Developing experimental methods for the tiniest tiny marine predators and their symbioses

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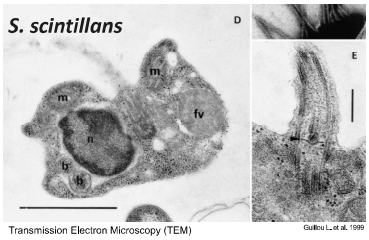
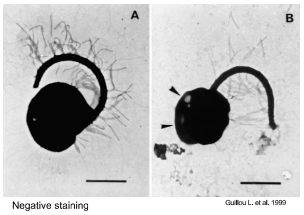
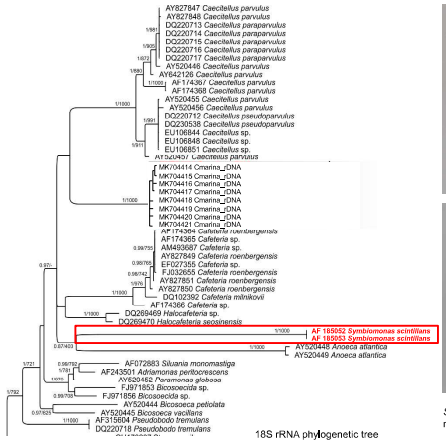
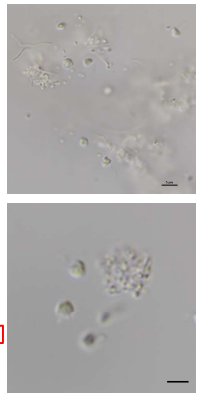
Introduction

Although nano- and pico-eukaryotes (0.2-10 μm) and especially heterotrophs/predators are ecologically extremely important in the marine environment (they mainly feed on bacteria), they are surprisingly understudied. There are also very few methods optimized for studying these tiny eukaryotes. Here, we present *Symbiomonas scintillans* as a helpful model to establish experimental methods for tiny eukaryotes housing bacterial endosymbionts.








Symbiomonas culture DIC images in light microscopy

General features reported previously

- Equatorial Pacific and Mediterranean Sea
- Heterotrophic flagellate
- ~1.3 μm in size
- Endosymbiotic bacteria associated with its nucleus

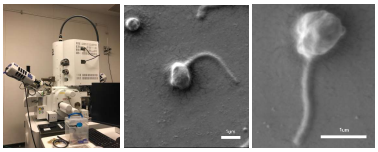
Main questions

- Why does such a tiny eukaryote need bacterial symbionts?
- How does it capture its bacterial prey and which bacteria does it feed on?

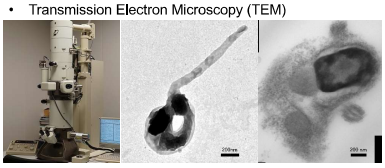
Approaches & Results

Method 1. Electron Microscopy

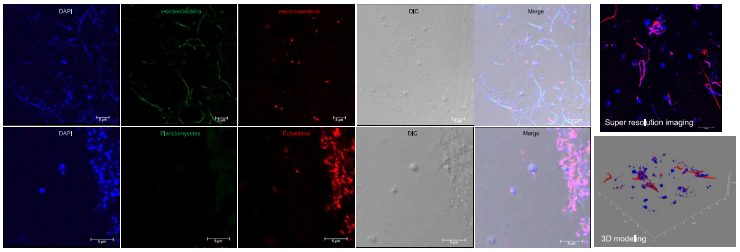
- Scanning Electron Microscopy (SEM)



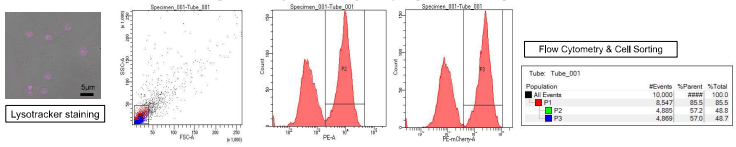
- Transmission Electron Microscopy (TEM)



Method 2. Confocal Microscopy & FISH (Fluorescent In Situ Hybridization)



Method 3. FACS & Staining with Specific Dyes (organelles, cytoskeleton, membranes, etc.)



Electron microscope

- Investigating ultrastructure of tiny eukaryotes
- Observing symbiont presence and absence and their localization

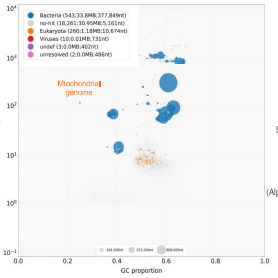
Confocal microscopy

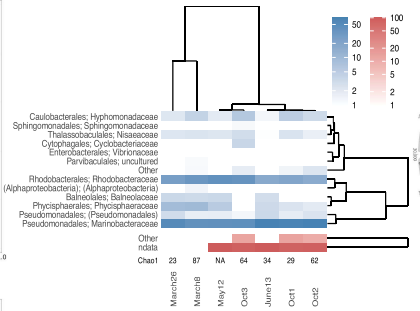
- Using DNA/RNA probes to detect free-living and symbiotic bacteria
- Super-resolution imaging for detecting localization of symbionts in the eukaryotes

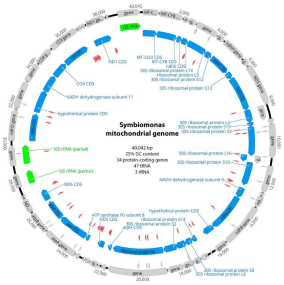
FACS

- Sorting tiny eukaryotes from bacteria co-existing in the culture for further applications

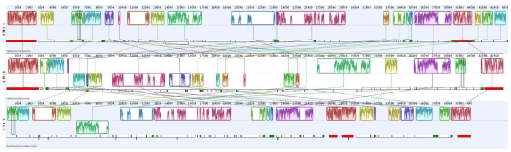
Method 4. Metagenomics

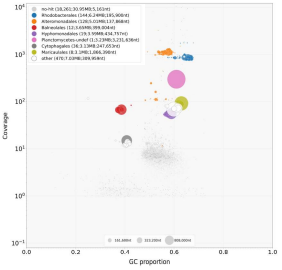


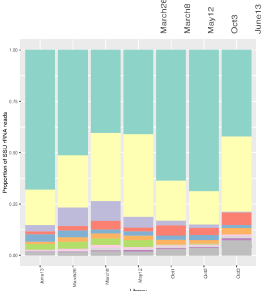




Mitochondrial genome assembly


Mitochondrial genome comparison with related species
Top: *Cafeteria*, Middle: *Cafileria*, Bottom: *Symbiomonas*





- Blobtools plots show eukaryotic and bacterial contigs
- <10 bacteria are present in the culture/metagenome

- 16S rRNA gene analysis showing presence of bacteria and *Symbiomonas* in the culture
- Identical bacteria are maintained among all our cultures even though proportion of bacteria changed depending on the culture age

Genomics highlights

- Draft genomes for all bacterial species in the culture now available
- Potential bacterial symbionts
- *Symbiomonas* has a gene-rich mitochondrion

Future directions

- Super-resolution 3D imaging to uncover interactions of nano- and pico-eukaryotes with their prey and symbionts
- Comparative genomics (lineages with and without symbionts)
- Cell enrichment with FACS and culturing improvements (1-2 prey spp.)
- Cell biology and development of genetic tools

References:
1. Jirosova D. et al. " Morphology, Ultrastructure, and Mitochondrial Genome of the Marine Non-Photosynthetic Bicosoecid Cafileria marina Gen. et sp. nov" Microorganisms 2019, 7, 240; doi:10.3390/microorganisms708024
2. Guillou et al. "*Symbiomonas scintillans* gen. et sp. nov. and *Picophagus flagellatus* gen. et sp. nov. (Heterokonta): Two New Heterotrophic Flagellates of Picoplanktonic Size" Protist 1999, vol. 150: 383-398
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