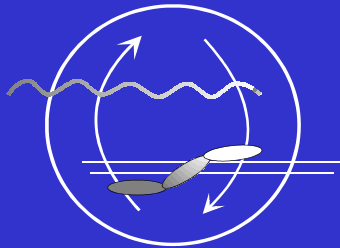


# Identification of free-living heterotrophic flagellates by fluorescence- in situ-hybridization

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## Common methods

- light microscopy → live counts
- electron microscopy → species identification
- epifluorescence m. → bulk measurements

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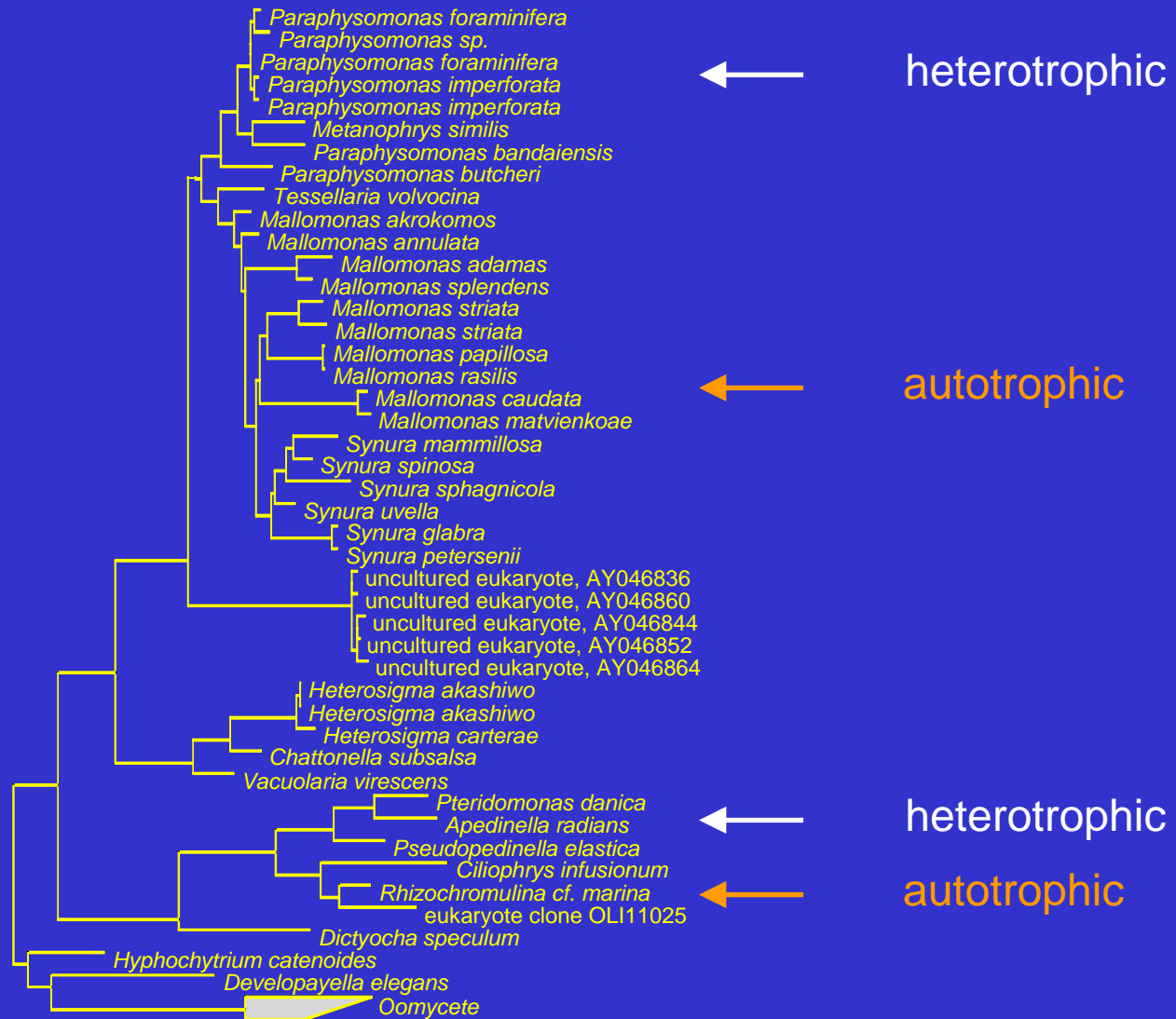
## New method

- FISH → quantitative phylogenetic analysis

# FISH in flagellates

- sensitivity:
  - fluorescent dyes
  - HRP-labeled probes with TSA
- autofluorescence of chlorophyll

# Different trophic modes in closely related species

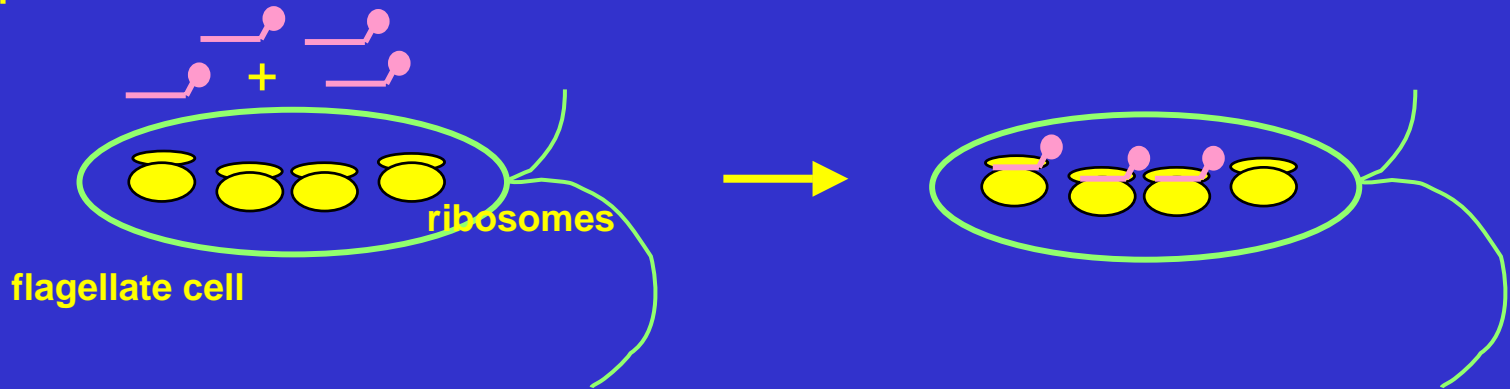


## FISH in flagellates

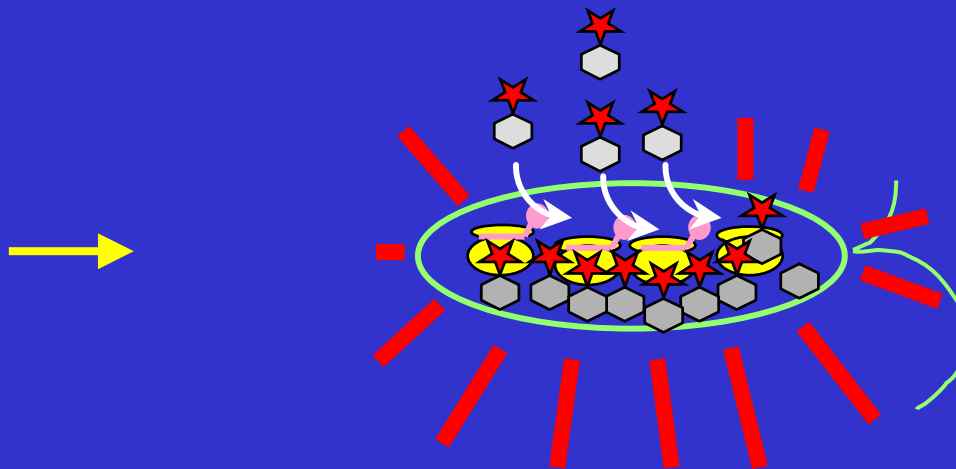
- sensitivity:
  - fluorescent dyes
  - HRP-labeled probes with TSA
- autofluorescence of chlorophyll
  - UV-excitable fluorochrome Alexa-350

# Hybridization with HRP-labeled probes:

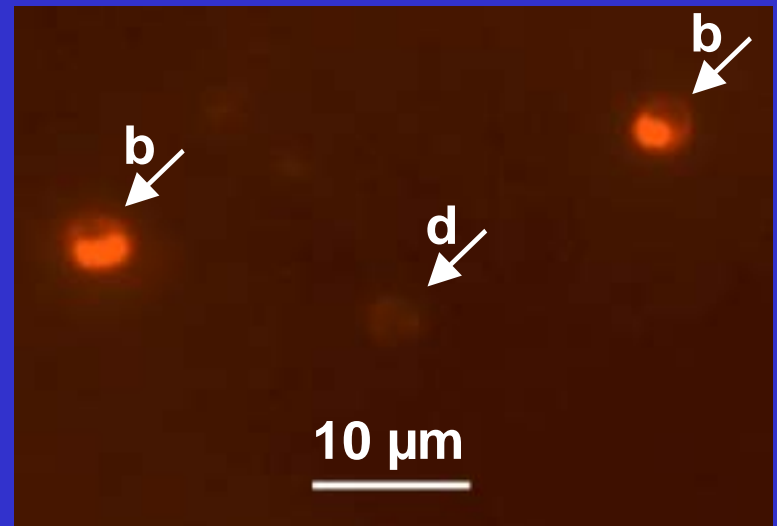
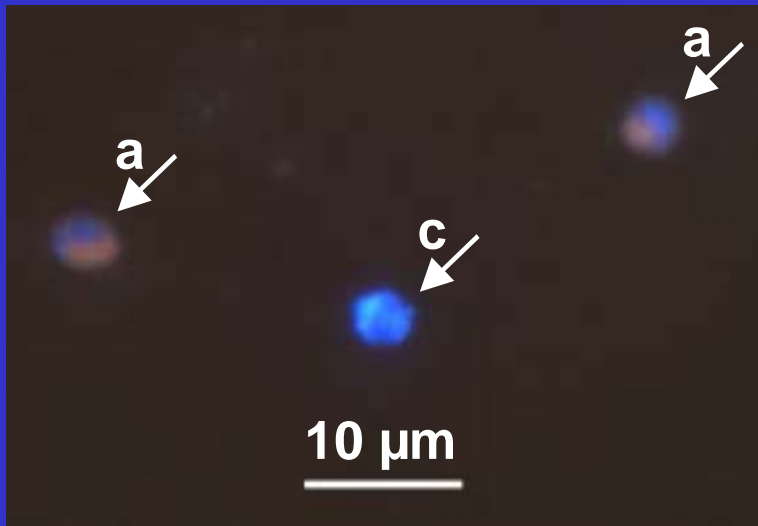
HRP-probes



# Incubation with Alexa350-labeled tyramides:



## Mixed culture of *Ochromonas* sp. and *Paraphysomonas imperforata*



Hybridization signal with EUK516

Chlorophyll autofluorescence

a+b: *Ochromonas* sp. (mixotrophic)

c+d: *Paraphysomonas imperforata* (heterotrophic)

# Development of group specific probes

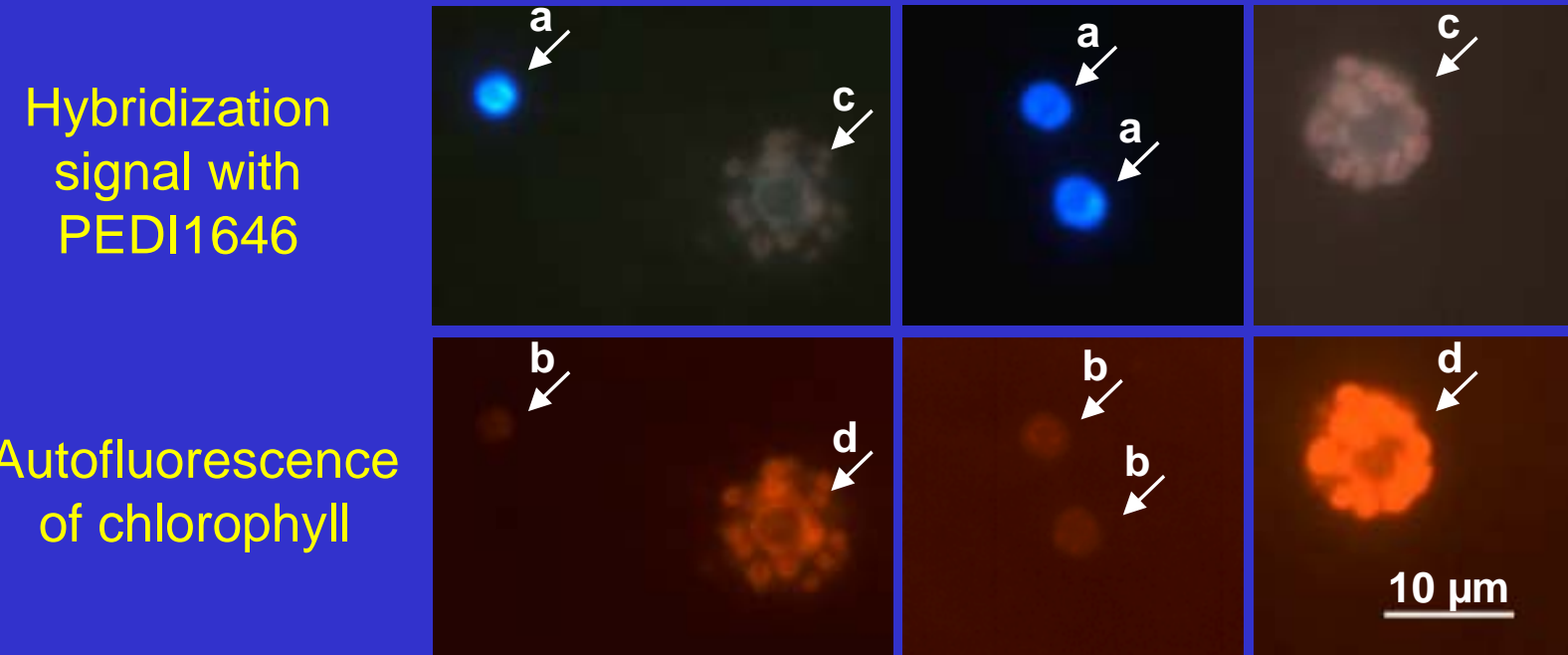


## Probe design:

PEDI1646 (5'-TTA CCT TCG ATC GGT AGG-3')

	mismatches	target sequence
PEDI1646		---CCUACCGAUCGAAGGUAA---
<i>Apedinella radians</i>	0	---CCUACCGAUCGAAGGUAA---
<i>Pteridomonas danica</i>	0	---CCUACCGAUCGAAGGUAA---
<i>Pseudopedinella elastica</i>	0	---CCUACCGAUCGAAGGUAA---
<i>Ciliophrys infusionum</i>	0	---CCUACCGAUCGAAGGUAA---
<i>Rhizochromulina cf marina</i>	0	---CCUACCGAUCGAAGGUAA---
Eukaryote clone OLI11025	2	---CCUACCGAUCGUAGGUGA---
<i>Willaertia magna</i>	3	---CCUACCGAUGGAAUGgAA---
<i>Plasmodium vivax</i>	3	---CCUACCGAUCGAAAGAUA---
<i>Dictyocha speculum</i>	5	---CCUACCGAUUGAAUGGCU---

# Mixed culture of *Pteridomonas danica* and *Dictyocha speculum*



a+b: *Pteridomonas danica* (heterotrophic)

c+d: *Dictyocha speculum* (autotrophic)

**Conclusion**

**FISH**

Phylogeny

## Conclusion

**FISH**

+

**HRP**

Phylogeny

Sensitivity

## Conclusion

**FISH**

+

**HRP**

+

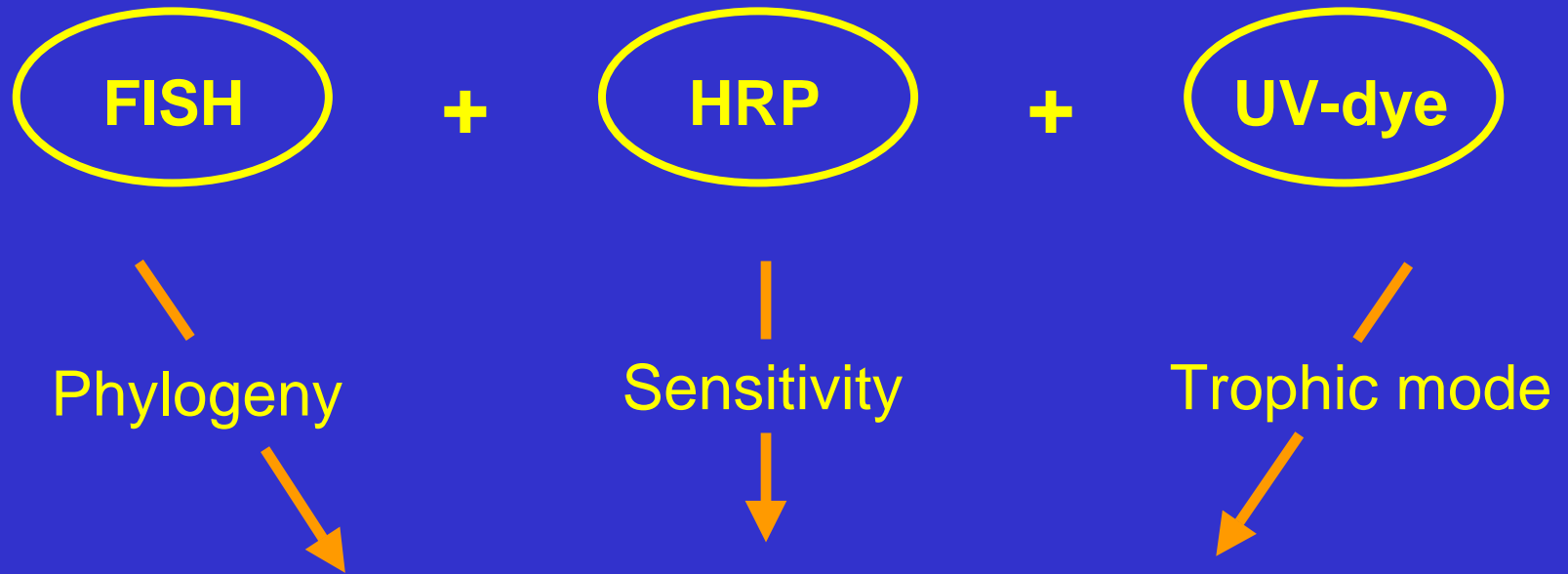
**UV-dye**

Phylogeny

Sensitivity

Trophic mode

## Conclusion



**Rapid analysis of structure and function  
of environmental flagellate communities**