Life style and cell architecture of *Achromatium oxaliferum*

Sina Schorn[1,2], Verena Salman-Carvalho[2], Niko Leisch[2], Sten Littmann[2], Hans-Peter Grossart[3,4], and Heribert Cypionka[1]

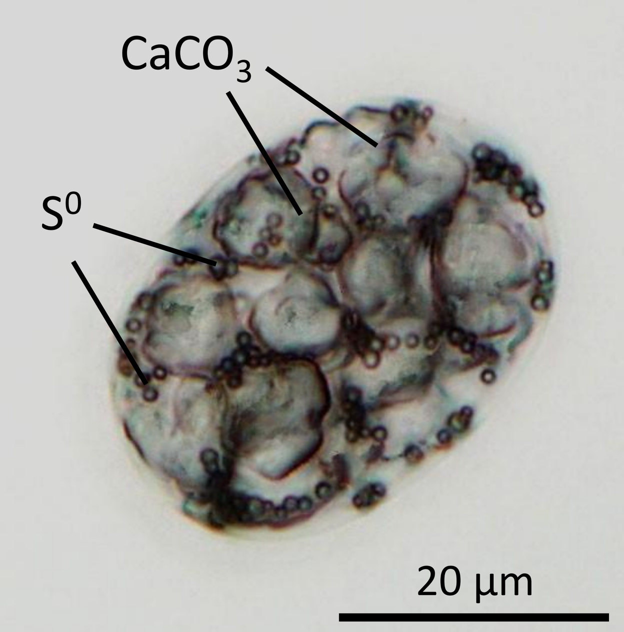
[(1)] Institute for Chemistry and Biology of the Marine Environment, Carl-von-Ossietzky University of Oldenburg, Oldenburg, Germany, [(2)] Max Planck Institute for Marine Microbiology, Bremen, Germany, [(3)] Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany, [(4)] Potsdam University, Institute for Biochemistry and Biology, Potsdam, Germany

Contact: cypionka@icbm.de or sschorn@mpi-bremen.de

Background

With a cell length of up to 200 µm *Achromatium oxaliferum* is the world's biggest freshwater bacterium. Large populations of this uncultured bacterium are found at the oxic-anoxic transition zone of aquatic sediments, within opposing gradients of oxygen and sulfide. The cells are filled with large calcite bodies of unknown function that give them a unique phenotype.

Aim: To study the particular cell architecture, ecophysiology and interactions of *A. oxaliferum* with other organisms.



Conclusion

- Calcite bodies are located in periplasmic pockets formed by invaginations of the cytoplasmic membrane
- Chemotactic behaviour in the sediment might be driven by negative chemotaxis to oxygen and sulfide
- *Achromatium* cells are grazed by various organisms although most of the cell consists of calcite

Results

Cell architecture of *Achromatium*

- Cell volume largely consists of calcite bodies and sulfur globules (Fig.1 a and b)
- Variable number of calcite bodies, their loss doesn't affect cell motility (Fig.1 c and d)

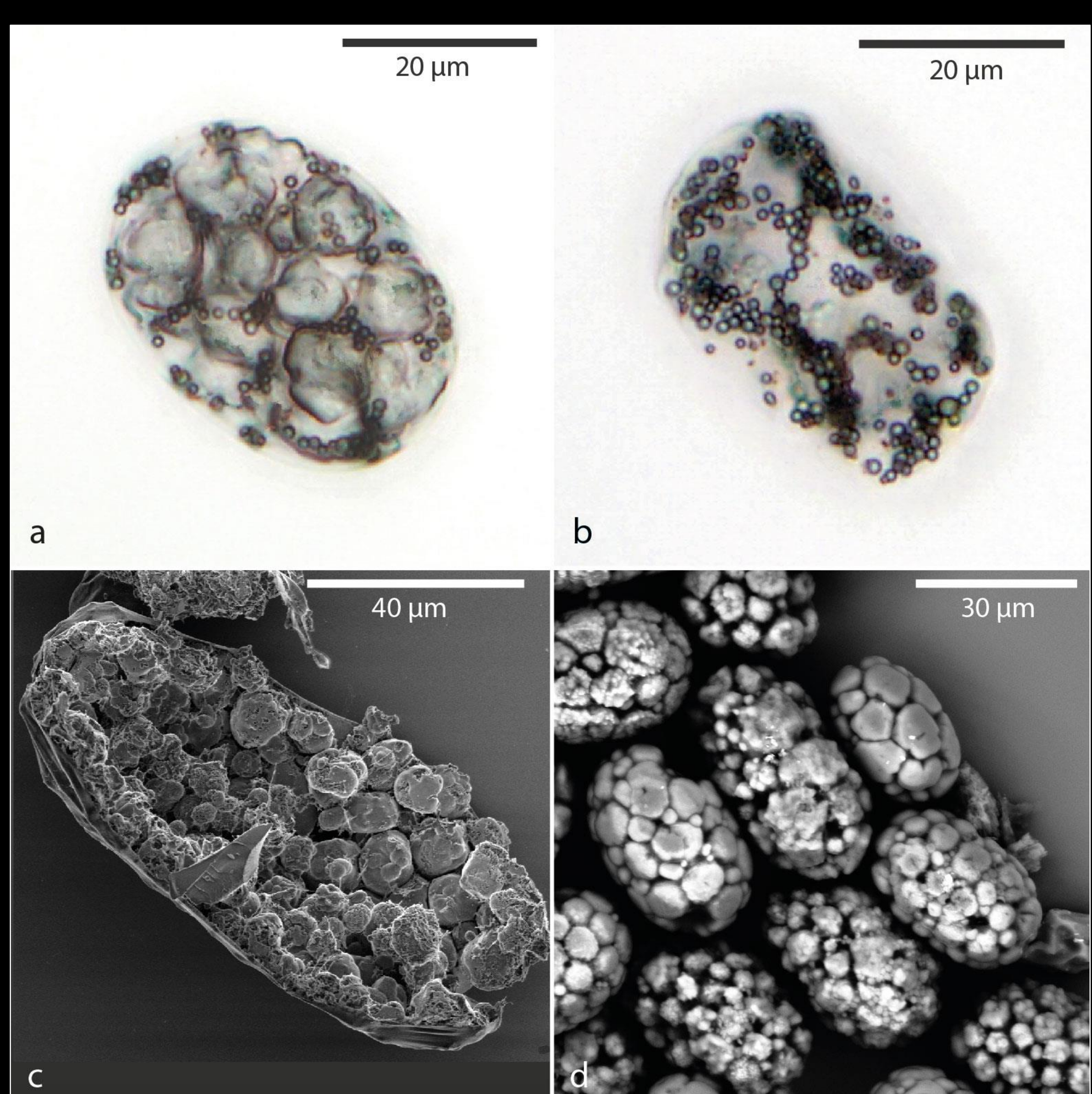


Figure 1 Morphological characteristics of *Achromatium* sp. (a) *Achromatium* cell with calcite bodies and sulfur; (b) cell with sulfur globules, only. (c) Scanning electron micrograph of a broken cell. (d) Cells with variable numbers of calcite bodies and some empty cavities.

- Condensed cytoplasm in the space between the calcite bodies (Fig.2 a and b, green)
- Multiple DNA hotspots (Ionescu *et al.* 2017) within the cytoplasm (Fig.2 c and d, green)

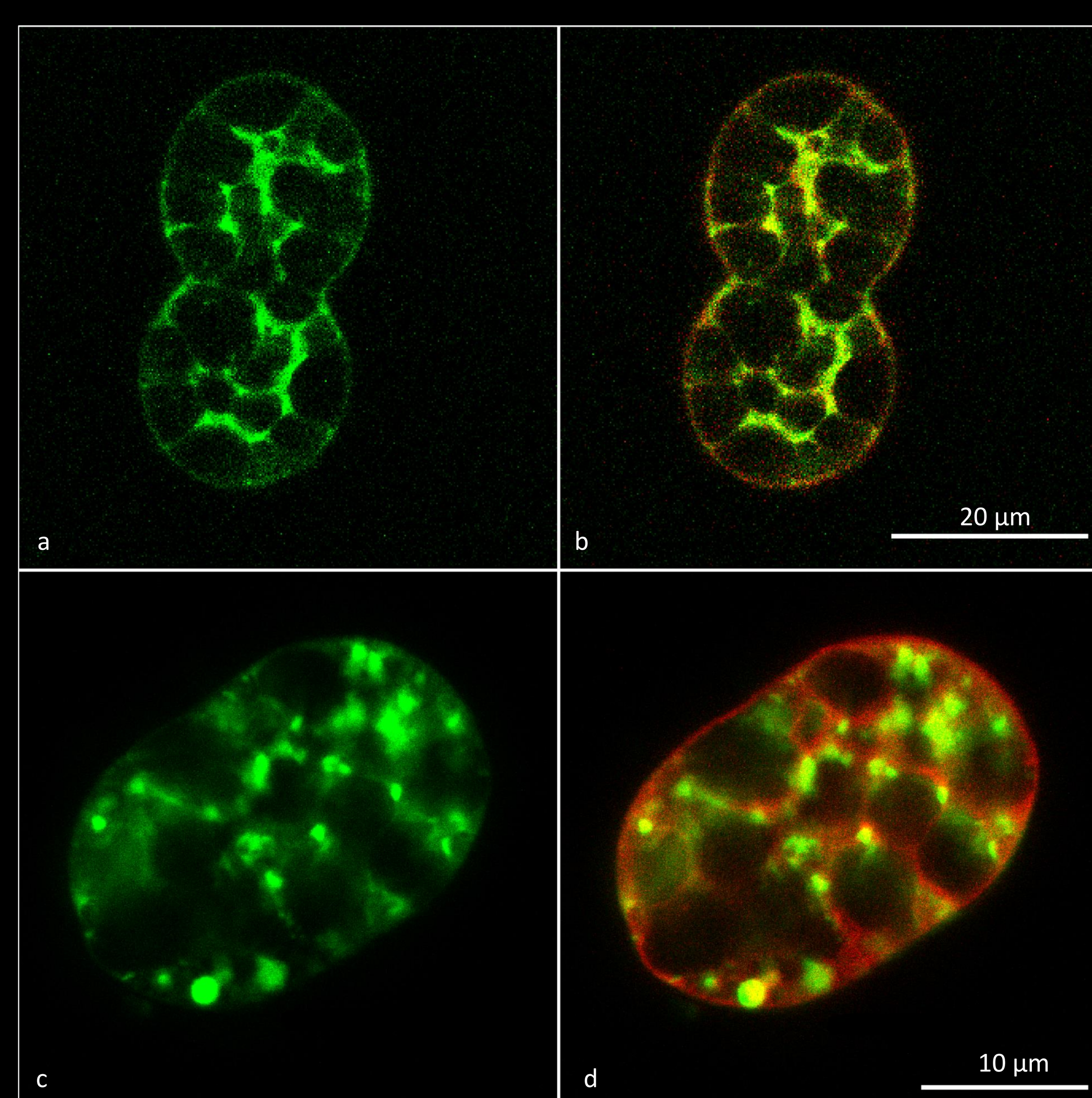


Figure 2 Intracellular structures. (a) Fluorescein isothiocyante (FITC) staining of cytoplasm, and (b) additional Nile Red staining of membranes. (c) DNA staining with Sybr Green I, and (d) additional staining with Nile Red.

- Hotspots of dissolved Ca^{2+} (Salman *et al.* 2015) are distributed across the entire cell (Fig.3 a and b)
- Calcite bodies are located in the greatly enlarged periplasm (Fig.3 c and d)

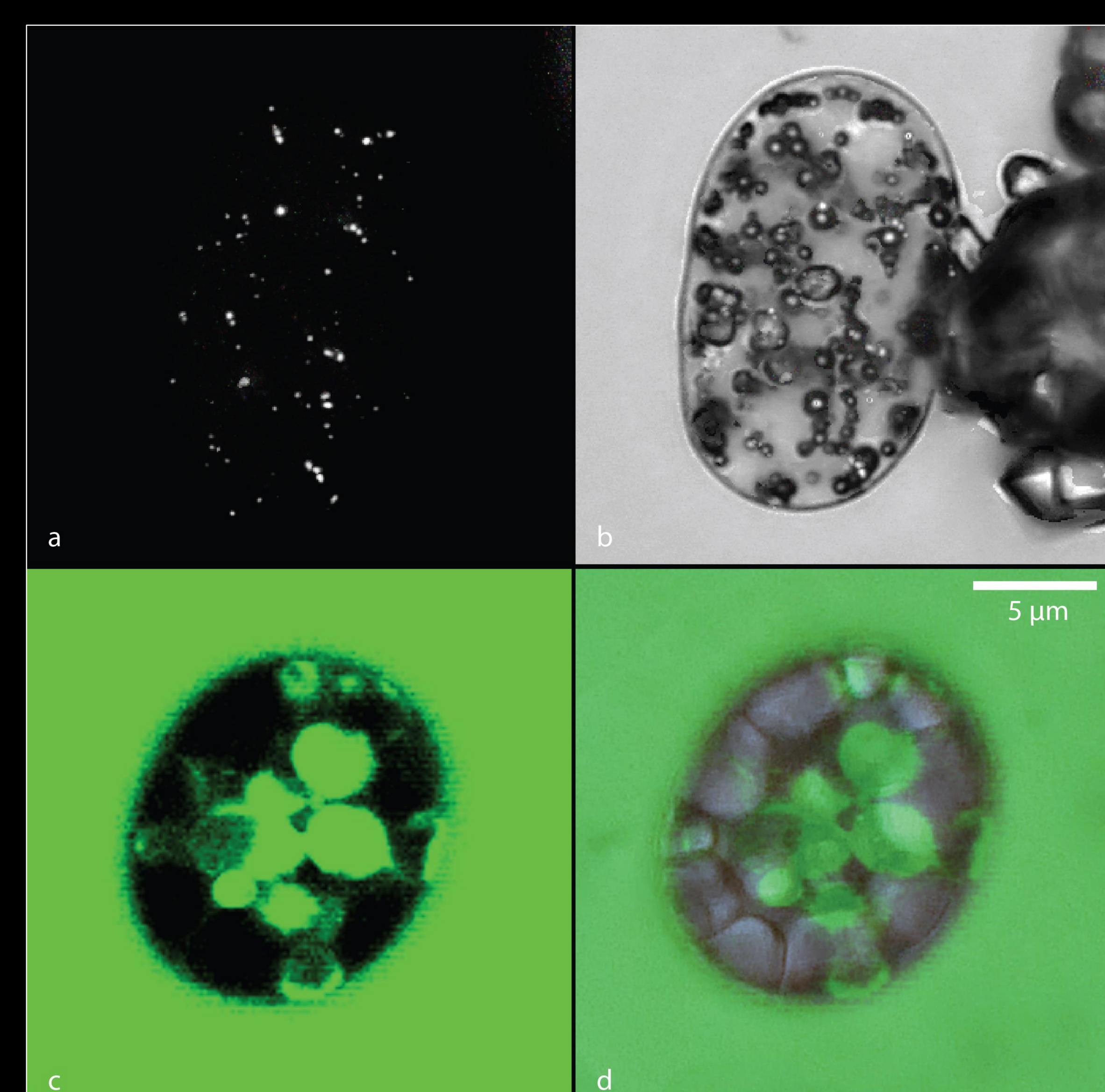


Figure 3 Enlargement of the periplasm. (a and b) Fura-2AM staining shows hotspots of dissolved Ca^{2+} inside the cells. (c) Fluorescein (hydrophilic dye that does not cross membranes) diffused into the cells and (d) stained empty calcite cavities.

Ecophysiology & interactions of *Achromatium*

Chemotactic behaviour

In diffusion chambers, *Achromatium* cells were exposed to oxygen and sulfide gradients to follow their migration under the microscope. The cells moved away from both oxidising (oxygen) and reducing (sulfide) conditions.

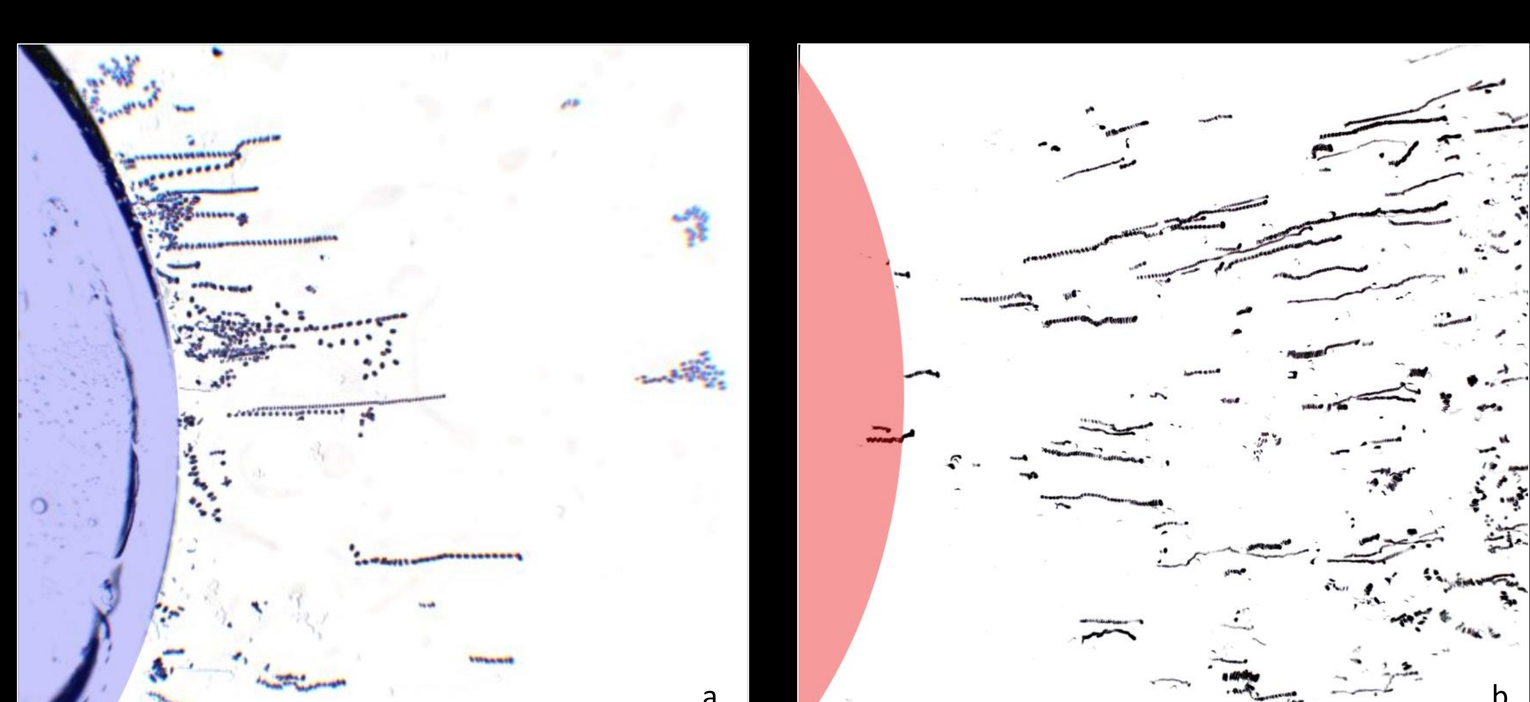


Figure 4 Chemotactic behaviour of *Achromatium* to oxygen (a) **and sulfide** (b). Chemotaxis experiments were performed with *Achromatium* cells exposed to oxygen (blue) or sulfide (red) on microscopic slides. The movement of cells was recorded over 30 minutes with pictures taken every 30 seconds. Thus, every dotted line represents the path of a single cell.

Grazers and parasites (Schorn and Cypionka, 2018)

In natural sediment, we observed various meiofaunal organism with ingested *Achromatium* cells. Furthermore, intracellular pear-shaped structures stained positive for chitin, showing infection of *Achromatium* sp. by aquatic fungi.

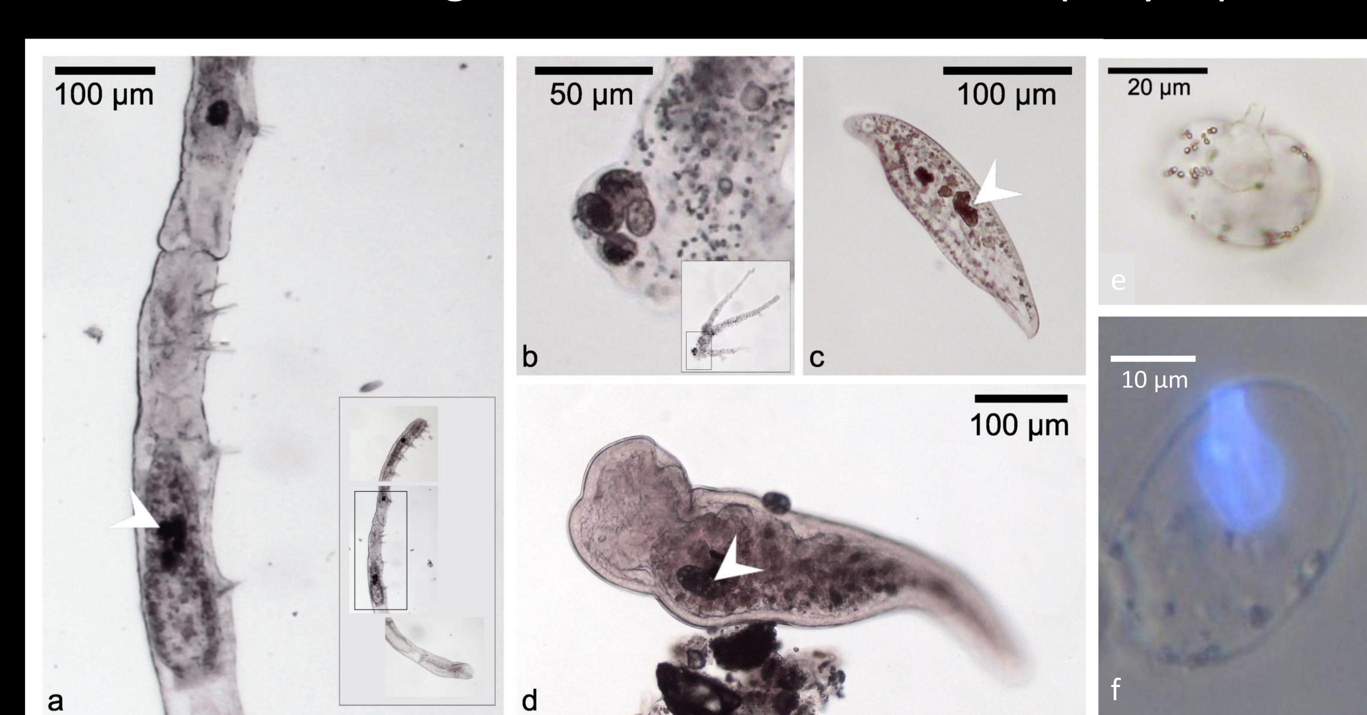


Figure 5 Grazers and parasitic fungi. Ingested *Achromatium* cells (marked with white arrows) were recognised due to transparent body structures of grazers. We observed oligochaetes (resembling *Chaetogaster diastrophus*, a), amoebae (b), ciliates (resembling *Amphileptus*, c), and plathelminthes (resembling *Stenostomum leucops*, d) with *Achromatium* cells in their intestines. Intracellular pear-shaped structures (e) stained positive for chitin, Calcofluor White staining (f) and showed that *Achromatium* sp. get infected by aquatic fungi.

References

Ionescu et al (2017) Community-like genome in single cells of the sulfur bacterium *Achromatium oxaliferum*. Nature communications
Salman et al (2015) Calcite-accumulating large sulfur bacteria of the genus *Achromatium* in Sippewissett Salt Marsh. ISME Journal
Schorn & Cypionka (2018) A crispy diet: Grazers of *Achromatium oxaliferum* in Lake Stechlin sediments. Microbial Ecology

Acknowledgements

We thank Hans-Dietrich Babenzien and Silke Van den Wyngaert (Leibniz Institute of Freshwater Ecology and Inland Fisheries, Stechlin, Germany), and Bert Engelen (ICBM) for valuable discussions and helpful comments on this work. Furthermore, we thank Wilko Ahlrichs (University of Oldenburg) for help with the classification of the grazers.