

Taxonomic Study on a New Granofilosean Strain JV1801

Julia Keiko Estes Vaughan (筑波大学 生物学類)

指導教員：石田 健一郎 (筑波大学 生命環境系)

Introduction

Cercozoans are a diverse group of protists that belong to the supergroup Rhizaria. They form a monophyletic group, but their morphological diversity and ambiguity in molecular analyses has clouded the phylogenetic relationships within the group. Cercozoans are all heterotrophic, except for the Chlorarachniophytes and three species of *Paulinella*. Their heterotrophy allows them to survive in various environments such as: soil, seawater, freshwater, and plant leaves. This diversity can also make them hard to culture; it is estimated that only 0.1-1% of bacteria and protists can easily be cultured. This culture bias leaves it unclear whether easily cultured protists play a major role in their environments or if they are simply easy to culture. Discovering new culturing methods and studying newly discovered species in depth can help us better understand the interactions that occur in these environments.

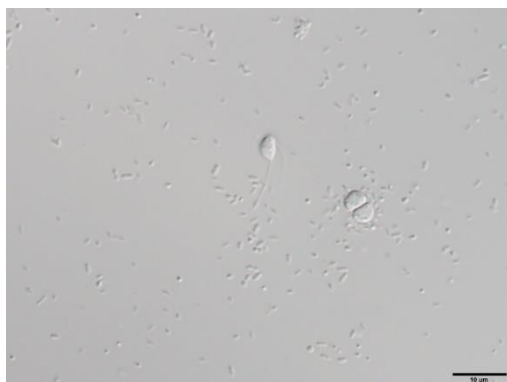


Figure 1: JV1801 in both amoeboid and flagellar stage.

In a course of searching for new protists, a cultured strain that seemed unidentifiable under a light microscope was established, although it was similar to some members of class Granofilosea which belongs to the Cercozoa. The strain was named JV1801.

Aim

My goal is to characterize the strain JV1801 and find its taxonomic home. This will expand our knowledge of cercozoan species that exist in marine environments, which are particularly understudied. To characterize JV1801, both phylogenetic and morphological analysis took place.

Materials and Methods

The strain JV1801 was maintained in ESM medium with contaminant bacteria as a food source and kept at 20°C under dark conditions. For morphological and ultrastructural characterization of the strain JV1801, a light microscope equipped with differential interference contrast (LM), a scanning electron microscope (SEM) and a transmission electron microscope (TEM) were used.

For phylogenetic analysis, genomic DNA of JV1801 was extracted using DNeasy Plant Mini Kit for DNA extraction, the 18S rRNA gene was amplified by PCR with KOD One Master Mix, which was sequenced directly. After acquiring the sequence, a maximum likelihood estimation was used to make a phylogenetic tree.

Results and Discussion

Microscopic observations (Figure 1, 2) revealed a biphasic lifecycle in JV1801, similar to *Massisteria spp.* JV1801 had a swimming flagellar stage, with one longer posterior flagellum of $11.1 \pm 0.25 \mu\text{m}$ and a shorter anterior flagellum of $3.79 \pm 0.21 \mu\text{m}$ ($n=6$). However, this flagellar stage was observed less frequently than its amoeboid stage. In its immotile amoeboid stage, cell bodies were $3.57 \pm 0.09 \mu\text{m}$ ($n=40$) in diameter. These cell bodies had extrusome-bearing pseudopodia radiating from them. The number of pseudopodia ranged from one to seven ($\bar{X}=4 \pm 0.33$) that were $14.03 \pm 1.18 \mu\text{m}$ in length ($n=40$). Unlike the morphologically similar *Massisteria spp.*, JV1801 did not have branching pseudopodia.

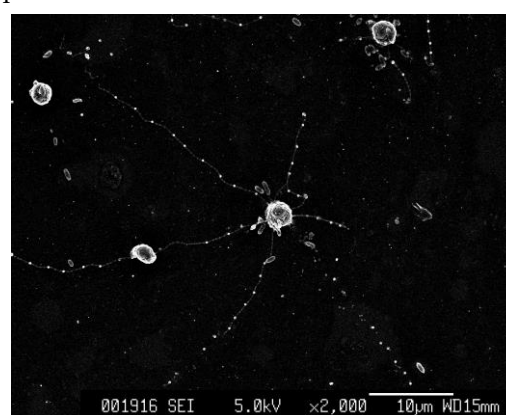


Figure 2: SEM image of JV1801

The phylogenetic tree revealed that JV1801 formed a monophyletic lineage with *Cathrulina elegans* and *Hedriocystis reticulata* in the Granofilosean clade (Fig 3).

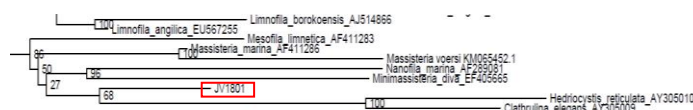


Figure 3: Partial phylogenetic tree containing JV1801 and other granofiloseans.

Conclusion

Considering the results of both phylogenetic analysis and careful characteristic assessment, I concluded that the strain JV1801 should be treated as new genus and species of Granofilosea. More studies should be done to further elucidate the species diversity of phylum Cercozoa.

References

- 1) Del Campo J, Not F, Forn I, Sieracki M, Massana R. 2013. Taming the Smallest Predators of the Oceans. ISME Journal. 7: 351-358.
- 2) Mylnikov A, Weber F, Jurgens K, Wylezich C. 2015. *Massisteria marina* has a sister: *Massisteria voersi* sp. nov., a rare species isolated from coastal waters of the Baltic Sea. EJOP. 51: 299-310.
- 3) Cavalier-Smith T & Chao E. 2003. Phylogeny and Classification of Phylum Cercozoa (Protozoa). Protist. 154: 341-358.