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A New Species of the Genus *Metacystis* (Ciliophora, Prostomatida, Metacystidae) from a Wastewater Treatment Plant

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ABSTRACT. Unusual prostomatid specimens were found in the biological reactor of a wastewater treatment plant in a health resort in Valencia, Spain. These ciliates were attached to flocs unlike other free-swimming prostomatid ciliates described to date in the mixed liquor of activated sludge plants. The morphological study of this species led to a typically different combination of characteristics: elongated cell shape, 20–30 somatic kineties, 2 perioral kineties, and 1 circumoral kinety, 1 large vacuole protruding at the terminal end, a lorica tapered toward the aperture with a smooth neck, and 11–16 annular ridges. These characteristics place this representative as a new species of the genus *Metacystis*—*Metacystis galiani* n. sp. This species became the dominant population within the biological reactor when high values of conductivity (4,244 mS/cm) and temperature (26.8 °C) were recorded.

Key Words. Activated sludge, high conductivity habitat, protist.

BIOLOGICAL reactors of sewage treatment plants receive inputs with different physical–chemical characteristics that determine variable biocenosis structures. The main components of this ecosystem are bacteria that reach 90–95% of the biomass, while protists and other microorganisms represent just 5–10% of the total biomass (Bitton 2005). The established community is organized as bioaggregates or flocs in which the biotic component is embedded in a complex matrix composed of organic and inorganic particles (contained in the sewage) and extracellular polymeric substances produced by the microbial activity (Barker and Stuckey 1999; Laspidou and Rittmann 2002).

Ciliates, often one of the most significant groups of protists within this ecosystem, have been divided into two categories depending on their ecological niche inside the reactor (Madoni 1988; Martín-Cereceda, Serrano, and Guinea 1996, 2001; Poole 1984): (i) associated with flocs are two functional groups—crawling ciliates with flattened bodies and a specialized ventral ciliature allowing the movement on the floc surface and sessile ciliates attached to the floc through stalks or adhesive structures; and (ii) free-swimming ciliates moving freely in the mixed liquor. The first category of ciliates is generally more abundant (Curds and Cockburn 1970; Madoni 1988; Martín-Cereceda, Serrano, and Guinea 1996).

Species of prostomatid ciliates are scarce in conventional activated sludge wastewater treatment plants (WWTP), never reaching higher numbers or reported as the dominant population under stable reactor conditions (Curds 1969; Esteban, Téllez, and Bautista 1991; Madoni 1988; Martín-Cereceda, Serrano, and Guinea 1996). Exceptionally the prostomatid swimming ciliate *Coleps hirtus* has been described in biological reactors showing high efficiency in nitrification processes (Bick 1972; Madoni, Davoli, and Chierici 1993; Poole 1984). However, our group showed recently that several prostomatid species could be stable components in the biological communities of activated sludge plants with advanced nitrogen removal systems (Pérez-Uz et al. 2009).

The aim of this work was to characterize a sessile prostomatid ciliate found as a stable population associated with flocs in the biological reactor of a WWTP from a health resort with a very specific set of physical–chemical conditions. We conclude that this is a new species *Metacystis galiani* n. sp.

MATERIALS AND METHODS

Sessile ciliates were sampled from the biological reactor of a WWTP of a health resort located in Cofrentes, Valencia, Spain (39°13′N, 1°04′W). Samples were collected in plastic bottles and taken to the laboratory for analysis. Fifty-microliter aliquots containing living cells were observed with a phase contrast microscope using either an Olympus BH-5 (Olympus Optical España S. A., Madrid, Spain) or a Zeiss Axiostar microscope (VWR International Eurolab S. L., Barcelona, Spain). Digital images were taken with a digital camera (Olympus Camedia C-5050 or a Digital Still Camera Sony DKC-CM30) attached to the microscope. Linear measurements were obtained from the digital images with Adobe Photoshop 6.0 after calibration with a 1-mm Zeiss stage micrometer.

Ciliates were stained using the pyridinated silver carbonate method of Fernández-Galiano (1976, 1994) and the protargol impregnation modification of Wilbert (1975). Permanent slides of both staining techniques were deposited at the Museo Nacional de Ciencias Naturales (Madrid, Spain) with accession number MNCN 39.04/1.

The specimens were also stained with Flutax-2, a fluorescent derivative of taxol that binds specifically to microtubules (provided by J.M. Andreu of the Centro de Investigaciones Biológicas, Consejo Superior de Investigaciones Científicas, Madrid) as described in Arregui et al. (2003) in order to acquire complementary data to those obtained with the silver impregnations since optimal silver impregnation images were difficult to obtain due to the presence of a lorica. Samples were observed under a Zeiss Axioplan 2 epifluorescence microscope using 40X and 63X objectives. Images were recorded with a CCD Spot Camera and then analyzed using the MetaMorph Imaging System software (Universal Imaging Corporation).

Physical–chemical parameters were measured according to standard methods (APHA 1989).

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