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Discomorphella pedroeneasi sp. nov. (Ciliophora, Odontostomatida): An anaerobic ciliate hosting multiple cytoplasmic and macronuclear endocytobionts

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Abstract

Odontostomatids are among the less studied representatives of the Ciliophora. They are anaerobic microeukaryotes usually occurring as rare species in sapropelic environments. Here we describe a novel species of *Discomorphella*, named *Discomorphella pedroeneasi* sp. nov., using light and electron microscopy observations. *Discomorphella pedroeneasi* displays many complex morphological features, for which new terms are introduced, such as the auricules, epistomial fringe spacer, frontal awning, odontostomatid ciliary sockets, oral lips and ventral flap. Remarkably, *D. pedroeneasi* has at least six types of endocytobionts, including intramacronuclear ones, and organelles ultrastructurally similar to the M/H bodies of the odontostomatid *Saprodinium dentatum*. Based on the new data and revision of the literature, we propose two new diagnostic characters for species separation within *Discomorphella*: the fringe spacer ratio and the posterior fringe ratio. The taxonomy of *Discomorphella* is revised and an identification key is provided.

Keywords: Armophorea; Brazil; Endosymbiosis; Odontostomatida; Plagiopylea; Taxonomy

Introduction

Free-living anaerobic ciliates occur in anoxic and chemically reducing environments, may be killed or metabolically inhibited by oxygen, and most are associated with eutrophized environments (Fenchel and Finlay 1991). Among such ciliates, the Odontostomatida Sawaya, 1940, are represented by minute, but morphologically complex, unicellular

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organisms which have been recognized as potential bioindicators for sapropelic ecosystems (Bick 1972; Foissner et al. 1992; Fenchel 1987; Lynn 2008). Although knowledge of their ultrastructure is still incipient, it is known that they lack typical mitochondria and/or hydrogenosomes, thus their metabolism is assumed to be linked with both bacterial and archaeal endosymbionts (Foissner et al. 1992). According to Schrenk and Bardele (1991), only about 36 species of odontostomatids have been described, and the meager knowledge of their biology is generally attributed to their rarity, with most descriptions made from a few specimens taken directly from their habitats.

Among the odontostomatids, *Discomorphella* Corliss, 1960 is the sole representative of the family Discomorphellidae Corliss, 1960 (Corliss 1960; Foissner et al. 1992; Lynn 2008; Tuffrau 1992). *Discomorphella* is characterized by having a long acute spine projecting from a clear, wide dorsal keel; a perizonal complex that includes five upper transversal kineties forming the epistomial and the posterior fringes; and the presence of long cirri at the posterior end of body (Jankowski 1964; Tuffrau 1992). We found a population of *Discomorphella* inhabiting stagnant water of an artificial pond in Rio de Janeiro, which differs from the type species, *D. pectinata* (Levander, 1894) Corliss, 1960, its subspecies *D. pectinata bidenticulata* (Kahl, 1932b) Corliss, 1960, and *D. lauterborni* (Wetzel, 1928) Corliss, 1960. Here we describe the new species *Discomorphella pedroeneasi* sp. nov., based on observations of live specimens, protargol impregnation, and both scanning and transmission electron microscopy. We discuss the morphology of the new species and its endocytobionts in comparison with the literature, and review the taxonomy of *Discomorphella*.

Material and Methods

Samples of water with bottom sediment were collected in sterilized glass flasks from an artificial pond located in the outer yard of the Rector office building on the campus of Universidade Federal do Rio de Janeiro, Ilha do Fundão, RJ, Brazil (22°51′47.3″S 43°13′27.6″W), at various times during the period from 2011 to 2015. Water characteristics were measured in freshly collected samples, using an electronic multiparameter device (Hanna Instruments, USA). *Discomorphella pedroeneasi* usually disappeared from samples one day after collection, and attempts at cultivation failed. Fortunately, specimens appeared frequently in fresh samples, making it possible to study their morphology in detail.

The ciliates were studied initially in vivo under the stereomicroscope, and then under bright field and DIC at 100×, 200×, 400× and 1000× (oil immersion) to document their behavior and general morphology. Additional data were gathered from protargol-impregnated slides, prepared according to Wilbert (1975), and both scanning (SEM) and transmission (TEM) electron microscopy preparations, made according to Silva-Neto et al. (2012) and Schnepf et al. (1982), respectively. Morphometric data (Table 1) were obtained from protargol-impregnated specimens measured at 1000×. A 1.6× optovar device was used to facilitate counting ciliary structures. Statistics were performed with the computer program GraphPad Prism 4 (Motulsky 1999). Schematic diagrams of *D. pedroeneasi* sp. nov. are reconstitutions made with Adobe Photoshop CS5 (Adobe Systems Inc., USA), and were based on several photographs of specimens in vivo, after protargol-impregnation, and SEM images. Scale bars were used when necessary (Foissner and Xu 2007).

Table 1. Morphometric characterization of *Discomorphella pedroeneasi* sp. nov. based on protargol-impregnated specimens. Measurements are in μm.

Character	Mean	M	SD	SE	CV	Min	Max	N
Body length	45.8	46.0	3.0	0.6	6.7	40.0	50.0	25
Body width	35.7	36.0	2.9	0.6	8.2	31.0	42.0	25
Number of pectinelles in F1	14.4	14.0	0.9	0.2	6.3	12	16	25
Number of papillary dikinetids left of F1	5.6	5.0	0.8	0.3	15.1	5	7	10
Number of pectinelles in F2	5.5	5.5	0.5	1.0	9.3	5	6	24
Number of adoral membranelles	10.0	10.0	0	0	0	10	10	20
Number of supplementary ventral dikinetids	2.0	2.0	0	0	0	2	2	10
Number of dikinetids in V1	7.4	7.0	0.5	0.2	7.0	7	8	10
Number of dikinetids in V2	6.3	6.0	0.5	0.2	7.7	6	7	10
Number of precaudal dikinetids	3.0	3.0	0	0	0	3	3	15
Number of caudal cirri	2.0	2.0	0	0	0	2	2	15
Distance from front-most pectinelle in F2 to posterior end of body	5.7	5.5	0.8	0.2	13.4	5.0	7.0	24
Distance from hind-most pectinelle in F2 to posterior end of body	11.5	11.0	1.3	0.6	11.1	10.0	15.0	24
Length of macronucleus	12.2	11.5	2.0	0.6	16.8	10.0	16.0	10
Width of macronucleus	17.3	16.0	2.5	0.8	14.4	15.0	22.0	10

Abbreviations: CV = coefficient of variation in %; F1 = dextro-frontal fragment of epistomial fringe; F2 = left fragment of epistomial fringe; M = median; Max = maximum value observed; Mean = arithmetic mean; Min = minimum value observed; N = sample size; SD = standard deviation; SE = standard error; V1 = anterior part of ventral ciliary row; V2 = posterior part of ventral ciliary row.