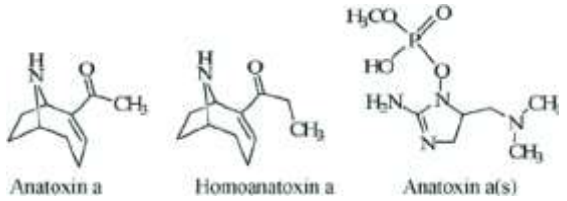
Integrative Characterization of the Anatoxin-A-Producing Benthic Cyanobacterial Genus *Microcoleus* in the Shenandoah River

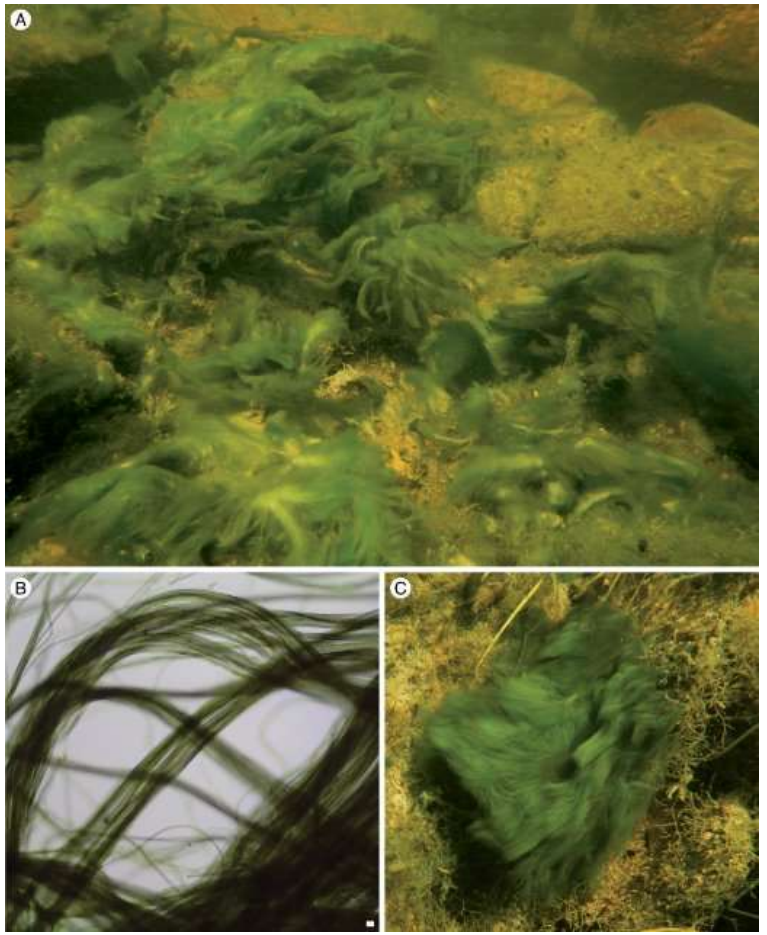
A. Bruce Cahoon

UVAWISE

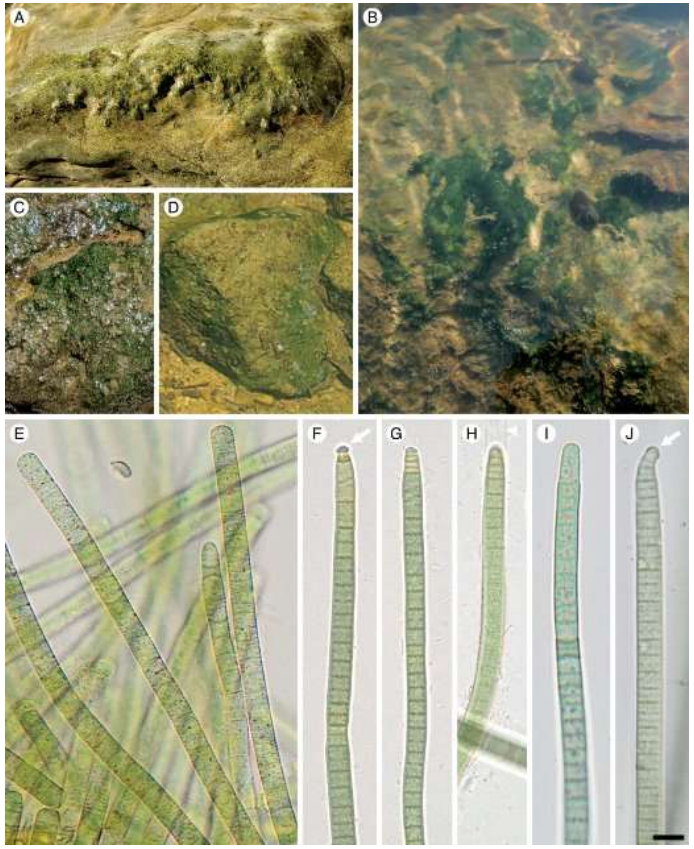
The Over-arching Question(s)

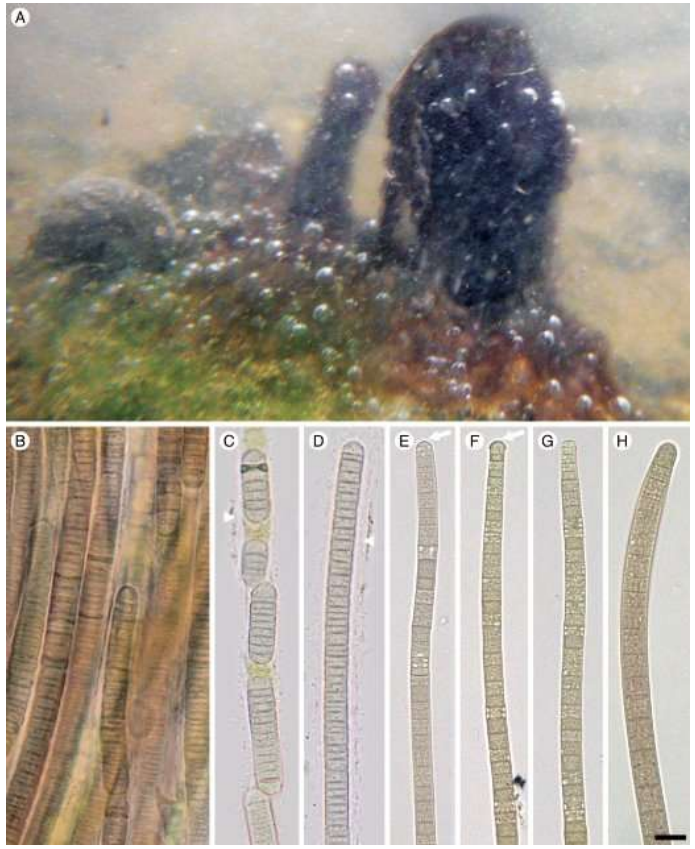
- What are the mat-forming cyanobacteria in the Shenandoah River?
  - Do they produce anatoxins?

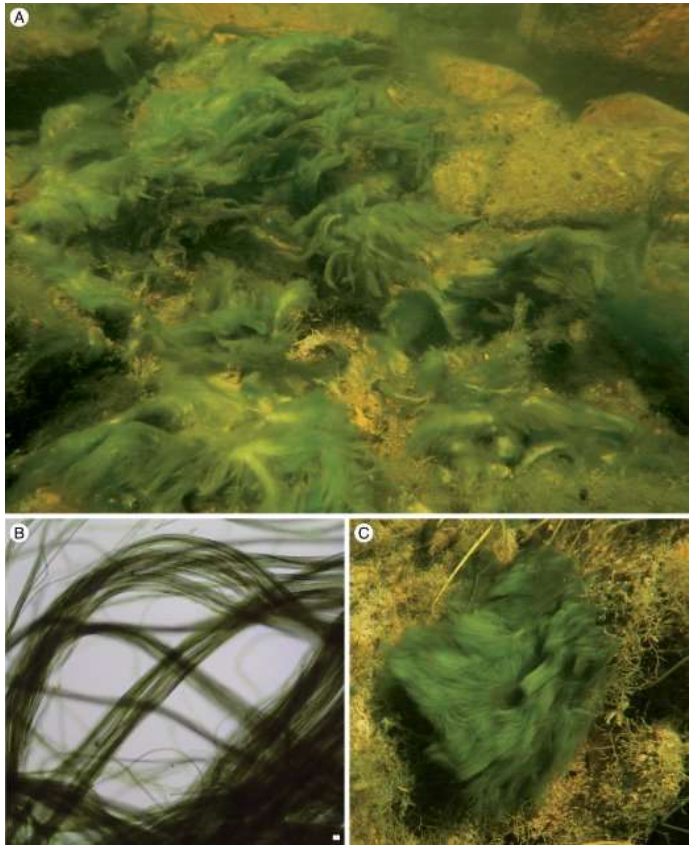




The Christova Lab (GMU) Has Identified Three Mat-Forming Cyanobacterial Morphotypes







Images from:
FIELD GUIDE TO COMMON MACROALGAE IN THE SHENANDOAH RIVER by
Rosalina Christova, Sydney Brown, Jacob Mormando, Katia Holguin, Gordon M. Selckmann –
for details see the poster session

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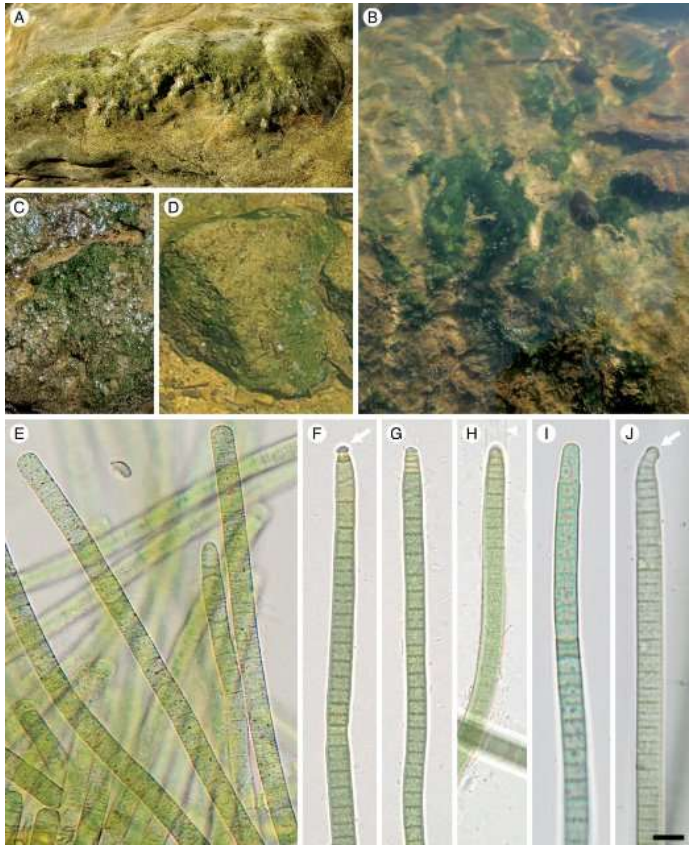

Sp. 1

Olive-green filaments attenuating at the tip, with short, not-constricted cell walls, apical cell with calyptra, single trichome in a sheath (cryptic - many possible species)

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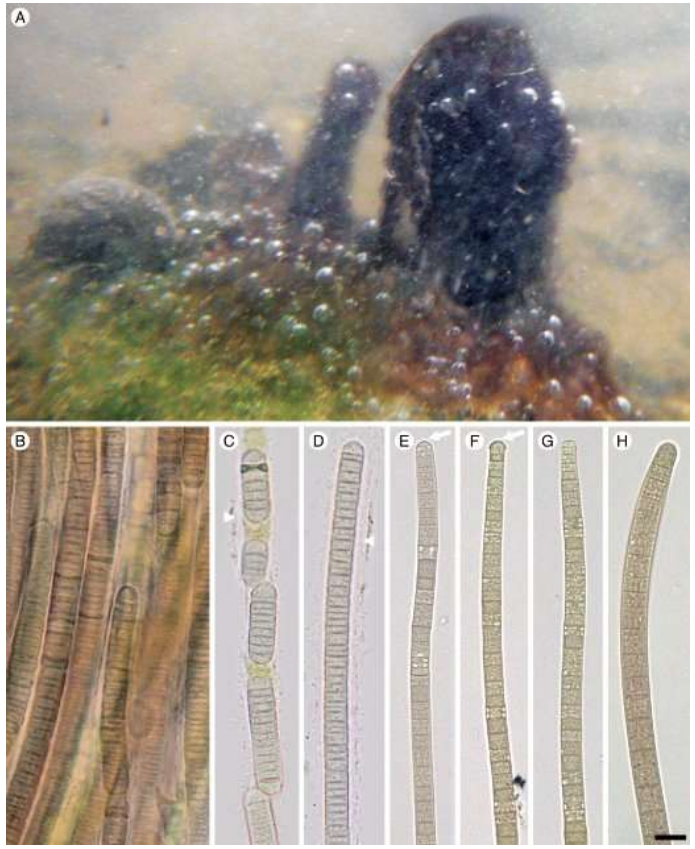
Sp. 2

Brownish filaments, slightly attenuating or not at the tip, with short, ± constricted cell walls, apical cell with calyptra, single trichome in a sheath (possibly *M. anatoxicus*)



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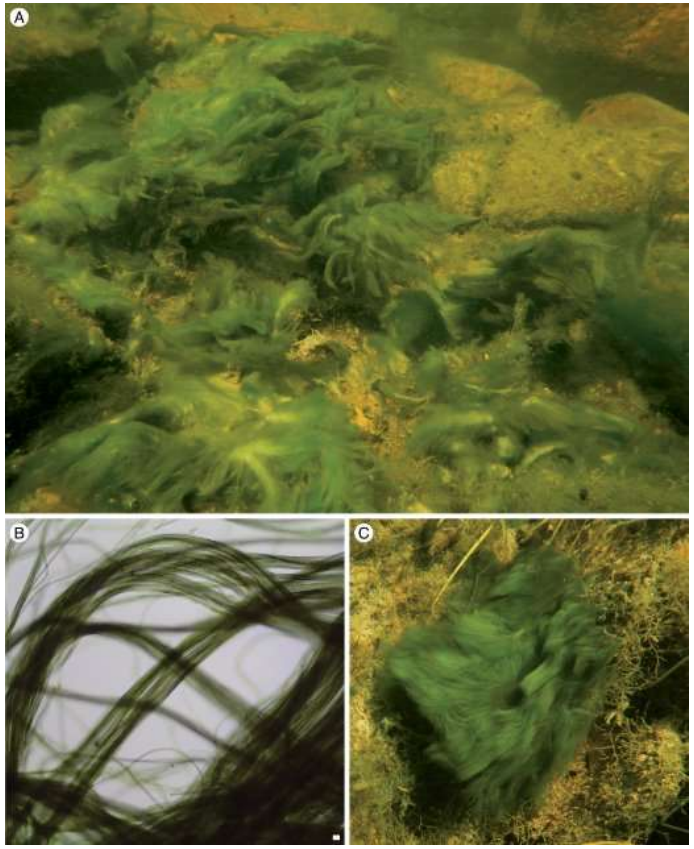
Sp. 3

Emarald-green filaments with long, constricted cells, apical cell without calyptra, multiple trichomes in a common sheath (cryptic, Microcoleus?)



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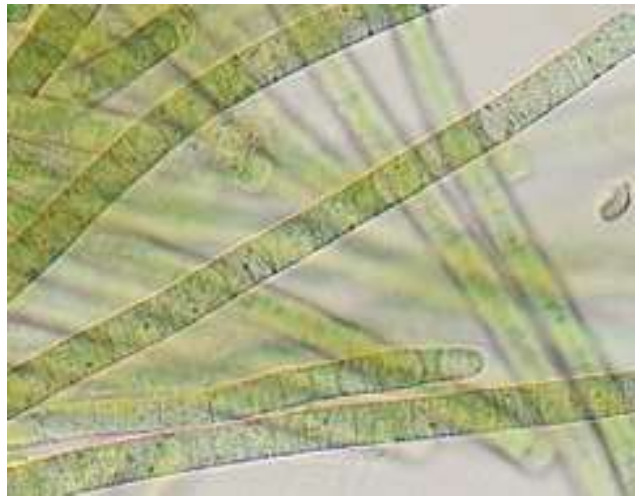
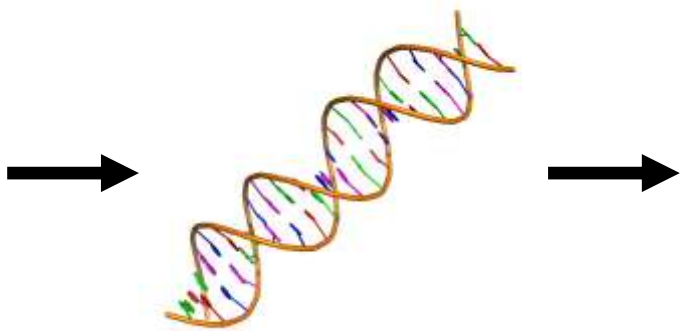
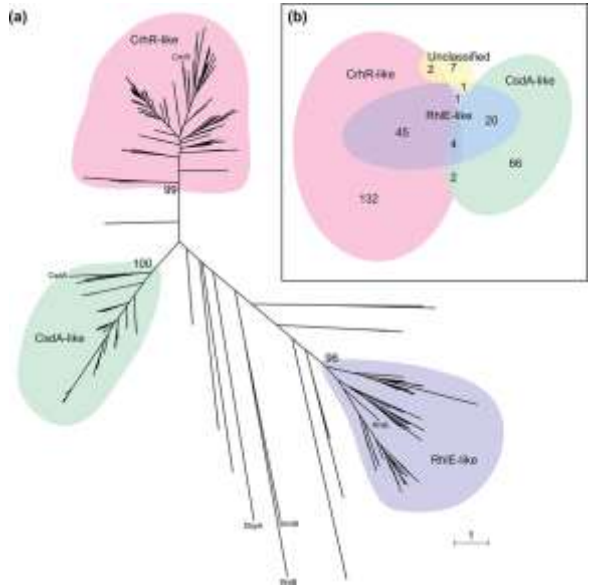
The Purpose of the Collaboration

- It is impossible to definitively identify most cyanobacteria with morphological analysis alone as there are many cryptic morphotypes.
- Molecular genetic analyses was needed to identify the mat forming cyanobacterial samples collected from the Shenandoah River.







Technical Challenges

- Is it possible to extract enough high-quality DNA from an environmental sample to identify a specific cyanobacterium of interest?
- Environmental Samples are often a mixture of morphotypes.
- These cyanobacteria have thick cell walls and mucopolysaccharide sheaths which creates an extra challenge for DNA extraction.

Images from: FIELD GUIDE TO COMMON MACROALGAE IN THE SHENANDOAH RIVER

Attempt 1

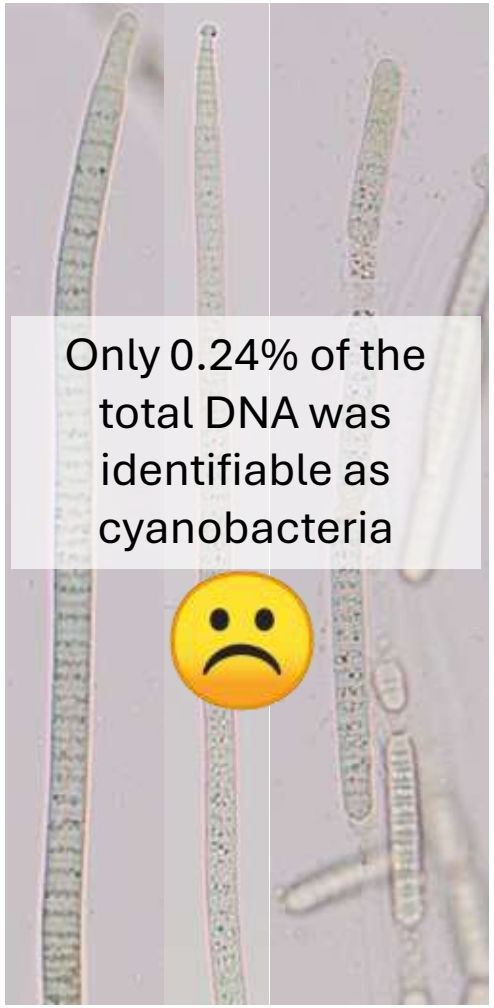
Samples for DNA extraction sent to Dr. Gillevet Lab on 8/28/2024

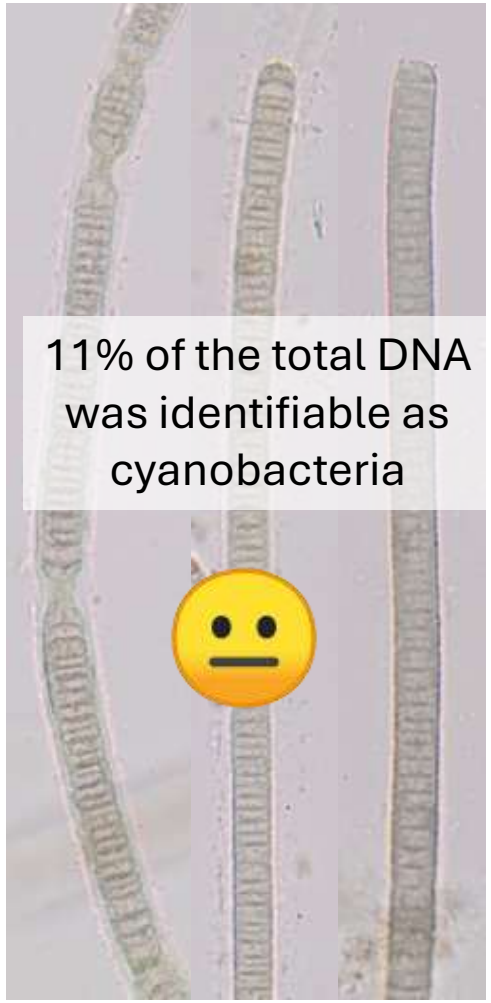
Sp. 1

Non-toxic strains (B3.4)
LCMS/MS: ATX not detected

Shenandoah South Fork

SF1, Alma, (38.588729, -78.566347)
collected on 09.20.2023





Sp. 3

Toxic strain (B1.2)
LCMS/MS: ATX and dhATX detected

Shenandoah North Fork

(NF10, Red Banks, (38.7823259, -78.60042925)
collected on 08.08.2023

Attempt 2

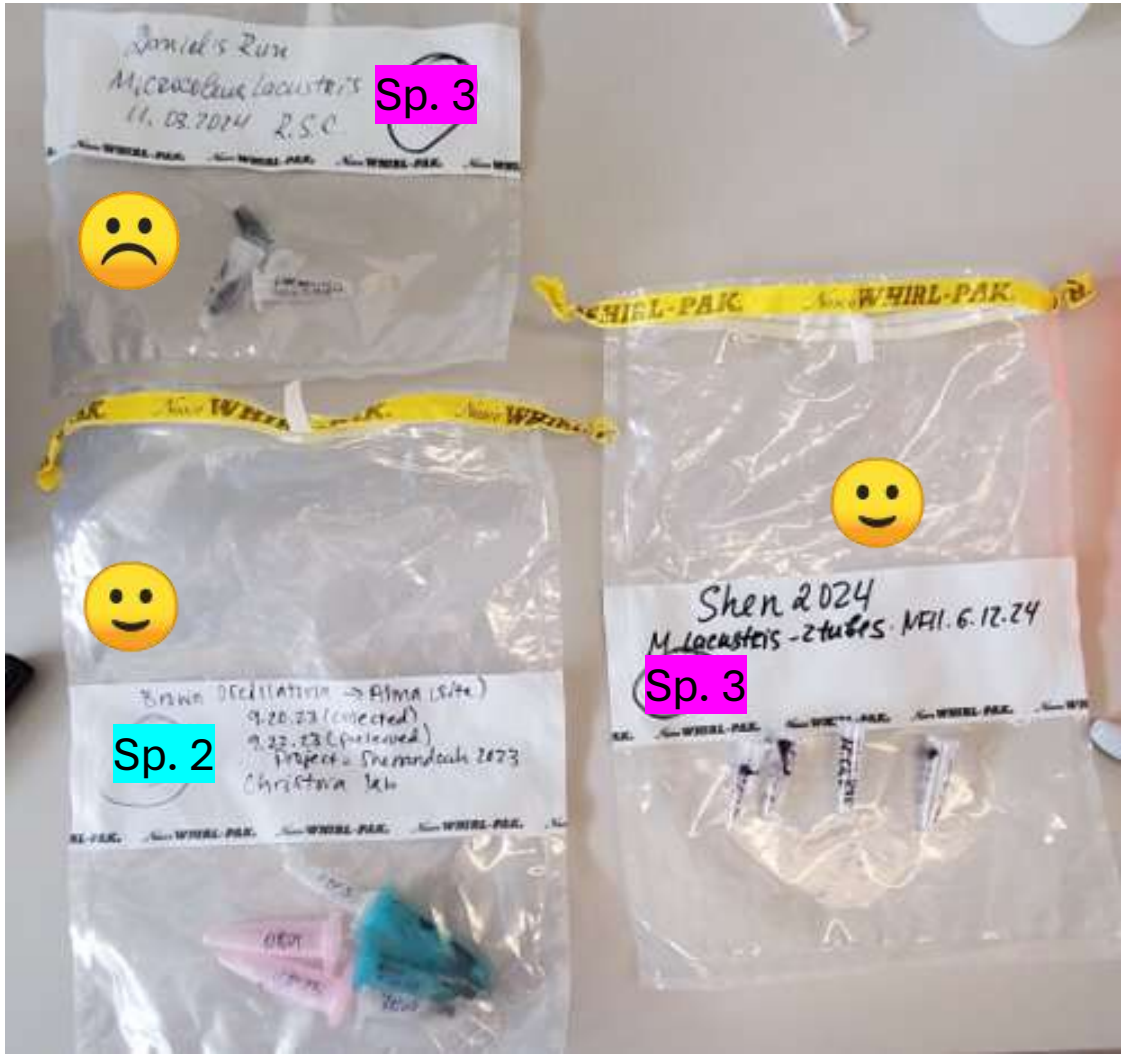
Bruce Cahoon visited the Christova lab and attempted to extract DNA from frozen samples

Sp. 3

High enough yield from initial extraction for genomic sequencing but genomic sequencing failed

Sp. 2

Low yield
Genomic DNA amplified using REPLI-G (Qiagen)



Sp. 3

Low yield

Genomic DNA amplified using REPLI-G (Qiagen)

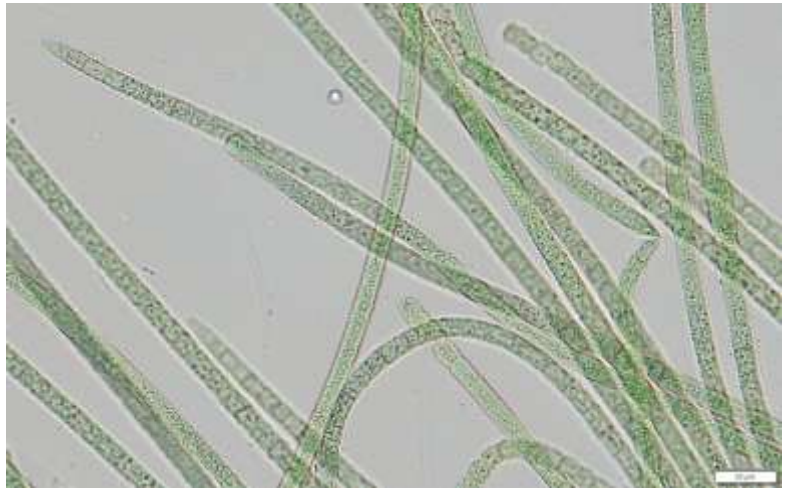
Attempt 3

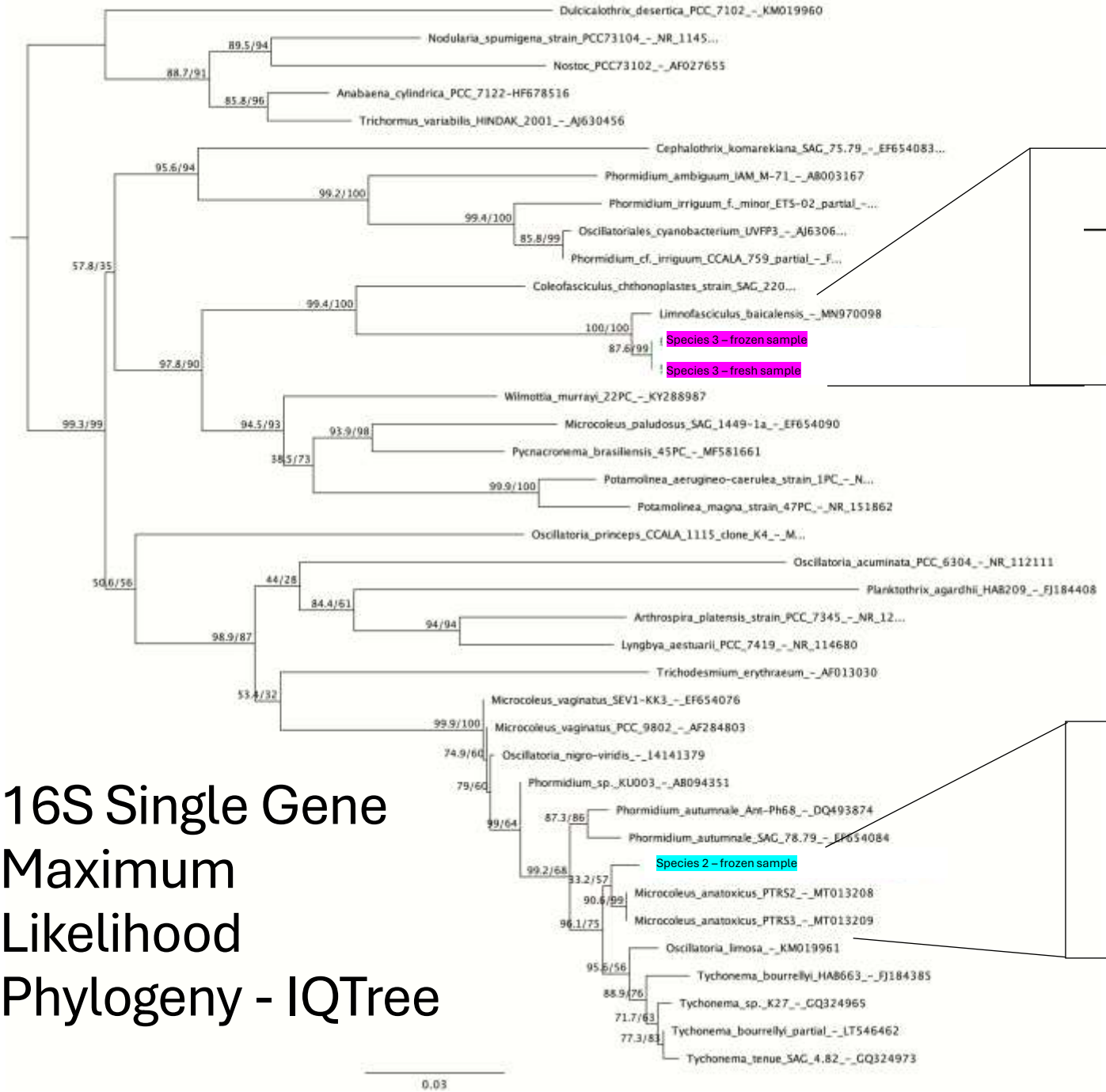
Fresh, field collected samples were overnighted to the Cahoon Lab

Sp. 3

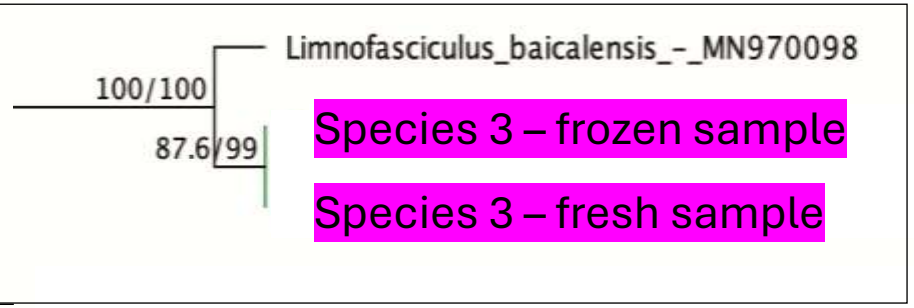
Coordinates for the location of the mats are South Fork Shenandoah River, near Front Royal, 38.86766394471952, -78.28067848600591 38.86683813351128, -78.26833895163502 Collected by Jake Mormando on 08/13/2025

High Yield, Good Purity, 98% single Cyanobacterium 🙂

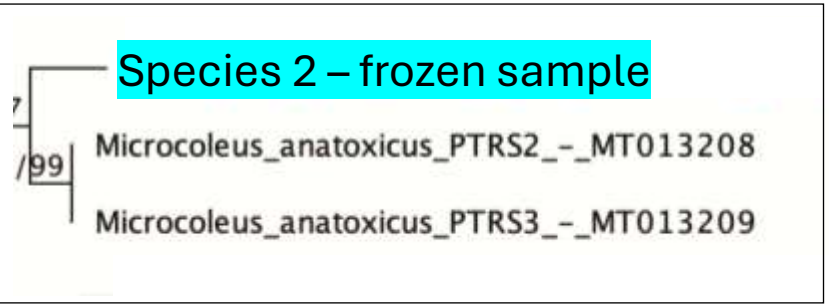




16S Single Gene Maximum Likelihood Phylogeny - IQTree



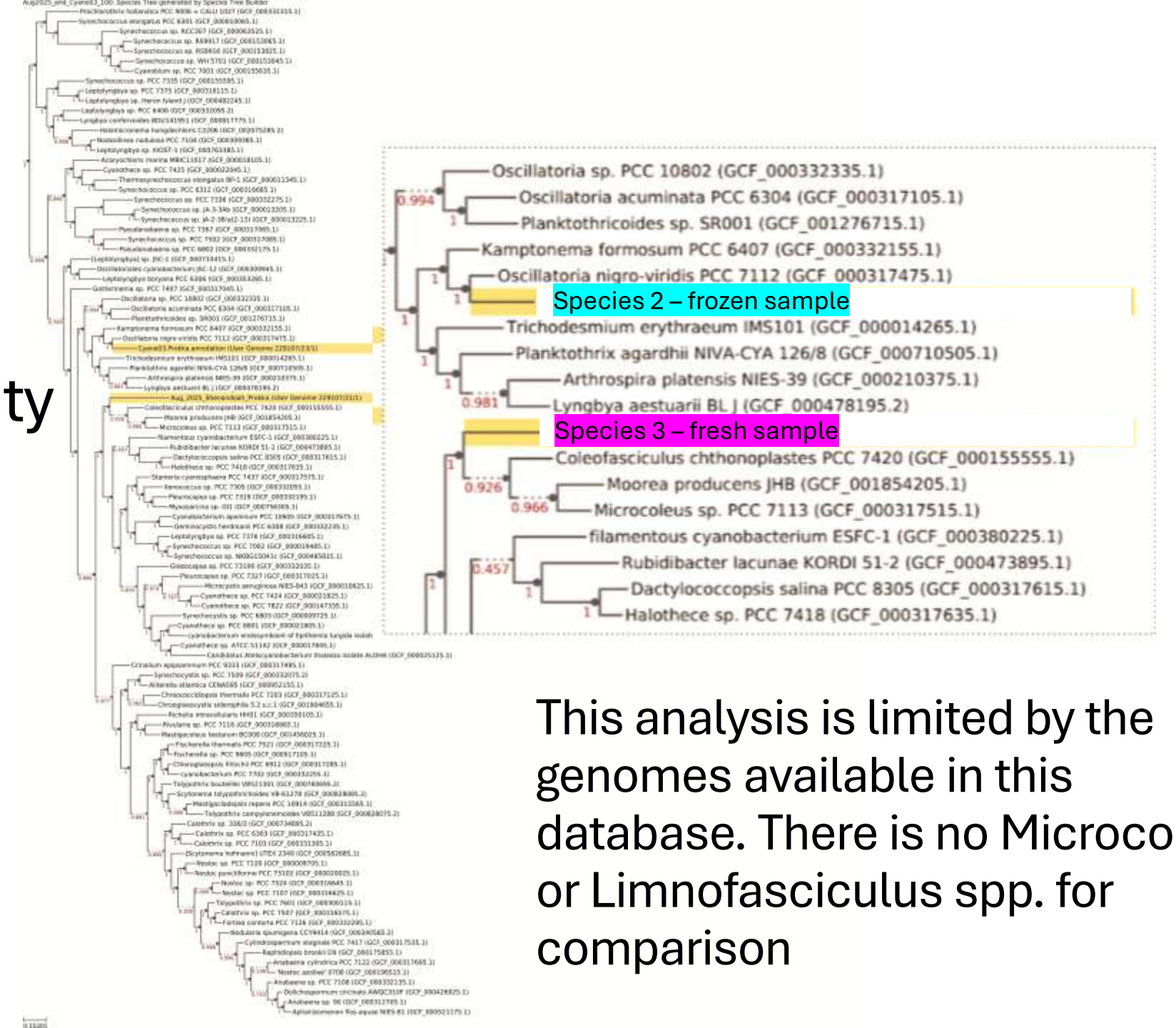
PRELIMINARY ID



Multi-gene phylogenetic Analysis is required for the greatest certainty

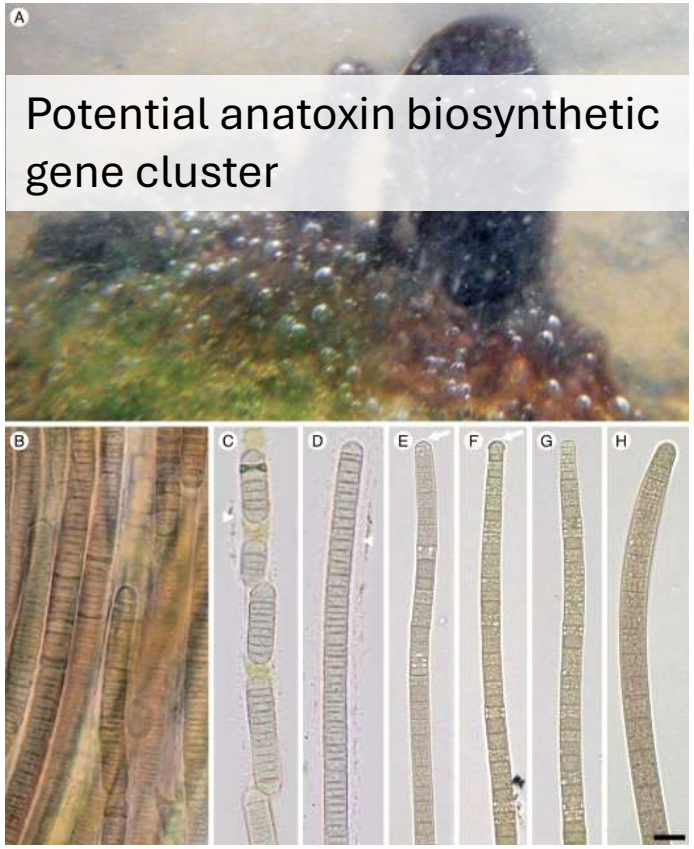
49 gene phylogeny

Kbase InsertGenome application



This analysis is limited by the genomes available in this database. There is no Microcoleus or Limnofasciculus spp. for comparison

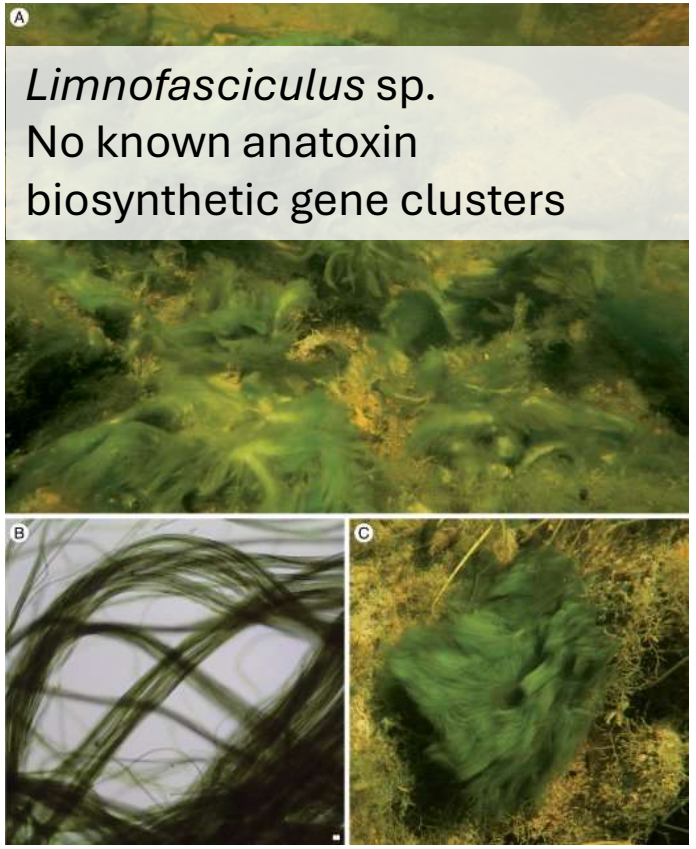
With Phylogenetic Analyses + GTDB-tk Taxonomic Classification



Sp. 2

Microcoleaceae family

Most likely *Microcoleus* sp.

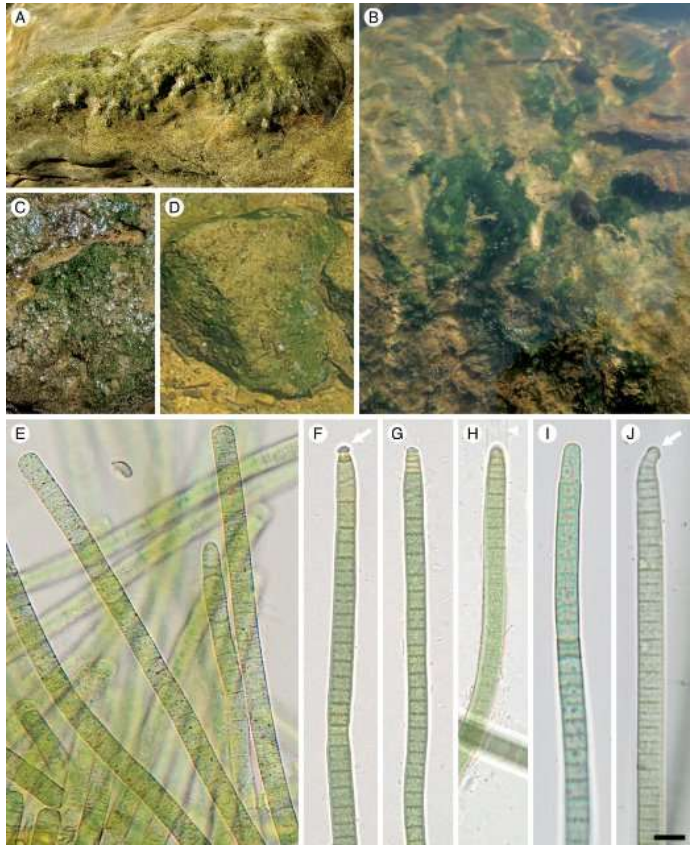


Sp. 3

Limnofasciculus sp. NOV - a recently described genus from Lake Baikal (Sorokovikova et al. 2023) and detected in Nova Scotia (Valadez-Cano et al.)

Conclusions

- When extracting DNA from environmental cyanobacterial mats, fresher is better.
- We have preliminary IDs from one, possibly two morphotypes.
- Both may be undescribed species.
- Sp. 1 is still unknown



Sp. 1
Needs more attention

The Team

Fearless Leader

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4-VA
Advancing The Commonwealth