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Morphology and phylogeny of *Apertospathula oktemae* n. sp. (Ciliophora, Haptoria, Spathidiida) from Lake Van, Turkey

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Abstract

A new spathidiid ciliate, *Apertospathula oktemae* n. sp., was isolated from bottom sediments on the eastern shore of Lake Van, Turkey. The living cells are clavate and 45–80 × 17–30 μm in size. This species is characterized by a lasso-shaped circumoral kinety composed of more than 100 dikinetids, 16 meridionally arranged somatic ciliary rows, a three-rowed dorsal brush with 1.5–2.0 μm long bristles, an oblong and curved macronucleus with an ellipsoidal or globular micronucleus, numerous refractive granules in the anterior portion of the cell, and a single posterior contractile vacuole. This new species lives in alkaline brackish water. *Apertospathula oktemae* differs from congeners by their body shape, number of oral dikinetids, presence of refractive granules, and their habitat. This is the first study to investigate the 18S rRNA gene sequence of a member of the genus *Apertospathula.* Phylogenetic trees show that *Apertospathula oktemae* is most closely related to *Arcuospathidium* sp. There is a discrepancy between the morphological classification system of the spathidiid ciliates and their molecular phylogeny. To overcome this problem, more molecular data, obtained from more taxa from various geographical regions of the world, are needed.

Keywords: *Apertospathula oktemae* n. sp.; Morphology; Phylogeny; SSU rDNA; Turkey

Introduction

Spathidiids mainly inhabit terrestrial and semi-terrestrial habitats across the world (Foissner and Xu 2007; Jang et al. 2017). There are over 250 species distributed throughout approximately 20 genera (Foissner and Xu 2007). The genus *Apertospathula* was established by Foissner et al. (2002) within the family Spathidiidae, which contains three species (*A. inermis, A. armata, A. dioplites*) that originated from Namibian soils. This genus is characterized by a highly flexible body, a continuous circumoral kinety open ventrally and shortened on the left side of the oral bulge, and by a dorsal brush composed of three rows of similar length. Later, Foissner et al. (2005b) described a new species (*A. verriculifera*) from Venezuelan soils, and two new related genera (*Longispatha* Foissner et al. 2005b and *Rhinothrix* Foissner et al. 2005b) were established. The authors stressed that members of the genera *Apertospathula, Longispatha* and *Rhinothrix* have a ventrally open, loop-like oral bulge and circumoral kineties unlike other spathidiid ciliates and, therefore, transferred them to the newly established family Apertospathulidae. In the same study, they also transferred the species *A. dioplites* into the newly constructed genus *Longispatha*, emphasizing that the right branch of the circumoral kinety was very long and almost reached the posterior body end. Subsequently, Foissner and

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Xu (2007) described five new species (*A. similis, A. longiseta, A. cuneata, A. lajacola* and *A. pelobia*) from various soil habitats, and one species, previously described as *Spathidium swarezewskyi,* was transferred into the genus *Apertospathula. Apertospathula* is, at the present state of knowledge, represented by nine nominal species reported from various soil habitats (Foissner et al. 2005a,b; Foissner and Xu 2007). In this study, which was conducted on coastal sediments of Lake Van, a new population of the genus *Apertospathula* was isolated from aquatic environments. Detailed investigations, using both in vivo and silver impregnation methods and gene sequence analyses confirmed the validity of this new species.

Material and Methods

Apertospathula oktemae n. sp. was collected from bottom sediments from marina of Van Yuzuncu Yıl University in Lake Van, Turkey (38°33′38″N, 43°16′39″E, depth 1–3 m, water temperature 6–10 °C, salinity 19‰, pH 9.8) from March 2015 to May 2017. Sediment samples were taken from the upper 5 cm layer, together with some water, by a sediment sampler. Specimens were either observed immediately or maintained for several days (10–15 °C) in glass bottles for further study. Live cells were first observed in vivo under a stereo microscope (Leica S8) at low (20–80×) magnification and then under a compound microscope at higher magnifications (400–1000×) (Carl Zeiss Axio Imager 2) using bright field and differential interference contrast optics. Photomicrographs were captured using a CCD camera (Axio Cam HRc, Carl Zeiss). The ciliary pattern was revealed by silver proteinate and silver carbonate impregnation methods (Foissner 1991; Foissner and Xu 2007; Vdacny and Foissner 2012). Because silver proteinate preparations were impregnated very faintly, good photomicrographs could not be captured. Therefore, all photomicrographs were taken from silver carbonate-impregnated specimens. Counts and measurements of important morphological characteristics of impregnated specimens were performed at a magnification of 1000×. Drawings of live and impregnated specimens were made in Photoshop CS5 software on the basis of free-hand sketches and photomicrographs.

Species identification and terminology followed Kahl (1930), Foissner et al. (2002, 2005b), Foissner and Xu (2007) and Jang et al. (2017).

After species identification, individuals of the new species were collected, washed several times in distilled water to remove potential contaminants, transferred to 200 μL PCR tubes with a minimum volume of water (1–2 μL), and stored in a cryogenic freezer (−55 °C) for molecular investigations. To test the quality and reliability of sequences, six microtubes, each containing one or two cells, were prepared. Genomic DNA was extracted using the REDExtract-N-Amp Tissue PCR kit (Sigma, St. Louis, MO, USA) according to the manufacturer's instructions, modified such that only 1/10 of the suggested volume for each solution was used (Gong et al. 2007). The SSU rRNA gene was amplified using the eukaryotic universal primers forward EukA (5′-AAC CTG GTT GAT CCT GCC AGT-3′) and reverse EukB (5′-TGA TCC TTC TGC AGG TTC ACC TAC-3′) (Medlin et al. 1988) with the following conditions: initial denaturation at 94 °C for 2 min followed by 40 cycles of 45 s at 95 °C, 1 min at 60 °C, 3 min at 72 °C, and the final extension step at 72 °C for 8 min. PCR products were sequenced with PCR primers and the internal SR7R (5′-AGT TAA AAA GCT CGT AGT GT-3′) primers by Macrogen Europe (Amsterdam, The Netherlands). A total of 18 sequence fragments obtained from six different PCR products were imported into CodonCode Aligner ver. 7.0.1 (CodonCode Corporation) to check for data quality, trim the 5′ and 3′ ends, and assemble individual clone sequences into a consensus contig.

The SSU rRNA gene sequence of the *Apertospathula oktemae* was aligned with the sequences of 48 other ciliates retrieved from GenBank (see Fig. 4 for accession numbers) using Clustal W implemented in Mega 6.0 (Tamura et al. 2013). Four pleurostomatid ciliates, *Loxophyllum rostratum, L. jinni, Litonotus paracygnus* and *Amphileptus aeschtae*, were used as outgroups. Ambiguously aligned regions were masked using Gblocks ver. 0.91b allowing gap positions within the final blocks (Castresana 2000; Talavera and Castresana 2007). GTR + I + G was the best evolutionary substitution model found for the alignment using jModelTest ver. 2.0.1 under the Akaike Information Criterion (Posada 2008). Maximum-likelihood (ML) analyses were performed with 1000 replicates using RAxML Black Box (Stamatakis 2014; Stamatakis et al. 2008) on the online portal of the CIPRES Science Gateway ver. 3.3 (https://phylo.org). Bayesian inference (BI) analysis was performed in MrBayes ver. 3.2.2 (Ronquist and Huelsenbeck 2003; Ronquist et al. 2012) with two parallel runs. The Markov chain Monte Carlo (MCMC) was run for 3,000,000 generations with sampling every 100th generation and a burn-in of 25% (the first 7500 sampled trees were discarded). The remaining 75% of the trees were used to generate a consensus tree and to calculate its posterior probabilities.

Results

Phylum: Ciliophora Doflein, 1901

Subphylum: Intramacronucleata, Lynn, 1996

Class: Litostomatea Small and Lynn, 1981

Subclass: Haptoria Corliss, 1974

Order: Spathidiida Foissner and Foissner, 1988

Family: Apertospathulidae, Foissner et al., 2005b

Genus: *Apertospathula Foissner*, Agatha and Berger, 2002

Apertospathula oktemae n. sp. (Figs. 1a–c, 2a–d, 3a–d; Table 1