Protist, Vol. 168, 439–451, August 2017
http://www.elsevier.de/protis

Protist

ORIGINAL PAPER

Pseudophyllomitus vesiculosus (Larsen and Patterson 1990) Lee, 2002, a Poorly Studied Phagotrophic Biflagellate is the First Characterized Member of Stramenopile Environmental Clade MAST-6

Takashi Shiratori[a,1], **Rabindra Thakur**[b], and **Ken-ichiro Ishida**[a]

[a]Faculty of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan
[b]Graduate School of Life and Environmental Sciences, University of Tsukuba, Tsukuba, Ibaraki 305-8572, Japan

Submitted March 2, 2017; Accepted June 21, 2017
Monitoring Editor: Michael Melkonian

There are many eukaryotic lineages that are exclusively composed of environmental sequences and lack information about which species are included. Regarding stramenopiles, at least 18 environmental lineages, known as marine stramenopiles (MAST), have been recognized. Since each MAST lineage forms deep branches in the stramenopiles, the characterization of MAST members is key to understanding the diversity and evolution of stramenopiles. In this study, we established a culture of *Pseudophyllomitus vesiculosus*, which is a poorly studied phagotrophic flagellate of uncertain taxonomic position. Our molecular phylogenetic analyses based on small subunit ribosomal RNA gene sequences robustly supported the inclusion of *P. vesiculosus* in the MAST-6 clade. Our microscopic observations indicated that *P. vesiculosus* shared characteristics with stramenopiles, including an anterior flagellum that exhibits sinusoidal waves and bears tubular mastigonemes. The flagellar apparatus of *P. vesiculosus* was also similar to that of other stramenopiles in having a transitional helix and five microtubular roots (R1–R4 and S tubules) including R2 that split into two bands. On the other hand, *P. vesiculosus* was distinguished from other deep-branching stramenopiles by the combination of flagellar apparatus characteristics. Based on the phylogenetic analyses and microscopic observations, we established Pseudophyllomitidae fam. nov in stramenopiles.

Key words: Flagellar apparatus; MAST; phylogenetic analysis; stramenopiles; ultrastructure.

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[1]Corresponding author; fax +81 298 53 4533
e-mail wb.takashi@gmail.com (T. Shiratori).

http://dx.doi.org/10.1016/j.protis.2017.06.004

Introduction

Environmental DNA surveys focusing on small subunit ribosomal RNA (SSU rRNA) gene sequences have uncovered the great diversity of protists (Bass and Cavalier-Smith 2004; Lara et al. 2009; López-García et al. 2001). These environmental DNA surveys have revealed not only the cryptic diversity of previously described taxa (Cavalier-Smith and von der Heyden 2007; Holzmann et al. 2003; Lara et al. 2009), but also novel environmental lineages that could not be assigned to any described groups (Choi et al. 2017; Kim et al. 2016). Culture-independent studies, such as fluorescence in situ hybridization (FISH) and single cell-based genome sequencing are effective approaches to characterize these environmental lineages, and the methods have provided important information, including cell shape and size, presence or absence of plastids, and mode of nutrition (Jones et al. 2011; Yoon et al. 2011). However, traditional culture-based taxonomic studies are also important for improved characterization of these environmental lineages (del Campo et al. 2016; Moreira and López-García 2014). This approach provides detailed morphological and ultrastructural information that complements culture-independent studies and aids our understanding of the ecological role and evolutionary history of targeted organisms.

Stramenopiles are a large eukaryotic assemblage characterized by the presence of tripartite or bipartite tubular hairs (mastigonemes) on the anterior flagellum, and the group includes various organisms ranging from multicellular seaweeds (Phaeophyceae) and parasitic or saprotrophic fungus-like organisms (e.g., Hyphochytrea and Oomycetes) to diverse photosynthetic and heterotrophic protists (e.g., Bikosea, Chrysophyceae, and Bacillariophyceae). The stramenopiles also include several environmental lineages that are not classified in any described groups, which have been described as MArine STramenopiles (MAST) or Mystery Heterokont (MH) (Massana et al. 2004; Orsi et al. 2011; Richards and Bass 2005). Massana et al. (2014) rearranged newly and previously described environmental lineages into 18 MAST groups (MAST-1, -2, -3, -4, -6, -7, -8, -9, -10, -11, -12, -16, -20, -21, -22, -23, -24, and -25) by excluding synonyms and lineages that are represented by chimeric sequences.

Molecular phylogenetic analyses using SSU rRNA gene sequences indicated that most MAST lineages formed deep branches within the stramenopiles (Cavalier-Smith and Scoble 2013; Massana et al. 2014). Therefore, the characterization of MAST species is key to understanding the diversity and evolution of stramenopiles. Investigations of MAST species to date have been mainly performed using culture-independent approaches. Environmental DNA surveys showed that the abundance of MAST sequences reached around 20% of total picoeukaryotes in the surface water of open oceans (Massana et al. 2004), while some MAST lineages were specific to the anaerobic water column or to sediments (Massana et al. 2014). FISH experiments using lineage-specific oligonucleotide probes and incubation experiments revealed the approximate cell size, habitat, and prey of some MAST members (Kolodziej and Stoeck 2007; Massana et al. 2009; Piwosz and Pernthaler 2010). On the other hand, culture-dependent approaches for the identification of MAST members have not been frequently performed and only a few MAST species have been identified so far (MAST-3: *Incisomonas marina* and *Solenicola setigera*) (Cavalier-smith and Scoble 2013; Gómez et al. 2011).

In this study, we established a culture of *Pseudophyllomitus vesiculosus* (strain SRT537) from marine detritus on the tidal flat sediment of Amami Island, Okinawa, Japan. *Pseudophyllomitus* is a genus of heterotrophic free-living flagellates established by Lee (2002), and it includes four species that were reassigned from *Phyllomitus* based on morphological differences. *Pseudophyllomitus* has a sac-shaped cell body and two heterodynamic flagella that emerge from a subapical gullet or pocket (Lee 2002). Although *Pseudophyllomitus* spp. were reported in field surveys of marine sediment samples (Aydin and Lee 2012; Larsen and Patterson 1990; Lee 2015; Lee and Patterson 2000), molecular and ultrastructural data of this genus are still unavailable, thus the taxonomic position is uncertain. Our molecular phylogenetic analysis using SSU rRNA gene sequences showed that *P. vesiculosus* is a member of MAST-6. We also performed microscopic observations of *P. vesiculosus*, and compared the morphology and ultrastructure with other deep-branching stramenopiles for MAST-6 characterization.

Results

Light Microscopic Observation

The cells of *P. vesiculosus* strain SRT537 were flexible, sac-shaped, and slightly dorsoventrally flattened (Fig. 1A, B). Cell length was 9.3–18.3 μm