Investigating the Prey Range and Feeding Preferences of Groove-Bearing, Eukaryote-Eating Flagellates

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Background

- Phagotrophy is a widespread and ancestral eukaryote-exclusive trait.

- Ancestral eukaryotes are hypothesized to have fed similarly to living 'typical excavates', who eat bacteria by generating a feeding current using a vaned flagellum beating inside a groove[1,2,3].

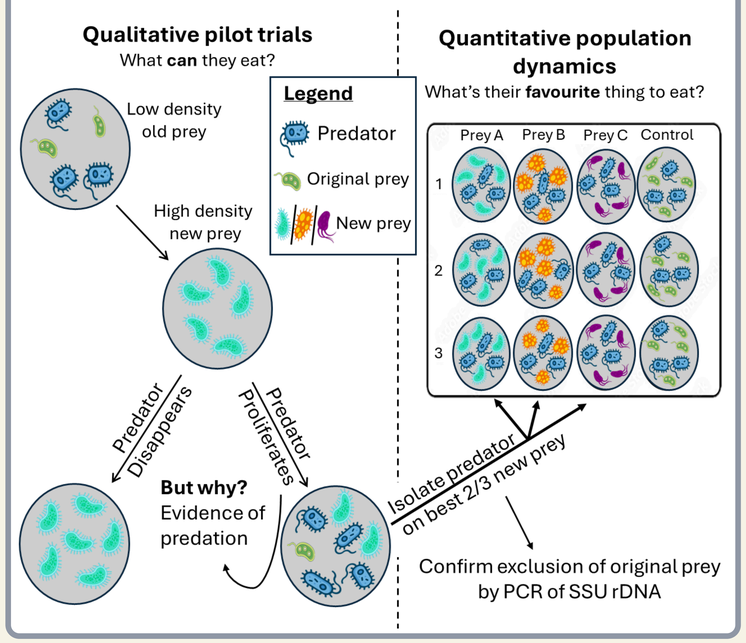
- Distantly related groups of predatory protists, like Colponemids and Provorans, possess similar looking vane-and-groove structures. However, because they feed on other eukaryotes, they must be using this structure differently.

- Only a single kinetoplastid prey species for each group of predators is known, limiting further research and impairing understanding of ecological role.

Aims

- Determine prey range of colponemids and provorans
- Create additional di-eukaryotic cultures for each group of predator by successfully isolating to exclude original prey.
- Identify prey species that leads to most rapid growth of predator

Materials and Methods



Results

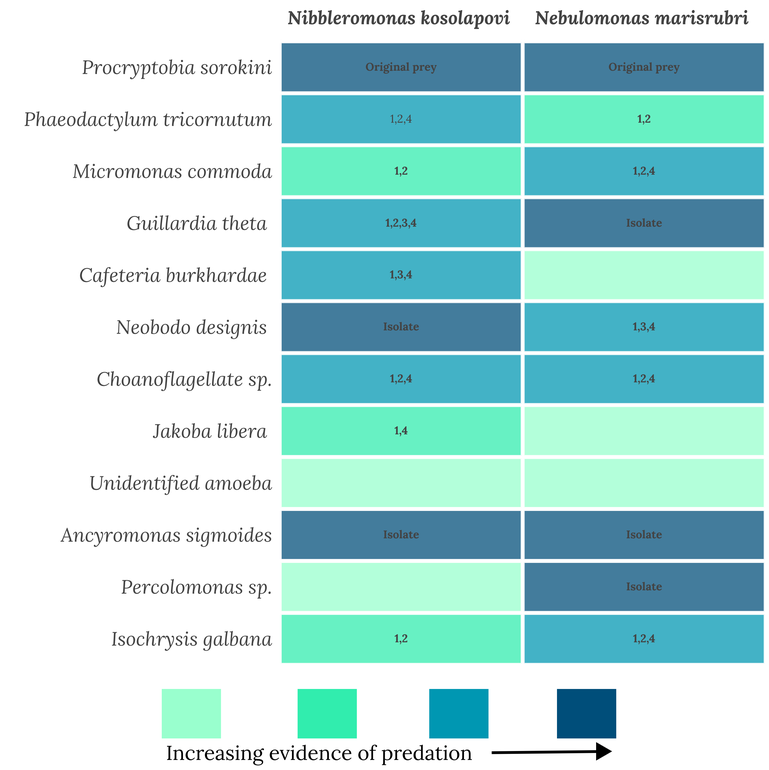


Figure 1 Heatmap displaying confidence that predation is occurring between each combination of provoran predator, with each type of prey. "Isolate" indicates growth after single-cell isolation. Numbers within each box correspond to the same number within the Evidence for Predation on New Prey below.

Single-cell Isolation

Confirmed absence of original prey following single-cell isolation using PCR primers designed to recognize SSU rDNA of *P. sorokini* in the cultures labeled "Isolate" listed in Figure 1.

Evidence for Predation on New Prey in non-isolated cultures

1 = Successive generations re-inoculated onto new prey
2 = Direct observation of vacuoles containing algal plastids
3 = Direct observation of predation
4 = Correlating density changes for predator/prey

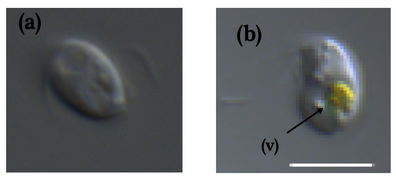


Figure 2 Differential interference contrast (DIC) images of live *Nebulomonas marisrubri* cells in culture with high density *Guilardia theta* under 160X magnification. (a) Cell without visible food vacuoles. (b) Food vacuoles (v) containing pigmented fragments of *G. theta*. Scale bars = 5 µm.

Discussion

- Provorans are generalist feeders. Their prey range is not limited to kinetoplastids.

- Provorans may specialize in feeding on prey species that associate with a substrate.

- Some new prey species grow to a higher density than the original prey, facillitating a higher rate of predator-prey combinations improving feasability of studying provoran feeding process.

- Culturing conditions expanded for both groups of predators, facilitating future studies.

- Freshwater Colponemids require new array of prey options.

Conclusions

- Expanded prey range of provorans
- Isolated both groups of provorans onto 2/3 new prey species.

Next Steps:

- Determine prey range of Colponemids
- Measure growth of predator in quantitative population dynamics

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References

1 = Burki F, Roger AJ, Brown MW, Simpson AGB. 2020. The new tree of eukaryotes. *Trends Ecol Evol.* 35(1):43–55.
2 = Suzuki-Teller M, Tikhonenkov DV, Mylnikov AP, Keeling PJ, Ishii A. 2024. Flagellar vane dynamics in excavates reveal conserved mechanisms of prey capture. *Curr Biol.* 34:1027–1039.
3 = Williamson C, Brown MW, Tikhonenkov DV, Simpson AGB. 2025. Revising the root of the eukaryotic tree with expanded phylogenomic sampling. *Proc R Soc B.* (in press).