Protist, Vol. 161, 133–147, January 2010
http://www.elsevier.de/protis
Published online date 5 November 2009

Protist

ORIGINAL PAPER

Ultrastructure, Life Cycle and Molecular Phylogenetic Position of a Novel Marine Sand-Dwelling Cercozoan: *Clautriavia biflagellata* n. sp.

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Submitted March 27, 2009; Accepted August 16, 2009
Monitoring Editor: Michael Melkonian

Clautriavia **is a genus of uncertain taxonomic affinity that was initially described as gliding cells with one prominent trailing flagellum and a mid-ventral groove. The genus has been classified either with euglenids on the basis of similar paramylon-like granules or with cercozoans, specifically *Protaspis* spp., on the basis of general similarities in cell morphology and behavior. We isolated and cultivated a novel species of *Clautriavia*, namely *C. biflagellata* n. sp., from marine sand samples collected from the west coast of Vancouver Island, Canada and characterized this isolate with high resolution microscopy (LM, SEM, and TEM) and small subunit (SSU) rDNA sequence. The gliding cells of *C. biflagellata* n. sp. were round to oval in outline (12-20 μm wide and 15-20 μm long), dorsoventrally flattened, and capable of engulfing other eukaryotic cells (e.g., diatoms). The cells possessed two recurrent flagella of unequal length that emerged from a subapical pit within a ventral depression: the longer prominent flagellum was about 2X the cell length; the shorter flagellum was inconspicuous and was confined to the ventral depression. Molecular phylogenetic analyses demonstrated that *C. biflagellata* n. sp. branched strongly within the Cercozoa, but was only distantly related to *Protaspis* spp. Instead, *C. biflagellata* n. sp. branched closely with the recently established Auranticordida clade, consisting of *Auranticordis quadriverberis* and *Pseudopirsonia mucosa*. This position was concordant with our ultrastructural data, which demonstrated several features shared by *A. quadriverberis* and *C. biflagellata* n. sp. that are not present in *Protaspis* spp.: (1) a dense distribution of pores on the cell surface; (2) a distinct layer of muciferous bodies immediately beneath the cell surface; (3) a robust microtubular root attached to the anterior end of the nucleus; (4) the absence of a thick cell covering; and (5) the absence of conspicuously condensed chromosomes.**

Key words: *Auranticordis*; Cercozoa; comparative ultrastructure; marine interstitial flagellates; phylogenetic analysis; *Protaspis*; SSU rDNA.

Introduction

Massart originally established *Clautriavia* in 1900 for gliding phagotrophic flagellates in interstitial environments with a non-metabolic cell, a single

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doi:10.1016/j.protis.2009.09.001

recurrent flagellum and a mid-ventral groove. Since that time, only three species of *Clautriavia* have been described with light microscopy: *C. mobilis* Massart, 1900 (the type species), *C. parva* Schouteden, 1907, and *C. cavus* Lee and Patterson, 2000. The cell morphology and behavior of these flagellates are essentially indistinguishable from species of *Protaspis* (Cercozoa), except that members of the latter group possess two heterodynamic flagella rather than only one prominent recurrent flagellum (Chantangsi and Leander in press; Hoppenrath and Leander 2006a). Accordingly, *Clautriavia* has been interpreted to be descendents of *Protaspis*-like ancestors that have subsequently lost the anterior flagellum (Larsen and Patterson 1990). However, the general morphological features of *Clautriavia* and *Protaspis* are also shared by several other very distantly related groups of eukaryotes living in the same environments, such as phagotrophic euglenids, cercozoans, dinoflagellates, and katablepharids; in fact, *Clautriavia* was once closely affiliated with euglenids based on the presence of paramylon-like granules within the cytoplasm (Walton 1915). Because of this phylogenetic uncertainty and the very poor state of knowledge about this group, *Clautriavia* is currently treated as "eukaryotes of uncertain taxonomic affinity".

Ultrastructural data and comparative analyses of DNA sequences are necessary to better understand the basic cellular organization, phylogenetic position, and evolutionary history of *Clautriavia* and the multitude of other heterotrophic flagellates thriving in marine interstitial environments. In this vein, we discovered, isolated, and successfully cultivated a novel species of *Clautriavia* living in marine sand samples collected from the eastern Pacific Ocean. We were then able to characterize the general ultrastructure, life cycle, and molecular phylogenetic position of this novel lineage using small subunit (SSU) rDNA sequence, scanning and transmission electron microscopy, and high-resolution light microscopy.

Results

General Morphology and Life Cycle

The cell shape of the *Clautriavia* isolate was circular to broadly ovate and was slightly concave ventrally, particularly near the flagellar insertion point (Fig. 1A-C, E-G). Two recurrent flagella of unequal length emerged from the same flagellar pit positioned on the anterior side of a shallow ventral depression (Fig. 1B, D, F-G). The shorter flagellum was thin, inactive and inconspicuous; the longer flagellum was thicker and involved in gliding motility along substrates. The short flagellum could only be observed with careful examination (Fig. 1D, F-G). The longer flagellum was about 2X the cell length and was vigorously motile when the cells were pipetted into the water column and during cell division (Fig. 1B, E). The cell surface of the *Clautriavia* isolate was covered with an interspersed distribution of minute pores (Fig. 1E-H).

Although a permanent oral or feeding apparatus was not present, the *Clautriavia* isolate fed on small diatoms and coccoid "green" algae through the ventral side of the cell (Fig. 2D-E, G). The formation of a common food vacuole was observed in the plasmodium stage (Figs 2D-E, 4A). The emergence of pseudopodia for locomotion and feeding was never observed in the culture condition. Reproduction was achieved by one of two possible methods depending on the density of prey cells in the culture as illustrated in Fig. 6: (1) binary division of a uninucleated parent cell, producing two uninucleated daughter cells (Figs 2A-C, 3); and (2) production of large plasmodia (i.e., multinucleated cells with upwards of 20 nuclei) that subsequently divide multiple times to form several uninucleated daughter cells (Figs 2D-G, 4). Binary fission of uninucleated parent cells occurred when prey cells in the culture dish were relatively scarce; the cleavage furrow formed along the mid-sagittal plane and proceeded from the anterior end of the cell toward the posterior end (Figs 2A-C, 3B-C). Large multinucleated plasmodia generated by multiple nuclear divisions formed when prey cells in the culture dish were abundant. Locomotion of the plasmodium stage varied depending on its shape. Flat plasmodia (Fig. 2D) were capable of gliding along the substratum by means of flagella; large spherical or irregular plasmodia (Fig. 2F) usually did not glide although flagellar beating was noticeable.

Main Cytoplasmic Components

The cytoplasm of the *Clautriavia* isolate was generally colorless except for food vacuoles containing pigmented prey cells and for a few pigmented granules (Figs 1A-D, 2, 3B, 4A, 5F). The cells also contained large lipid globules and numerous mitochondria with well-defined tubular cristae (Figs 1D, 3A-C, 4A-B, 4D, 5A, E-F). The