The Phylogeny and Taxonomic Position of GEM-kin, a Culture Representing The Evolutionarily Pivotal Kinetoplastid Taxon Allobodonidae

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Introduction

Kinetoplastids are unicellular heterotrophic eukaryotes that have one or two flagella arising from a flagellar pocket [1,2]. They may be free-living, endosymbiotic, or parasitic, and can live in freshwater, soil, marine, hypersaline and alkaline habitats [1,3]. They are united by having a 'kinetoplast', a unique mitochondrial DNA organisation [1,4].

There are five main groups of kinetoplastids: prokinetoplastids, neobodonids, parabadonids, eubodonids, and trypanosomatids[5]. A possible sixth group, Allobodonidae, has only one genus and species in it, *Allobodo chlorophagus* [6]. Allobodonid has an uncertain placement within kinetoplastids due to a lack of molecular data other than SSU rRNA sequences [6,7].

GEM-kin is a free-living allobodonid that consumes bacteria[8,9]. GEM-kin was suspected to be an allobodonid due to preliminary SSU rRNA gene sequencing[8]. As one of the few available cultures of an allobodonid, further study may provide insight into where allobodonids sit within kinetoplastids and thus can help us learn more about their deep level evolutionary history.

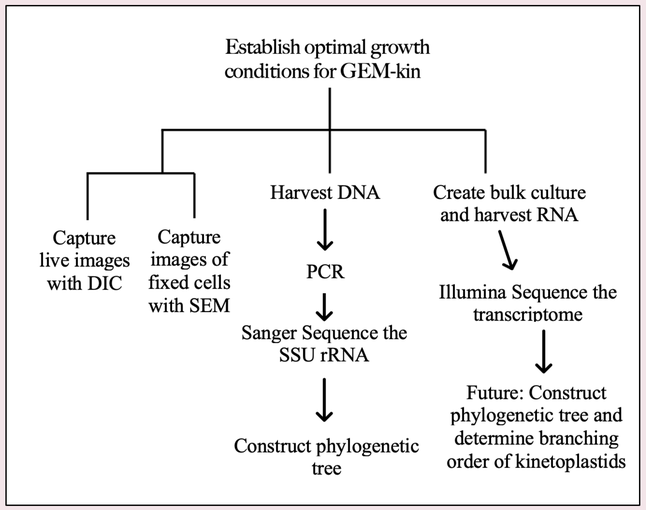
Aims

I. Describe the morphology of GEM-kin and compare with that of other allobodonids, and kinetoplastids at large.

II. Confirm if and where GEM-kin branches in Allobodonidae by inferring phylogenetic trees from SSU rRNA data.

III. Gather transcriptome data to allow a determination of where allobodonids branch within Kinetoplastidea.

Methods



Results

The highest abundance of GEM-kin was seen in CR10 media after 12 days growth. These conditions allow for bulk culture collection for RNA harvesting.

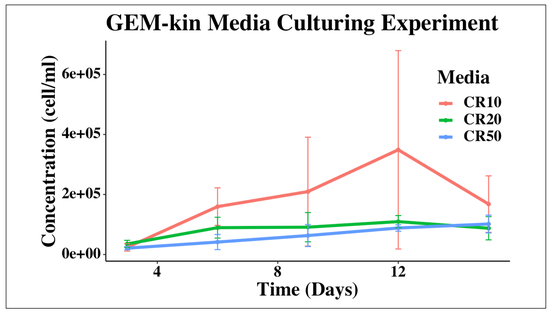


Figure 1 15-day observation study of the growth of GEM-kin in carbonate-rich (CR) media solutions using triplicates with three different salinities (labelled in parts per thousand). Abundance was measured using a hemocytometer and DIC microscope with a 40X objective.

GEM-kin has 2 flagella, a shorter anterior and a longer posterior arising from one flagellar pocket. Perpendicular to the flagellar pocket lies a very broad opening at the apex of the cell, this is the feeding apparatus. The cell has an average length of 7.9 µm (SD = 1.6, N = 30).

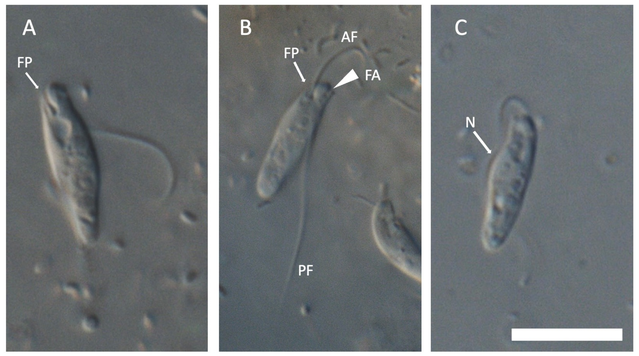


Figure 2 Differential interference contrast (DIC) images of live GEM-kin cells under 160X optical magnification. (A) Cell with feeding apparatus visible. (B) Cell with an anterior flagellum (AF) and posterior flagellum (PF) arising from flagellar pocket. Scale bar represents 10 µm for all images.

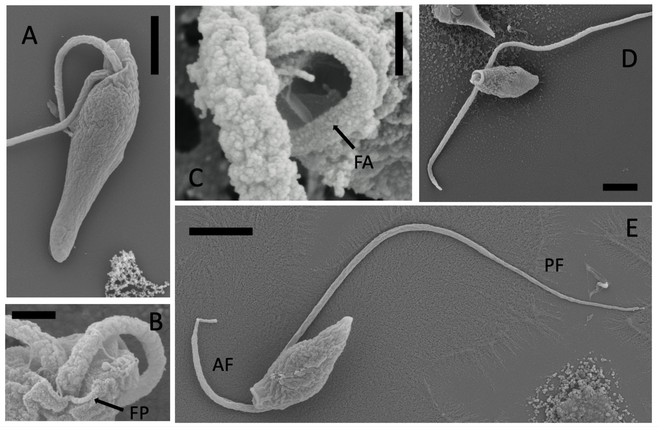


Figure 3 Scanning electron microscopy (SEM) images of GEM-kin. A) Ventral view of cell. B) Opening of flagellar pocket (FP). C) Feeding apparatus (FA). D) Anterior view of cell. E) Dorsal view of cell with the anterior flagellum (AF) and posterior flagellum (PF) in view. Scale bars are 2 µm (A, D, E), 600 nm (B) and 300 nm (C).

A BLAST search strongly suggests GEM-kin is an Allobodonid, with 94.22% identity to *A. chlorophagus*. A maximum likelihood phylogenetic analysis places GEM-kin in Allobodonidae with full bootstrap support. GEM-kin appears to be a member of the freshwater clade of Allobodonids.

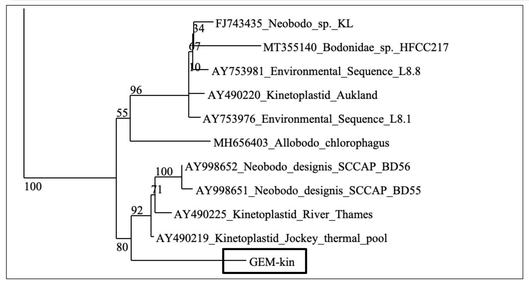


Figure 4 SSU rRNA phylogenetic tree constructed using a maximum likelihood method with 200 bootstrap replicates. Phylogenetic tree shows only the Allobodonid clade, but 154 other kinetoplastids were included in the analysis. Tree was constructed using 1877 sites with a GTR + Γ + I model in Seaview.

Discussion

- Morphological data shows us that the overall shape and size of GEM-kin is similar to that of *Allobodo chlorophagus*, but it may have a larger feeding apparatus than *A. chlorophagus.*
- GEM-kin seems most closely related to the freshwater clade of undescribed allobodonids rather than *A. chlorophagus* . This suggests that GEM-kin could represent a new genus within Allobodonidae.
- Future TEM imaging may reveal more detail about the internal cellular features of GEM-kin, such as the kinetoplast and the electron - dense globular bodies which appear in *A. chlorophagus.*
- The culturing experiment provided information on the optimal harvesting conditions of GEM-kin. This allowed us to harvest RNA which has been submitted for transcriptome sequencing.

Conclusion

I. The morphology of GEM-kin has been described to have 2 flagella, a flagellar pocket, and a large feeding apparatus.

II. GEM-kin branches in Allobodonidae, but not as a sister group to *A. chlorophagus,* and may represent a new genus.

III. Since GEM-kin is an allobodonid, pending transcriptome data can be useful for revealing where Allobodonids branch within kinetoplastids.

References

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Acknowledgements

I would like to thank Dr. Alastair Simpson for supervising me in this project, and for providing funding support, and graduate student Liz Weston for training me in lab procedures and for providing moral support during my honours year. Finally, I would like to thank my friends and family for their constant encouragement.