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Morphologic and molecular description of *Metopus fuscus* Kahl from North America and new rDNA sequences from seven metopids (Armophorea, Metopidae)

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Abstract

Most species in the large ciliate genus *Metopus* Claparède & Lachmann, 1858 lack detailed descriptions based on modern morphologic and molecular methods. This lack of data for the vast majority of species hampers application of a morphospecies approach to the taxonomy of *Metopus* and other armophorids. In this report we redescribe the large species, *Metopus fuscus* Kahl, 1927 based on in vivo observation, silver impregnation, scanning electron microscopy, and single-cell 18S rDNA sequencing of a freshwater North American (Idaho) population. *Metopus fuscus* invariably has a perinuclear envelope of endosymbiotic bacteria not found in other species. Unlike the original description of a single row of coarse granules between ciliary rows, the Idaho population has five loose rows of small interkinetal granules. We discuss the possible importance of this character in metopids. We also provide a phylogenetic analysis including seven other new metopid 18S rDNA sequences: *Brachonella spiralis*, *B. galeata*, *Metopus laminarius*, *M. setosus*, *M. striatus*, *M. violaceus*, *Palmarella lata*. *Metopus fuscus* and *M. setosus* form a fully supported clade, challenging previous morphospecies groupings. We discuss some ambiguities of armophorid morphologic terminology in the earlier literature. Our phylogenetic analysis of Idaho metopids indicates that the genera *Metopus* and *Brachonella* are both nonmonophyletic.

Keywords: Anaerobic ciliates; Armophorida; *Brachonella*; Cortical granules; Morphospecies; Silver impregnation

Introduction

Metopus Claparède & Lachmann, 1858 is a species-rich genus comprising a widely distributed group of hydrogenosome-bearing ciliates inhabiting anaerobic or microaerophilic freshwater, terrestrial and marine habitats (Corliss 1979; Lynn 2008). They are characterized morphologically by a leftward torsion of the anterior cell portion with a frontal lobe overhanging an obliquely situated adoral zone of membranelles (Esteban et al. 1995; Foissner et al. 1992; Jankowski 1964a, 1964b; Kahl 1927). Kahl (1927) separated the genus into six informal groups according to body shape and characteristics of the peristomial structures. Several attempts at revision (Esteban et al. 1995; Jankowski 1964b; Kahl 1927; Wetzel 1928) have resulted in more contention than clarity (Dragesco 1996; Foissner and Agatha 1999; Kahl 1929). Unfortunately, the detailed morphologic and morphometric data required for such efforts are still lacking for armophorids in general and *Metopus* in particular (Dragesco 1996; Foissner and Agatha 1999). An estimate (Roskov et al. 2013) at this writing includes 78 nominal species,

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23 infraspecific taxa and 26 supposed synonyms. Although protist species definitions, in general, remain problematic, the establishment of a coherent taxonomy of *Metopus*, will require much more detailed morphologic, molecular and ecological data (Boenigk et al. 2012).

One of the largest *Metopus*, *M. fuscus* Kahl, 1927, belongs to Kahl's third group that also includes the type species *Metopus es* (Müller, 1776) Lauterborn, 1916. We found a population of *Metopus fuscus*, during a broader study of free-living armophorids from Idaho, USA. In this report, we provide a detailed morphologic description, morphometrics, and 18S rDNA sequence of a North American population of *M. fuscus* and compare it with previous descriptions. A phylogenetic analysis includes the Idaho *M. fuscus* and seven other newly sequenced metopids. We also discuss the implications of our findings in relation to the shortcomings of the "morphospecies" concept as it has been applied to the free-living Armophorida (Esteban et al. 1995; Finlay et al. 1996).

Material and Methods

Collection data

We first found *M. fuscus* in sediments of a pond near Boise, Idaho (43°40′57.20″ N 116°15′15.44″ W; elev. 873 m) in June 2006. The locality is described in detail in the occurrence and ecology section below. The current report is based on this population collected from the original site and a subsample maintained for the past seven years in an open garden tub in Boise. The pond population and the garden tub population are indistinguishable. Thus, the results for both populations are combined. Attempts to establish pure cultures were unsuccessful. Conductivity measurements were done directly on water samples using an ExStik EC meter (Spectrum Technologies, Inc. Plainfield, IL, USA).

In addition to the perennial population of *M. fuscus*, this open mesocosm supports a diverse community of other armophorids including *Metopus striatus*, *M. laminarius*, *M. setosus*, *M. violaceus*, *Brachonella galeata*, *B. spiralis*, and *Palmarella lata* for morphologic and molecular studies. These taxa will be described in a separate report. Identifications were based on previous descriptions (Foissner et al. 1992; Jankowski 1964b; Kahl 1926, 1927, 1929, 1931, 1932).

Morphologic methods

Living cells were studied at magnifications of 40–1000× with brightfield, phase- and differential interference contrast illumination using a Zeiss Axioskop 2 plus microscope (Carl Zeiss Microscopy, LLC, Thornwood, NY, USA), a Flex digital camera, and calibrated Spot imaging software (Diagnostic Instruments, Inc., Sterling Heights, MI, USA). Video imaging was done using an Olympus BX53 microscope (Olympus America, Center Valley, PA, USA) and Canon 6D camera (Canon Inc., Tokyo, Japan). In vivo measurements were made from photomicrographs of freely swimming cells. Attempts to induce formation of resting cysts by starvation in filtered (0.22 μm pore size) site water were unsuccessful as cells quickly died. Protargol impregnation, methyl green-pyronin staining, and scanning electron microscopy (SEM) were done according to Vďačný and Foissner (2012). Cells were fixed in 10% formalin for protargol impregnation and a 1:1 mixture of 2% osmium tetroxide and aqueous 2.5% glutaraldehyde for SEM. Statistical analyses were performed using MedCalc for Windows, version 11.2 (MedCalc Software, Mariakerke, Belgium). All drawings were based on microphotographs.

DNA extraction, amplification and sequencing

Cells from the tub population were selected using a stereomicroscope (90X) and washed three times in filtered (0.22 μm pore size) Eau de Volvic mineral water. Single cells were placed in 0.2 ml PCR tubes with 50 μl of EB buffer (Qiagen, Valencia, CA, USA) and stored at −20 °C. DNA was extracted from each of four cells using a modified Chelex method (Strüder-Kypke and Lynn 2003) and the 18S rDNA was amplified and sequenced as previously described (Bourland et al. 2012). Chromatograms were manually edited using 4-Peaks (Griekspoor and Groothuis 2006) and assembled into contiguous sequences using CAP3 (Huang and Madan 1999).

Phylogenetic analyses

To determine the phylogenetic position of *M. fuscus*, we analyzed an alignment comprising 18S rDNA sequences of seven taxa (three belonging to Armophorida and four to Clevelandellida) from GenBank. *Metopus fuscus* and seven other armophorids were newly sequenced in the present study (Figs 46–52). Two spirotrich taxa (*Metaurostylopsis* and *Phacodinium*) were used as outgroup. Alignments were constructed using MAFFT (Katoh and Toh 2008) based on primary structure. Ambiguously aligned regions were edited by eye. jModelTest was employed to find the model of nucleotide substitution that best fit the data (Posada 2008). The General-Time-Reversible model with invariable sites and gamma distribution ($G+I+\Gamma$) was chosen under the Akaike Information (AI) Criterion. This model was implemented in MrBayes (Ronquist and Huelsenbeck 2003) on the CIPRES Portal V 1.15 (Miller et al. 2009), with support from four simultaneous MCMC chains run for five million generations sampling every 1000 generations. The first 25% of sampled trees were considered burn-in trees and were discarded prior to tree reconstruction. A 50% majority-rule consensus of the remaining trees was used to calculate posterior probability (PP) for Bayesian inference (BI). The maximum likelihood (ML) analysis was implemented on the CIPRES Portal, using RAxML with settings as described in Stamatakis et al. (2008). Support for ML analyses came from 1000 bootstrap replicates using heuristic searches. We considered bootstrap values <70 as low, 70–94 as moderate, and ≥95 as high support (Hillis