Protist, Vol. 169, 362–405, xx 2018
http://www.elsevier.de/protis
Published online date 21 April 2018

Protist

ORIGINAL PAPER

Tropidoatractidae fam. nov., a Deep Branching Lineage of Metopida (Armophorea, Ciliophora) Found in Diverse Habitats and Possessing Prokaryotic Symbionts

Johana Rotterová[a,1], **William Bourland**[b], and **Ivan Čepička**[a]

[a]Department of Zoology, Faculty of Science, Charles University, Viničná 7, 128 00 Prague, Czech Republic
[b]Department of Biological Sciences, Boise State University, Boise, ID 83725-1515, USA

Submitted February 14, 2018; received in revised form April 3, 2018; Accepted April 11, 2018
Monitoring Editor: Joseph Frankel

We report a discovery of a novel family of anaerobic ciliates, Tropidoatractidae fam. nov. Phylogenetic analyses based on the 18S rRNA gene show that the family Tropidoatractidae corresponds to the previously reported clade of environmental sequences closely related to the lineage consisting of orders Metopida and Clevelandellida. The family comprises two genera, *Tropidoatractus* and *Palmarella*, and five species, two of which are newly described herein. Tropidoatractidae are cosmopolitan Metopida with sparse somatic and oral ciliature, deep, cup-like buccal cavity, and hyaline cortex with interkinetal ridges. Moreover, all species occur in two morphotypes, slender and stout. They inhabit microoxic or anoxic freshwater, brackish, and marine sediments and possess anaerobic mitochondrion-related organelles and various prokaryotic symbionts. The discovery of Tropidoatractidae provides valuable information about the evolution of Armophorea and gives us insights to the diversity and ecological preferences of anaerobic ciliates in general.

Key words: Anaerobic ciliates; new species; phylogeny; protargol; 18S rRNA gene.

Introduction

The processes of anaerobiosis and the previously underestimated diversity of anaerobic microbial eukaryotes have been gaining increasing attention in recent years thanks to modern methods (e.g., Edgcomb et al. 2009; Leger et al. 2017; Orsi et al. 2012; Pánek et al. 2014; Stoeck and Epstein 2003; Stoeck et al. 2003, 2010; Takishita et al. 2007). Among ciliates (Ciliophora), possibly the most intensively studied group of free-living protists, anaerobiosis has arisen independently in several lineages (Embley et al. 1995). However, there are still large gaps in our knowledge of anaerobic protists in general, and ciliates in particular (Clamp and Lynn 2017; Lynn 2003; Warren et al. 2017).

[1]Corresponding author;
e-mail johana.rotterova@natur.cuni.cz (J. Rotterová).

https://doi.org/10.1016/j.protis.2018.04.003

Multigene and phylogenomic analyses repeatedly show ciliates of the phylum Intramacronucleata dividing into two superclades, CONThreeP (Colpodea, Oligohymenophorea, Nassophorea, Prostomatea including Plagiopylea, and Phyllopharyngea) and SAL (Spirotrichea, Armophorea, Litostomatea) (Gao et al. 2016; Gentekaki et al. 2014; Lynn and Kolisko 2017). In the latter, the anaerobic lifestyle is a particularly widespread behavior. Besides the exclusively anaerobic Armophorea, both Spirotrichea and Litostomatea contain some anaerobic species or subclasses (Embley 1995; Fenchel and Bernard 1993). The anaerobic class Cariacotrichea, recently discovered in a deep anoxic marine basin, also belongs to SAL according to the 18S rDNA analyses (Orsi et al. 2012).

According to the current classification of ciliates, the class Armophorea, established by Lynn (2003), comprises the endobiotic Clevelandellida de Puytorac and Grain, 1976 (several families), mostly free-living Metopida Jankowski, 1980 (families Metopidae Kahl, 1927 and Apometopidae Foissner, 2017), and entirely free-living Armophorida Jankowski, 1964 (family Caenomorphidae) (see Foissner 2016; Lynn 2008). It has been called a "riboclass" (Lynn 2008) due to the lack of any obvious morphologic synapomorphy. However, phylogenetic analyses based on the 18S rRNA gene showed that Armophorea is likely polyphyletic, because Armophorida seems not to be closely related to the rest and forms an independent lineage within SAL instead (Bourland et al. 2017a,b; Li et al. 2017; Paiva et al. 2013). In addition, Metopida is clearly paraphyletic with respect to Clevelandellida, and *Metopus* Claparède & Lachmann, 1858, the largest genus of Metopida, is highly polyphyletic (Bourland et al. 2017a,b; Omar et al. 2017; Paiva et al. 2013).

Although many armophoreans have been known for a long time, their internal phylogenetic relationships are only recently starting to get attention and relatively few representatives have been studied in detail using modern morphological methods (Bourland et al. 2014, 2017a,b; Bourland and Wendell 2014; Li et al. 2017; Omar et al. 2017; Paiva et al. 2013; Vďačný and Foissner 2017a,b). In addition, a plethora of past synonymizations and taxonomic reassignments, as well as a confused definition of species and genus boundaries in this group, have compounded the taxonomic chaos (Esteban et al. 1995; Jankowski 1964a,b, 2007; Kahl 1932; Wetzel 1928).

Recent studies have recovered environmental 18S rRNA gene sequences of marine, brackish, and salt-lake ciliates that formed a clade sister to Armophorea with exclusion of Caenomorphidae (Bourland et al. 2017a,b; Edgcomb et al. 2009; Stoeck et al. 2003; Stoeck and Epstein 2003; Takishita et al. 2007). Inspecting this clade would provide valuable information about the evolution of Armophorea and would give us insights into the diversity and ecological preferences of anaerobic ciliates in the SAL group. In this study, we have studied 33 populations of ciliates belonging to this as yet environmental clade. Our results show that this early-branching clade is extensively diversified and represents a novel family of Metopida, Tropidoatractidae, fam. nov.

Results

Origin of Studied Organisms

Representatives of the family Tropidoatractidae fam. nov. and *Platymetopus contractus* comb. nov. are common cosmopolitan taxa that frequently inhabit microoxic or anoxic sediments in freshwater, brackish, and marine environments, as typical for Metopida. Information about the origin of the organisms included in this study is shown in Table 1. Most of the strains were maintained in long-term monoprotist or mixed cultures (usually with archamoebae, preaxostylids, heteroloboseans, or other anaerobic protists present in the minority) with the presence of unidentified bacteria, as previously described in Bourland et al. (2017a). In case of populations GLW, SAGL (*Tropidoatractus acuminatus*), GL1, BLUFF8, SHOAKOI (*T. spinosus* comb. nov.), and SMTUB (*Platymetopus contractus* comb. nov.), the cells were isolated from fresh samples and processed without establishing cultures.

General Observations and Morphology of Members of Tropidoatractidae fam. nov.

The morphology of selected strains of each species was studied in detail by light microscopy of in-vivo and protargol stained cells and by scanning electron microscopy (Figs 1–15). Measurements and morphometrics are listed in Tables 2–6. Studied strains were assigned to two genera and five species according to the morphology and phylogenetic analyses: *Tropidoatractus acuminatus* Levander, 1894, *T. ariella*, sp. nov., *T. levanderi*, sp. nov., *T. spinosus* (Kahl, 1927) comb. nov. (formerly *Metopus spinosus*), and *Palmarella salina* (Gajewskaia, 1925); for diagnoses see the Taxonomic Summary. The two genera differ ecologically