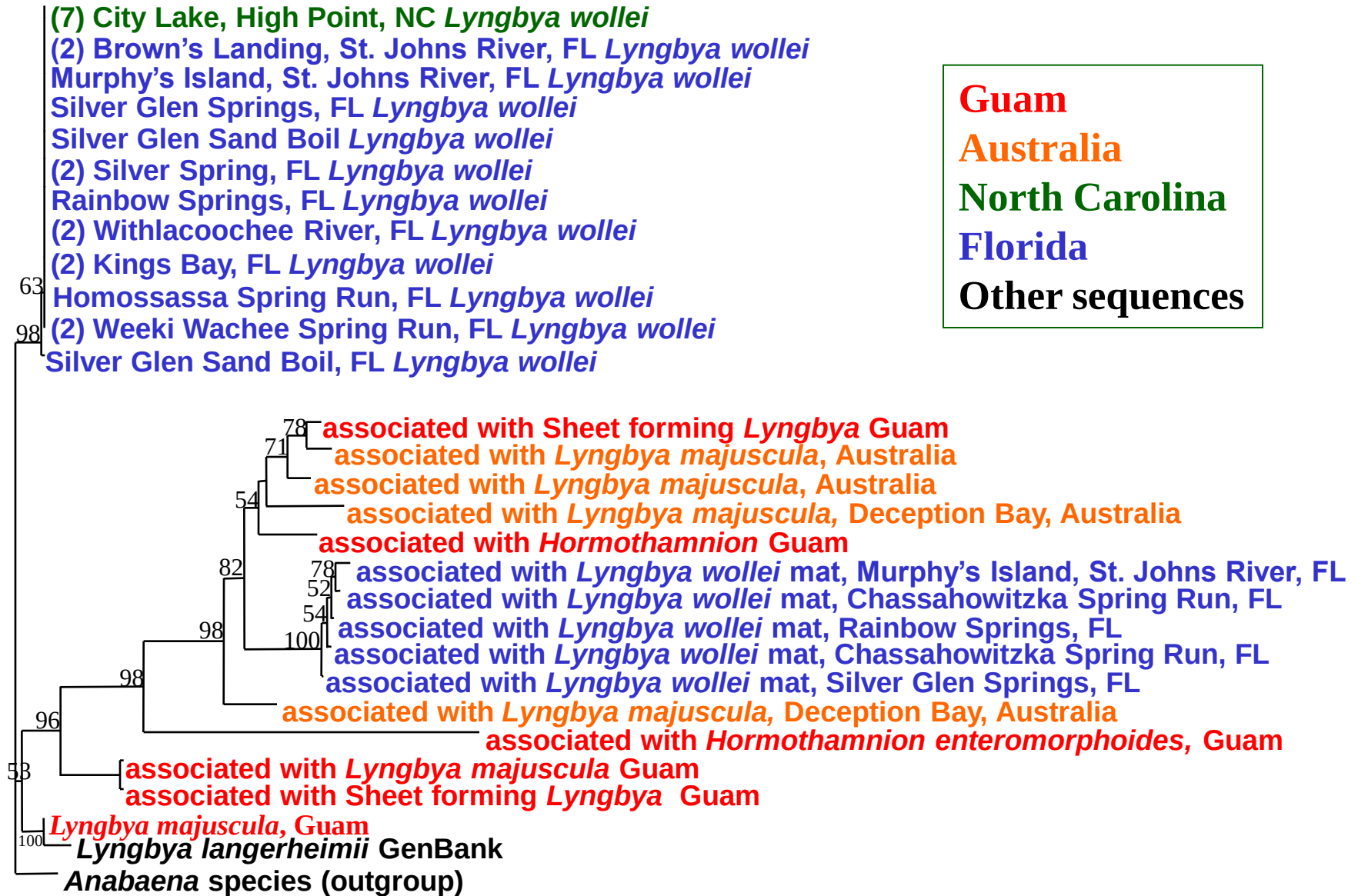
➤ **molecular analyses on samples twice a year**

➤ **toxin analyses on samples quarterly**

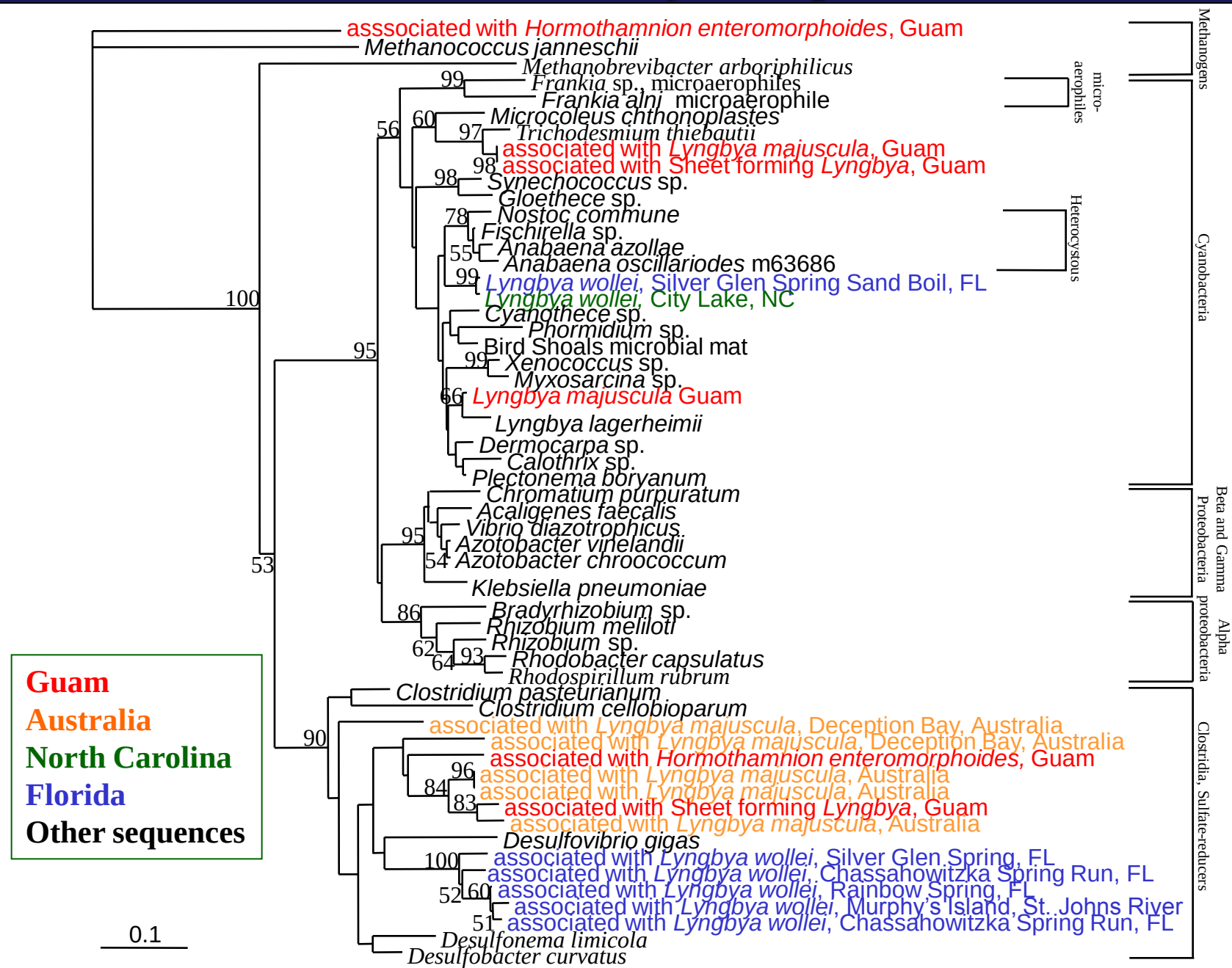
sample from 8 spring sites

- **Salt Spring**
- **Ichetucknee Springs**
- **Silver Glen Spring**
- **Silver Spring**
- **Rainbow Spring**
- **Crystal River Springs**
- **Homosassa Spring**
- **Weeki Wachee Spring**

nifH Sequences extracted from *Lyngbya* blooms



Diverse *nifH* sequences

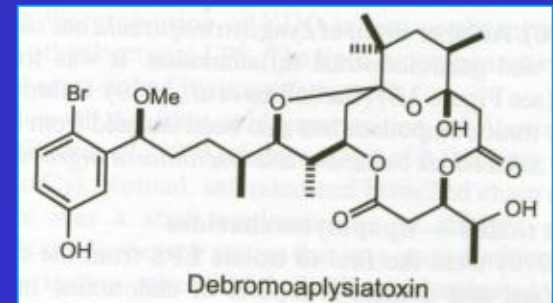
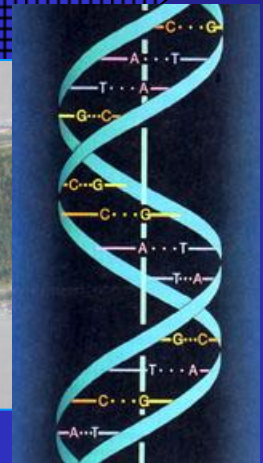
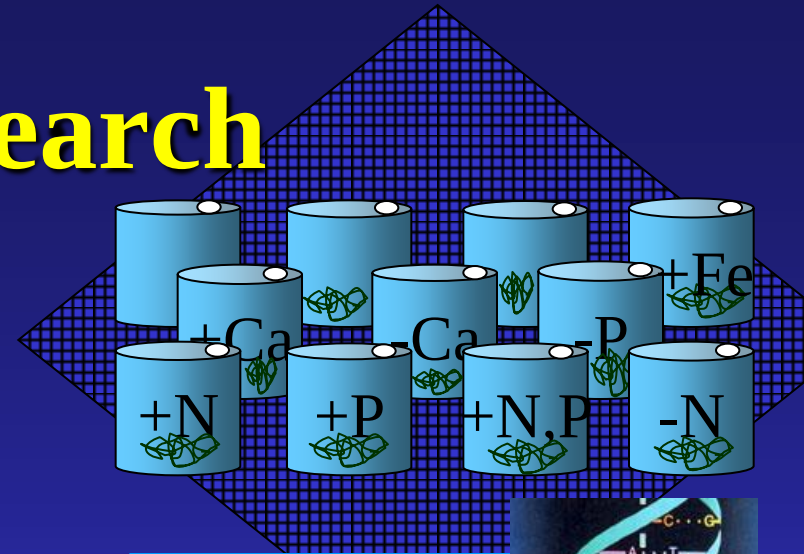


Conclusions

- *L. wollei* is present in Florida springs and contains toxins
- *In situ* bioassays of *L. wollei* are necessary for wise management practices
- Molecular characterization research will bring about quick detection of *L. wollei* and possibly of toxic strains

Future Research

- Bioassays in Florida springs
- Molecular characterization of *L. wollei* with *nifH* gene as well as 16S gene
- Compare analyses of toxins found in specific strains with the gene sequences



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Paerl lab