



**Flow cytometric mapping and  
isolation of freshwater  
autotrophic picoplankton.  
(FWF P14238)**

**Nicholas Crosbie (in collaboration with  
Thomas Weisse). Institute for Limnology,  
Austrian Academy of Sciences.**

## Aquatic flow cytometry: crude literature search using Current Contents, 1993 to present day.

(search focussed on autotrophic picoplankton)

- flow cytometr\$ and (picocyano\$ or picoprok\$ or picoplankt\$ or picoeuk\$ or Synechoc\$ or Prochlor\$ or coccoid cyanobacteri\$ or chroococcoid cyanobacteri\$) +
- ocean\$ or sea\$ or coastal or marine
- lake\$ or river\$ or freshwater\$ or limnolog\$ or limnetic or inland water\$ or reservoir\$

Oceanography = 151

Limnology = 16

Why?



## Flow cytometry.....

First applied to aquatic samples by oceanographers.

Is required to count *Prochlorococcus* cells....has been/is a fundamental technology for studies involving these important organisms.

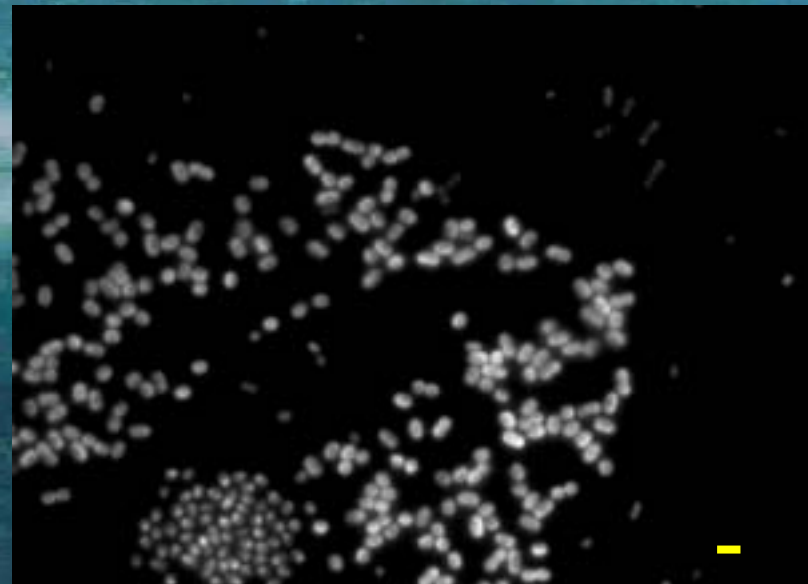
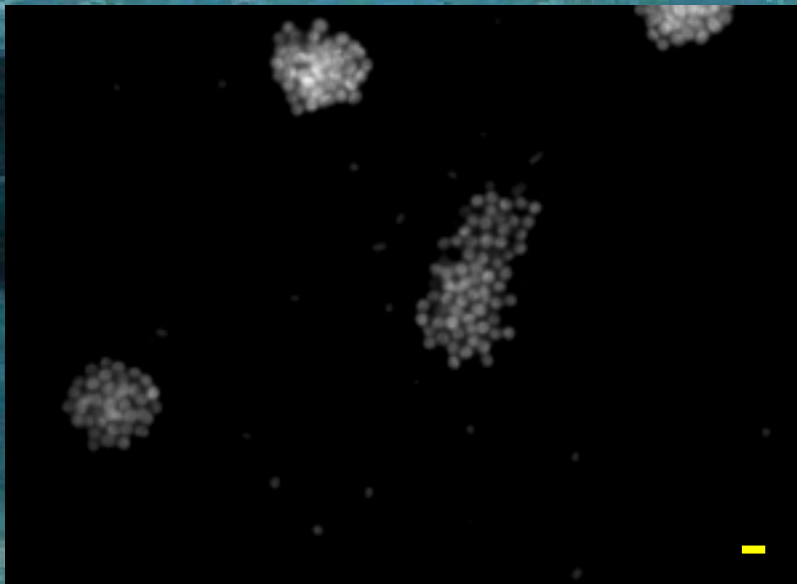
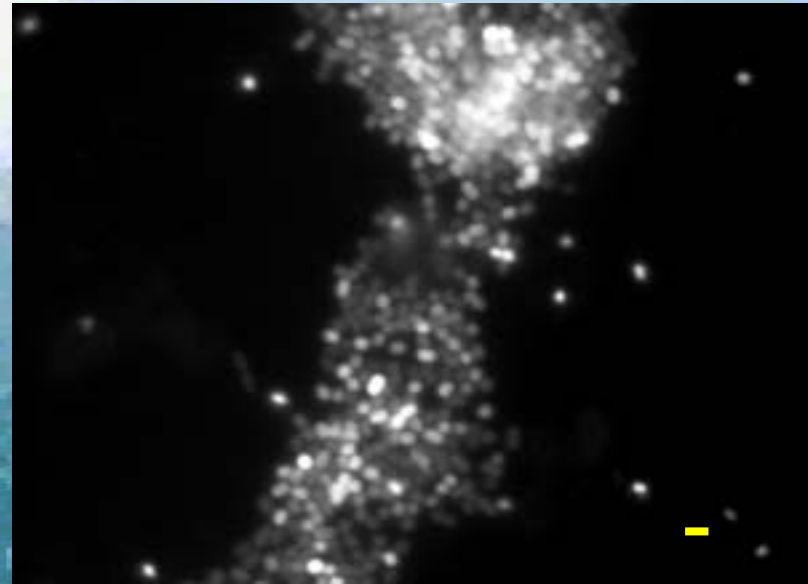
There's more money available to oceanographers?

Freshwater APP "signatures" are more complex.....



Mondsee 40m, September  
11th 2001.

colony size =  $90 \times 75 \mu\text{m}$



# Salzkammergut lakes district

Irrsee, 32 m,  
meso-eut.

Traunsee  
191 m,  
oligo.

Attersee, 169 m,  
ultra oligo.

Mondsee, 68 m,  
oligo-meso.

Hallstättersee, 125 m,  
oligo-meso

20 km  
Salzburg

20 km

10 km







# **Freshwater autotrophic picoplankton (APP) and flow cytometry.....**

**The identity of APP populations counted by flow cytometry was verified by.....**



## **1. natural samples:**

**A. microscope counts of APP**

**B. sorting of APP followed by microscopy and flow cytometry (whole and size-fractionated samples).**

## **2. flow-cytometric „signatures“ of:**

**Salzkammergut isolates (EAP, PC-rich Pcy, PE-rich Pcy, PC- and PE-rich microcolonies) obtained by single-cell/colony sorting.**



**FACS Vantage SE**


**Samples taken  
from Lake  
Mondsee: Spring,  
Summer and  
Autumn 2001.**

**Sorted 1,000 to  
100,000 cells or  
colonies per  
population per  
depth.**



# "Attractors" set for freshwater APP

 YG bead doublets/triplets

 YG beads

 YG bead junk

 Not Pcy

 High FL2-A

 High FL2, FL3

 Microcolonies

 EAP-A

 EAP-B

 Pcy-A

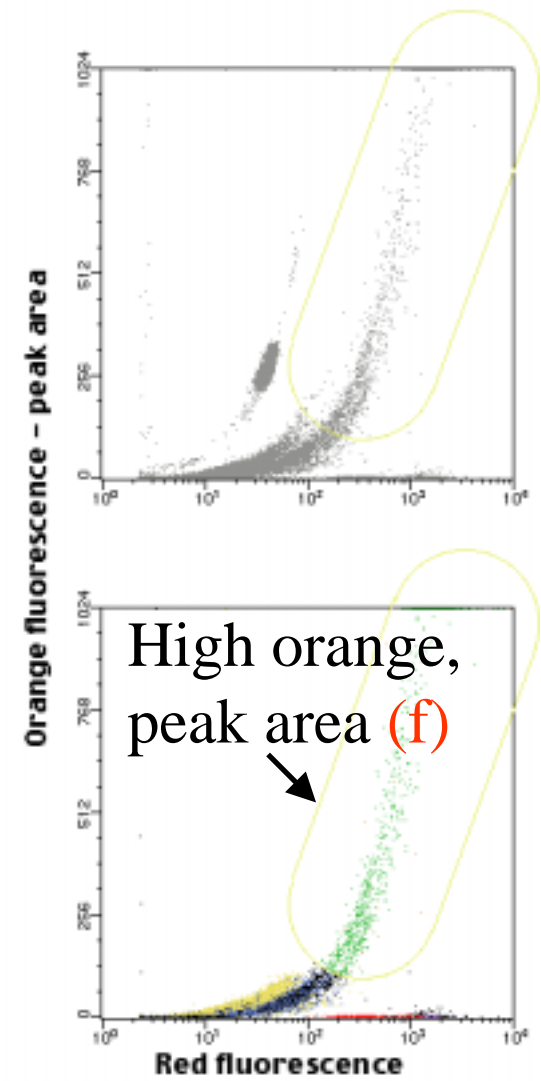
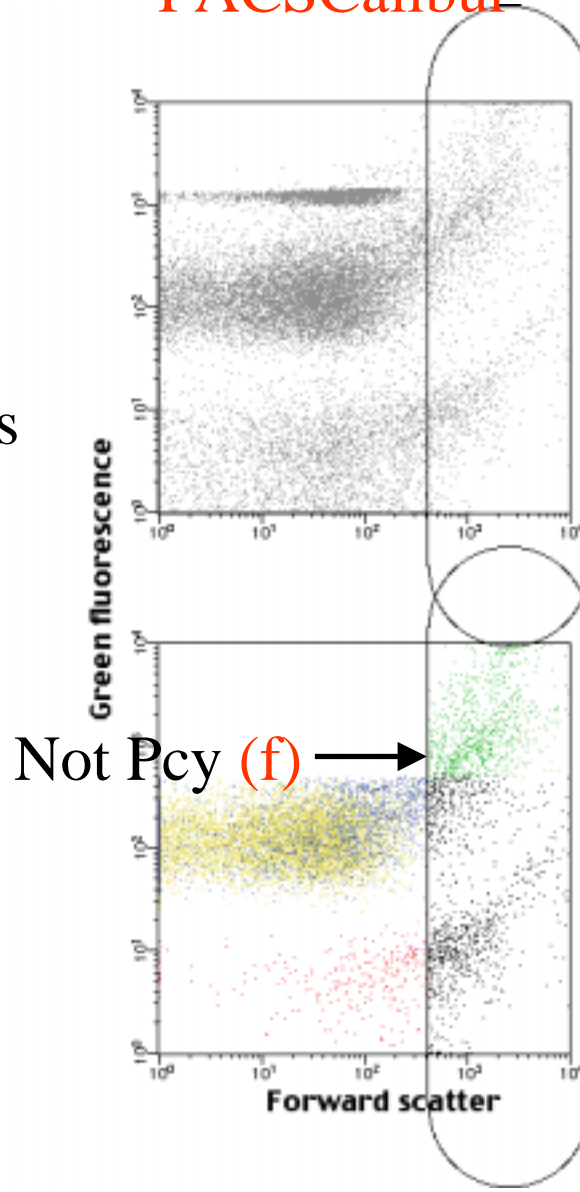
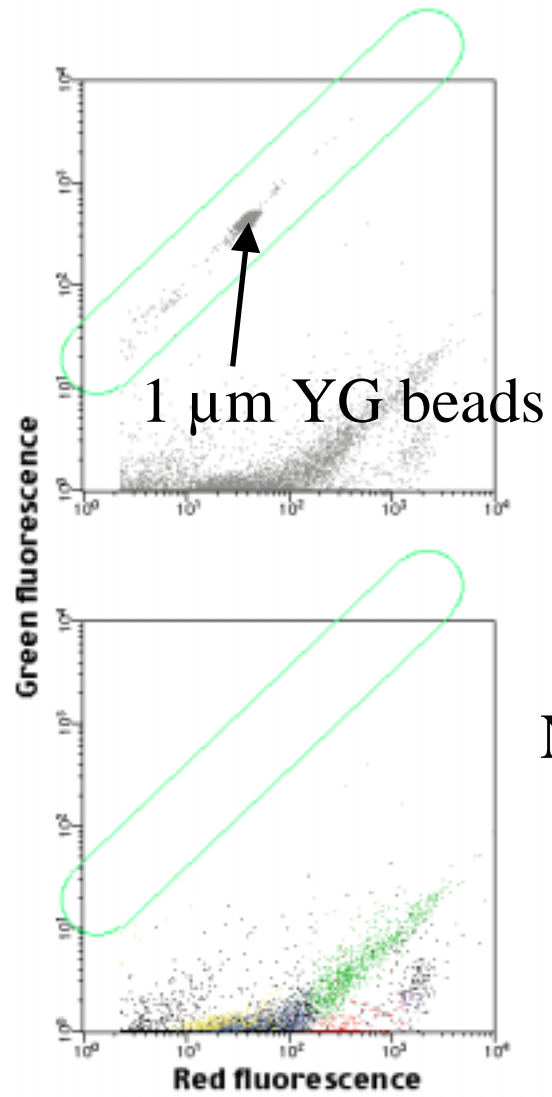
 NOT Pcy or microcolony

 Pcy-B

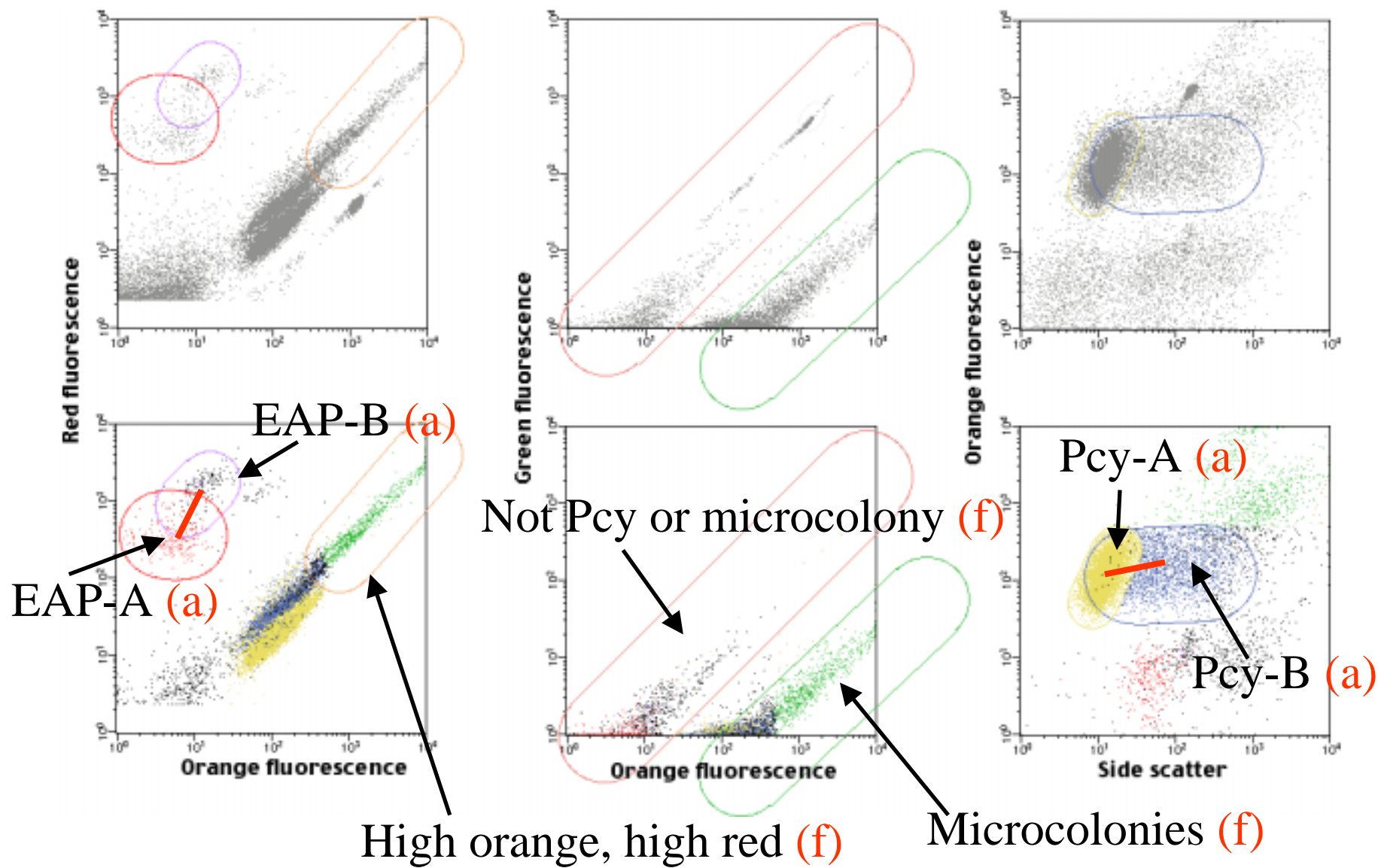
Pcy = "solitary"  
picocyanobacteria

EAP = eukaryotic APP

# FACSCalibur



# FACSCalibur





## **Pcy-B cells.....dislodged microcolony cells....real or handling artifact?**

**High purity preparations of microcolonies (sorting from natural samples) gave high purity microcolony signatures on FACSCalibur (i.e. few Pcy-B cells).**

**Sonification of sorted microcolonies for 5 min. was required to obtain significant enrichment of Pcy-B cells.**

**Physical disturbance (i.e. stormy period in summer of 2000) was accompanied by an increase in Pcy-B cells in surface layers.**



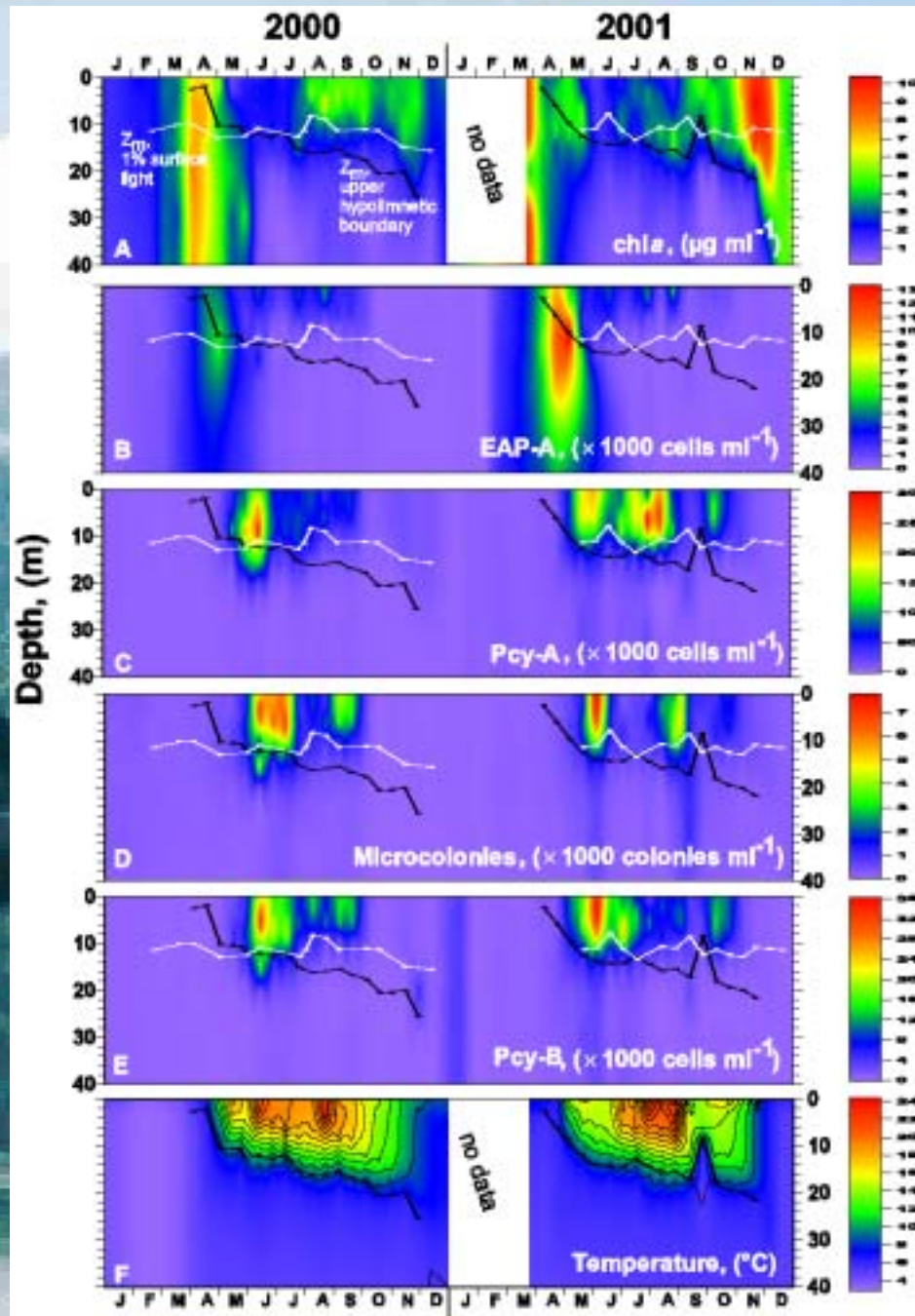
## PC-rich picocyanobacteria.....?

Were isolated from enrichment cultures, but were either very rare or could not be observed in sorted EAP, Pcy-A or Pcy-B preparations.

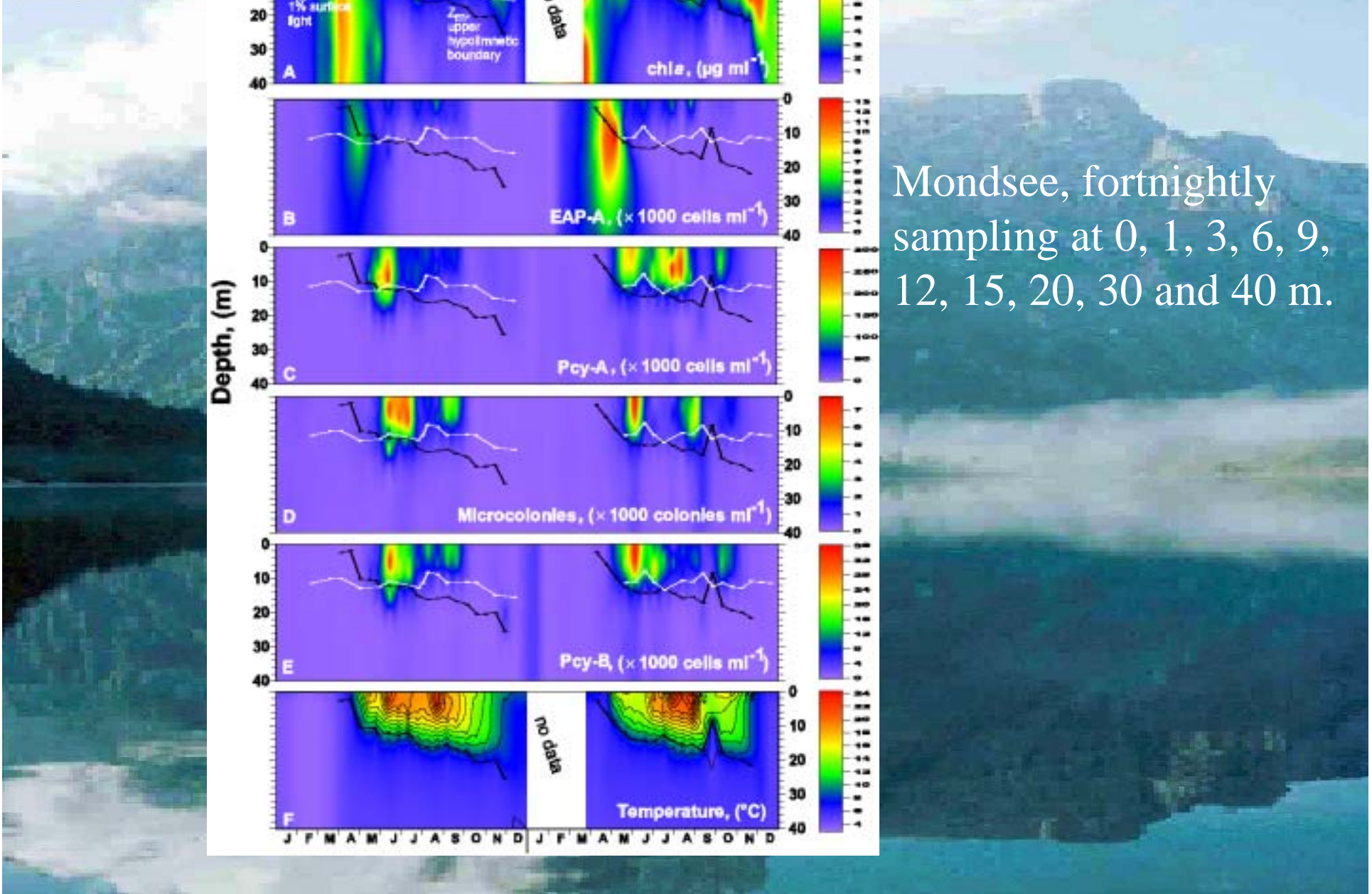


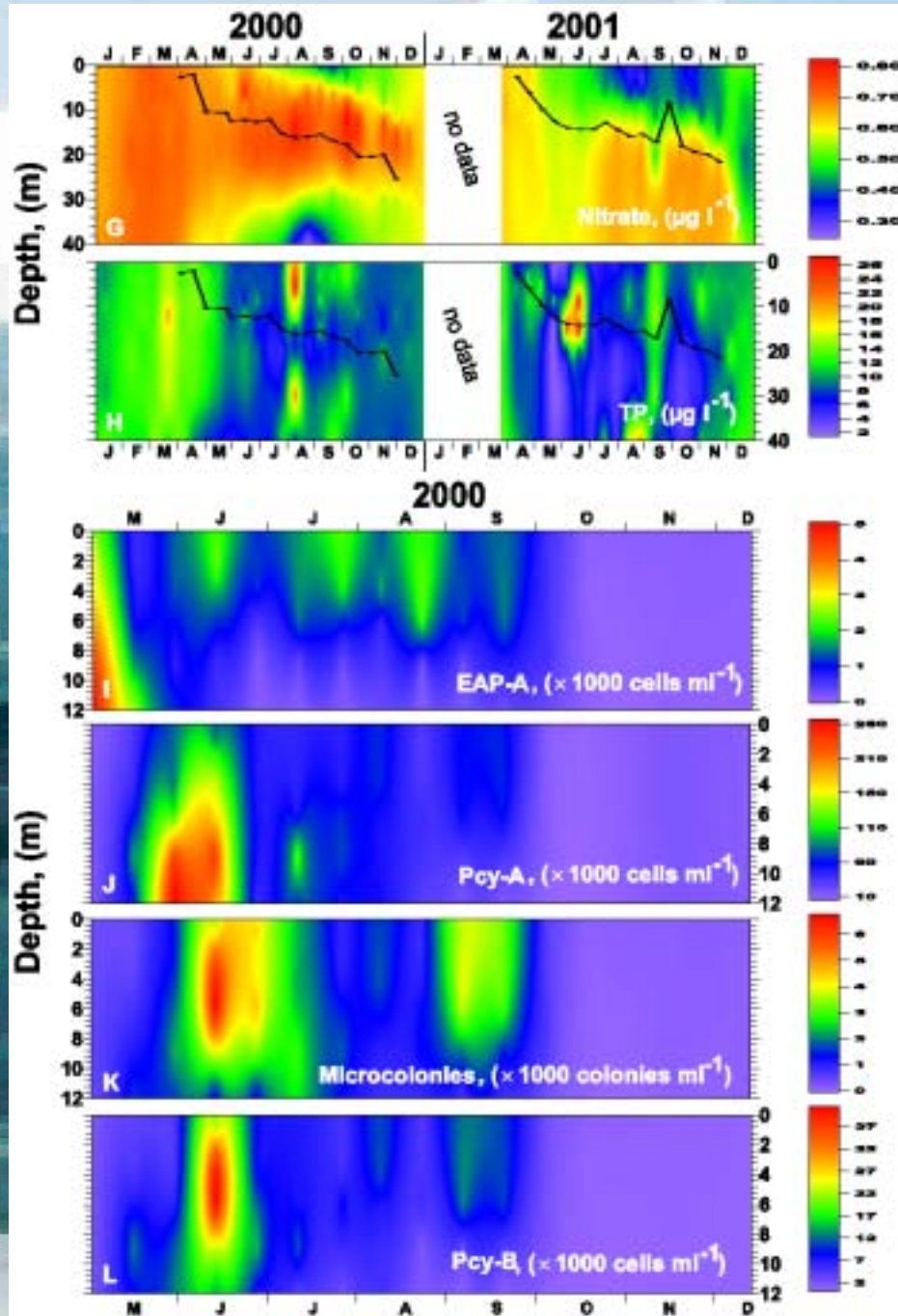
## **Some results....**

**Seasonal and vertical  
patterns of APP abundance  
in Mondsee, and isolation  
by single-cell/colony  
sorting.....**



Mondsee, fortnightly sampling at 0, 1, 3, 6, 9, 12, 15, 20, 30 and 40 m.

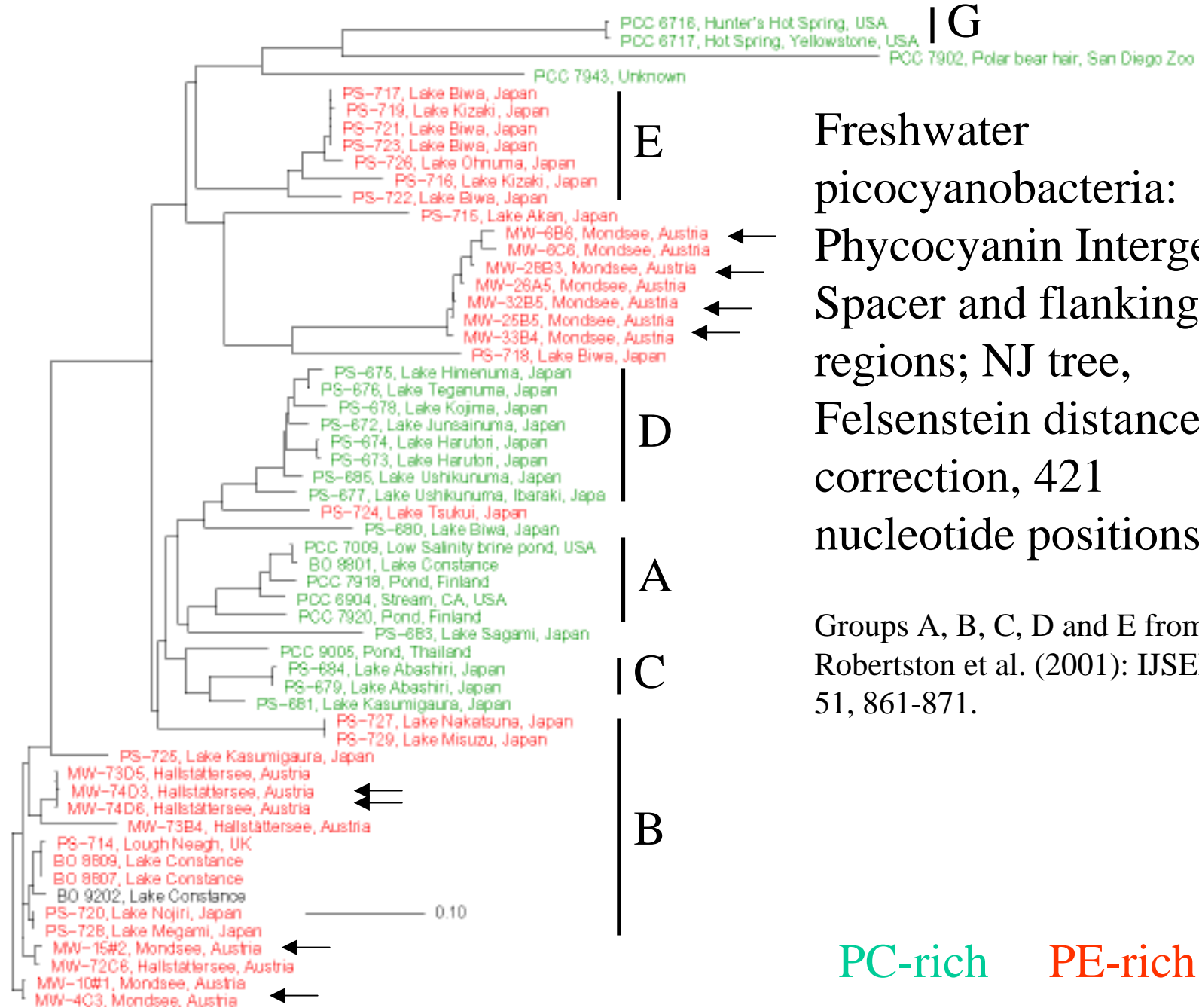




Mondsee, fortnightly sampling at 0, 1, 2, 4, 6, 9 and 12 m.



Single-cell/colony **sorting** is  
an efficient method to obtain  
**isolates** of freshwater  
APP.....

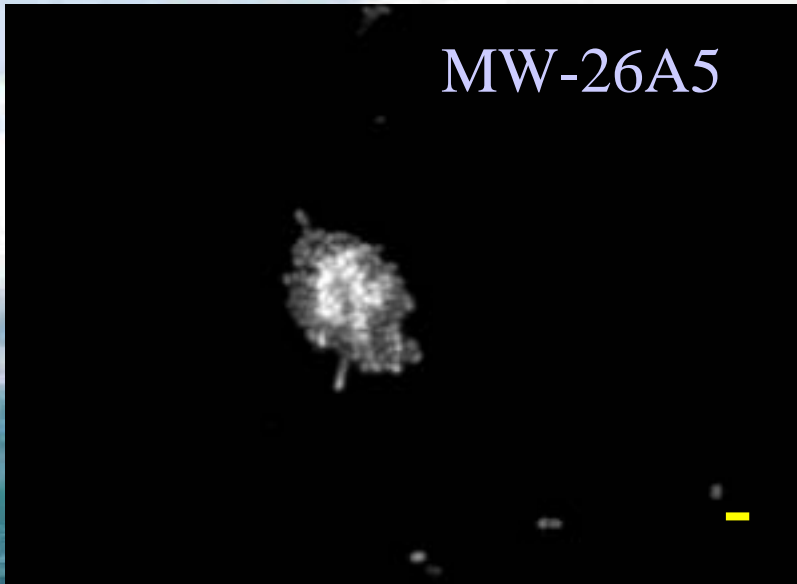


Freshwater  
picocyanobacteria:  
Phycocyanin Intergenic  
Spacer and flanking  
regions; NJ tree,  
Felsenstein distance  
correction, 421  
nucleotide positions.

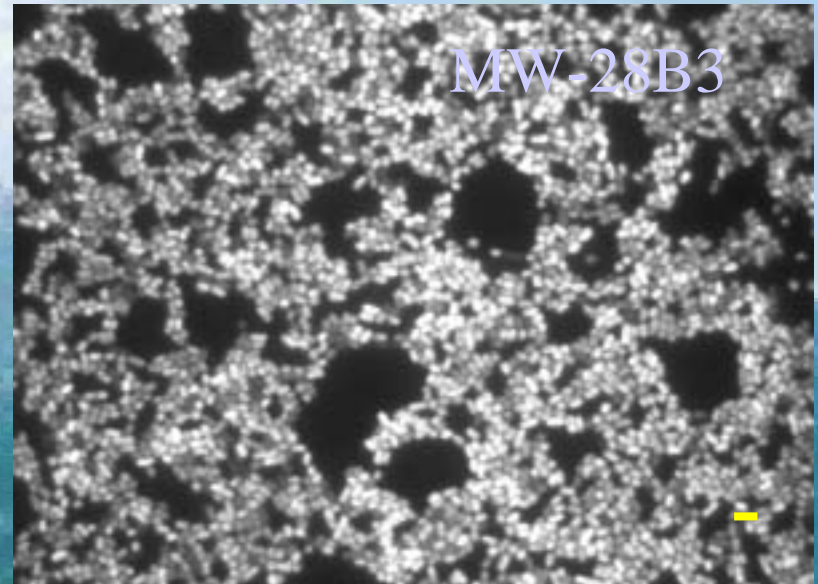
Groups A, B, C, D and E from  
Robertston et al. (2001): IJSEM  
51, 861-871.



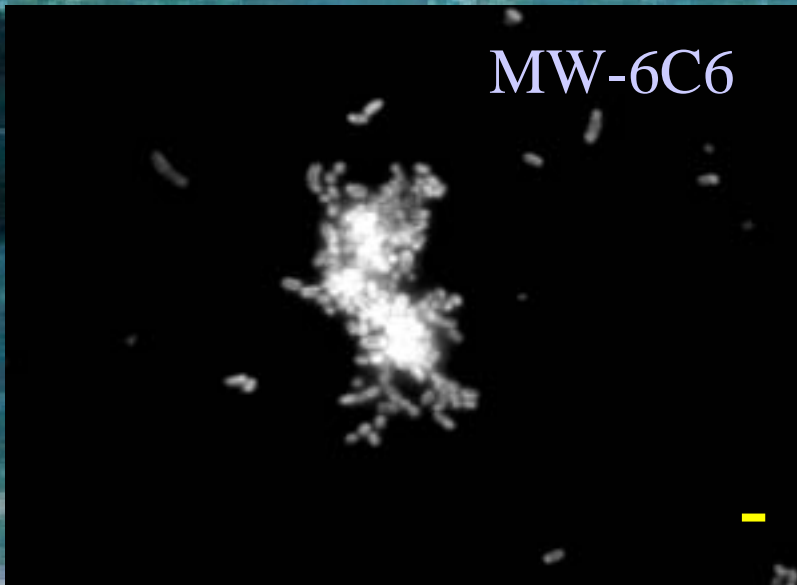
MW-26A5

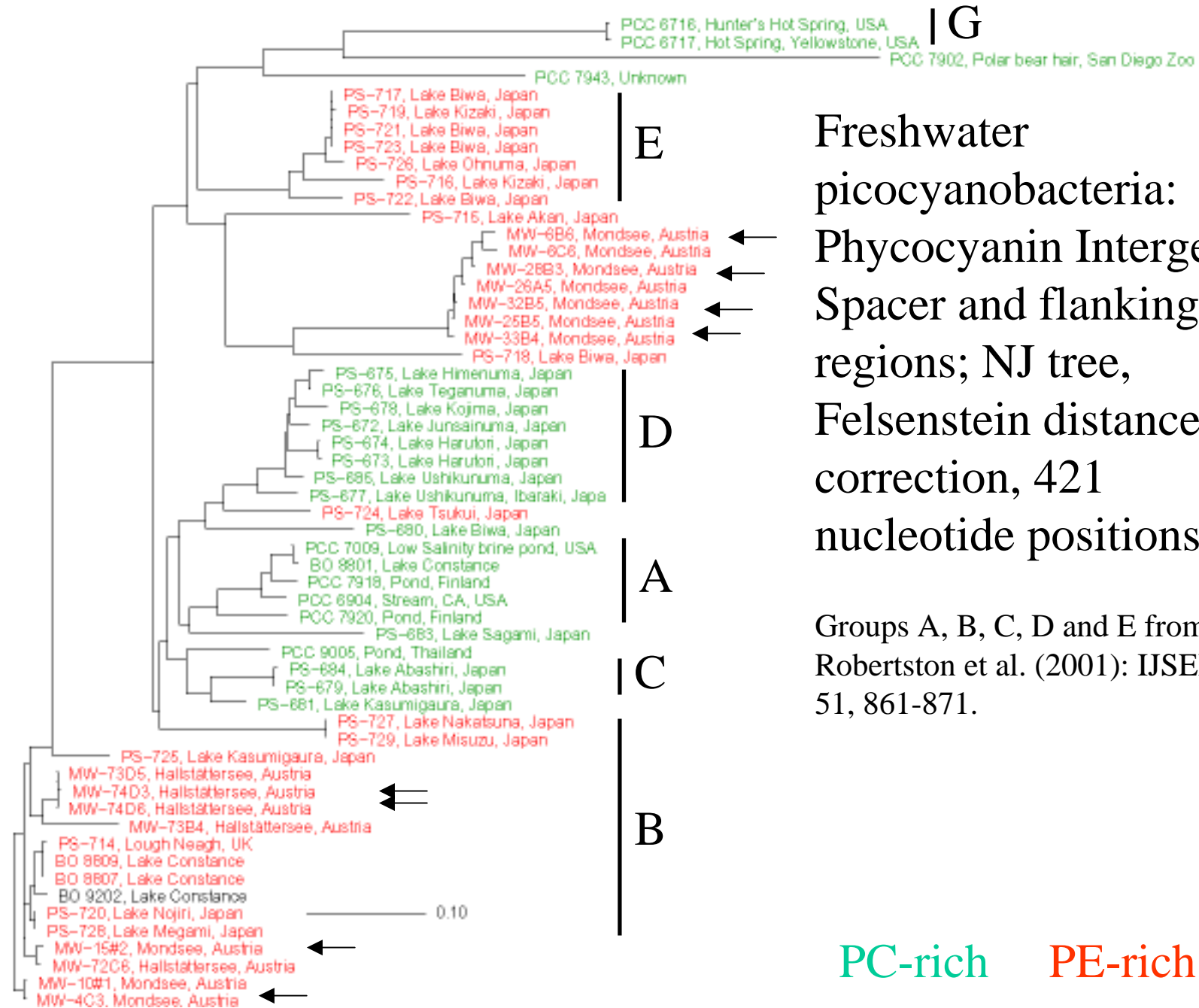


MW-28B3



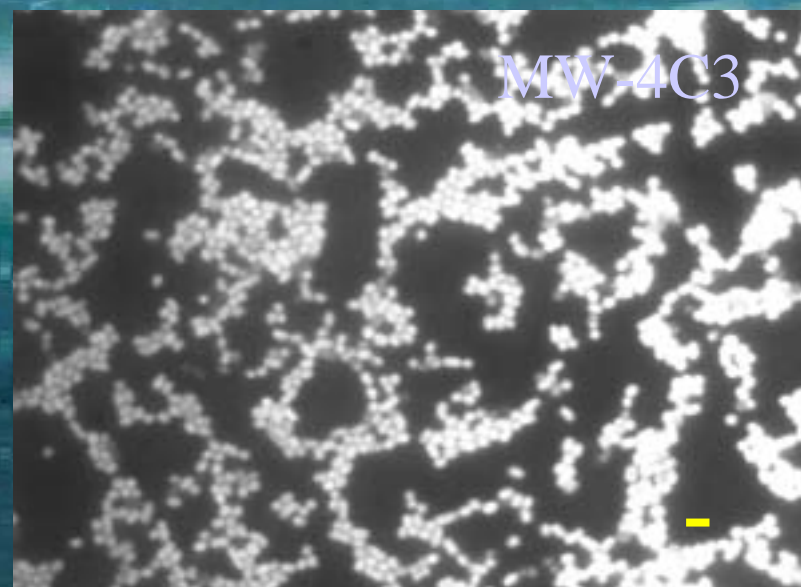
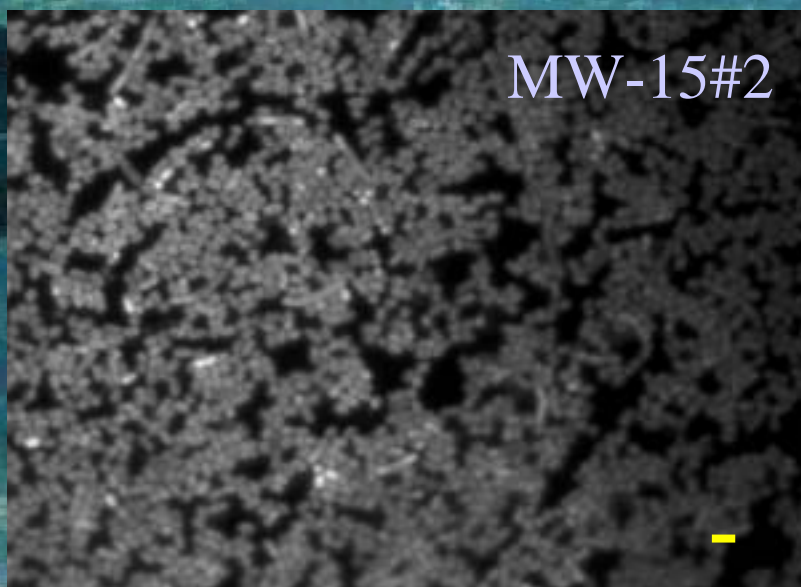
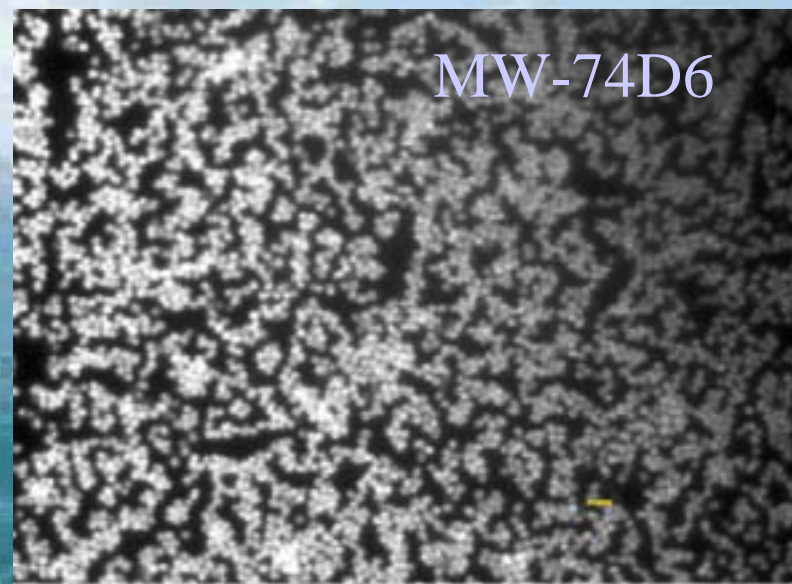
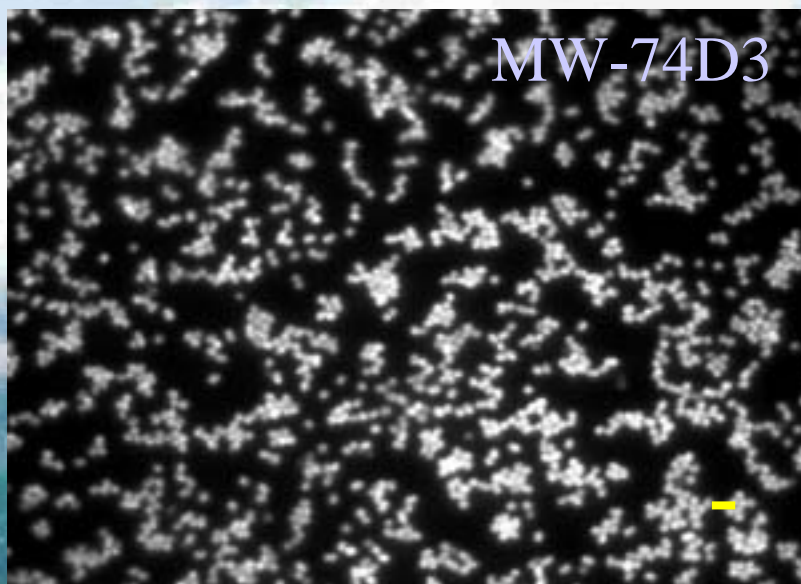
MW-6C6





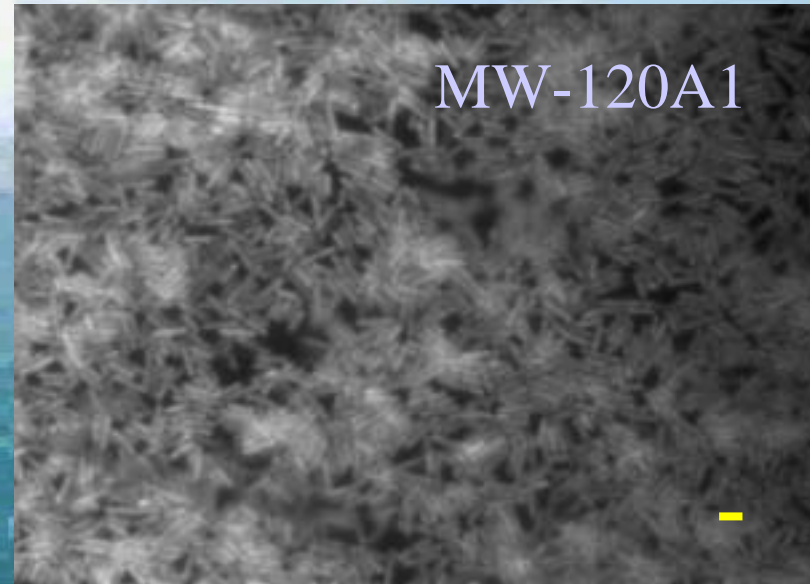
Freshwater  
picocyanobacteria:  
Phycocyanin Intergenic  
Spacer and flanking  
regions; NJ tree,  
Felsenstein distance  
correction, 421  
nucleotide positions.

Groups A, B, C, D and E from  
Robertston et al. (2001): IJSEM  
51, 861-871.

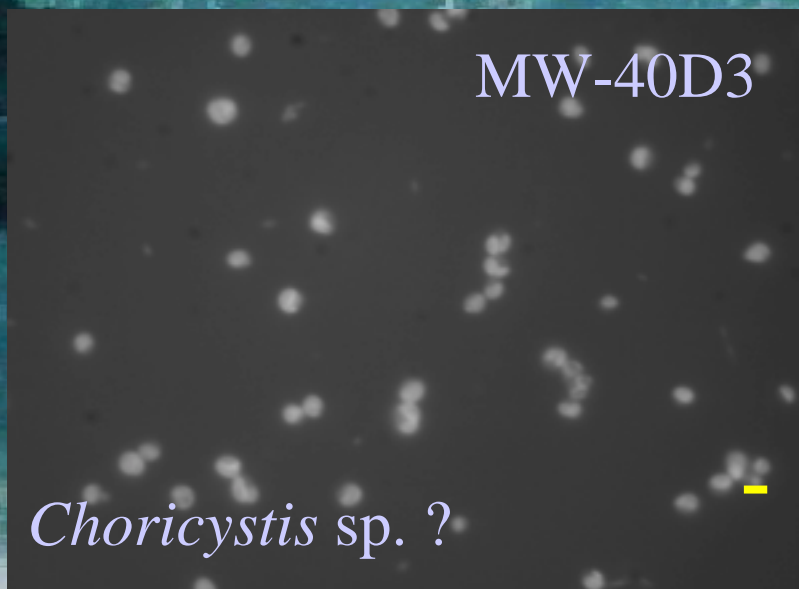




MW-24B5

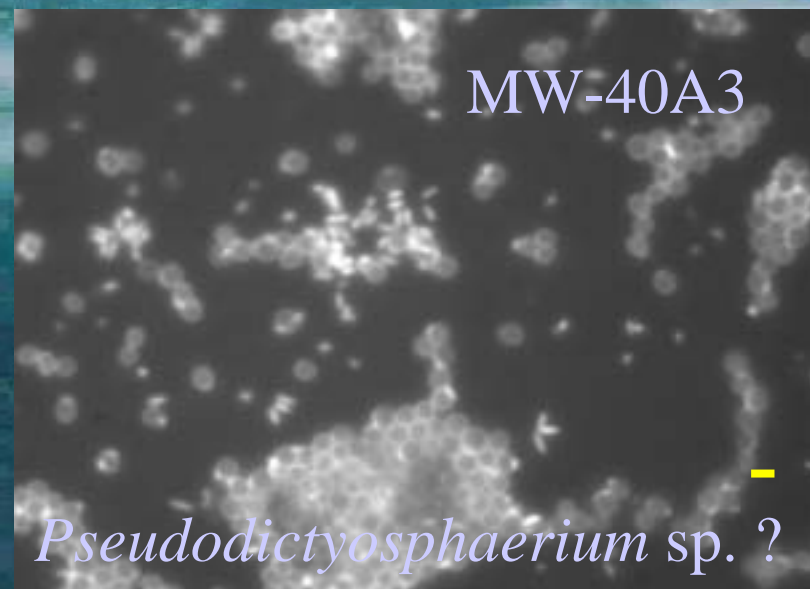


MW-120A1



MW-40D3

*Choricystis* sp. ?



MW-40A3

*Pseudodictyosphaerium* sp. ?



## Work in progress.....

- Sequencing of cyanobacterial 16S rDNA, ITS and CPC genes/regions.
- Development of DGGE screen(s).
- *In situ* and lab growth and grazing experiments.

## Planned.....

- FISH and quantitative PCR probes.



# Ways to improve “environmental flow cytometry”.....APP cells are NOT “about 10 $\mu\text{m}$ in diameter”!

(van den Engh in “Emerging Tools for Single-Cell Analysis”, Wiley-Lis 2000)

## Hardware

- more signals: e.g. non-standard scatter angles, analog, finer color resolution.
- “friendly” alignment.
- complex sort logic.
- Disposable/autoclavable fluidics.

## Software

- manual and automatic gating.
- better data exploration options....e.g. “view whole profile”.
- better record keeping.
- useful export options!



# Many thanks to.....

- Peter Stadler
- Thomas Weisse
- Martin Dokulil
- Martin Hahn
- Heike Heckermann
- Claudia Skolaut
- Liselotte Eisl
- Sonja Greisberger
- Michael Schauer
- Katrin Teubner
- Sabine Lettner
- Matthias Pöckl

....and the Austrian Science Fund (FWF).