A survey of ciliates in a small tributary to Shades Creek, Jefferson County, Alabama, using environmental sampling

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Abstract

We have used environmental sampling and molecular techniques to identify ciliates in a small tributary to Shades Creek on the Samford University campus. Primers specific for ciliate small subunit ribosomal DNA (SSU rDNA)(Dopheide, A. *et al.* 2008) were used in PCR-amplification of DNA isolated from whole water samples. Ciliates were then identified by cloning amplicons using the TOPA-TA cloning kit (Invitrogen) followed by colony sequencing (GeneWiz). Alternatively, we have used denaturing gradient gel electrophoresis (DGGE) to try to identify unique ciliates prior to sequencing. We discovered 61 unique ciliate sequences representing approximately 22 families.

We have undertaken a project that has as its goal to gain an understanding of the ciliate communities in a small, local freshwater stream. Ciliates are important in such systems as they are important primary consumers of bacteria. A complete understanding of such freshwater systems must include the roles of ciliates.

Materials and Methods

Water samples were collected from a small freshwater stream on the campus of Samford University, Jefferson County, AL (Figure 1). Microorganisms were concentrated either by filtration or centrifugation. DNA was isolated using the Wizard Genomic DNA Purification Kit (Promega). SSU rDNA was PCR-amplified using the ciliate-specific primers 384-F and 1147-R (Dopheide *et al.*, 2008). Gel electrophoresis was performed using either standard 1.5-2% agarose gels or 2% agarose in the E-Gel system (Life Technologies). For sequencing, amplicons were purified on a CloneWell 0.8% agarose gel (Life Technologies) and cloned using the TOPO-TA cloning kit (Invitrogen) followed by colony sequencing (GeneWiz). Geneious software (Drummond *et al.*, 2011) was used for sequence analysis, alignment, and tree building. Some SSU rDNA amplified DNA was subjected to denaturing gradient gel electrophoresis (DGGE) using a DGGE-2001 (CBS Scientific) in an attempt to determine the diversity of amplified samples.

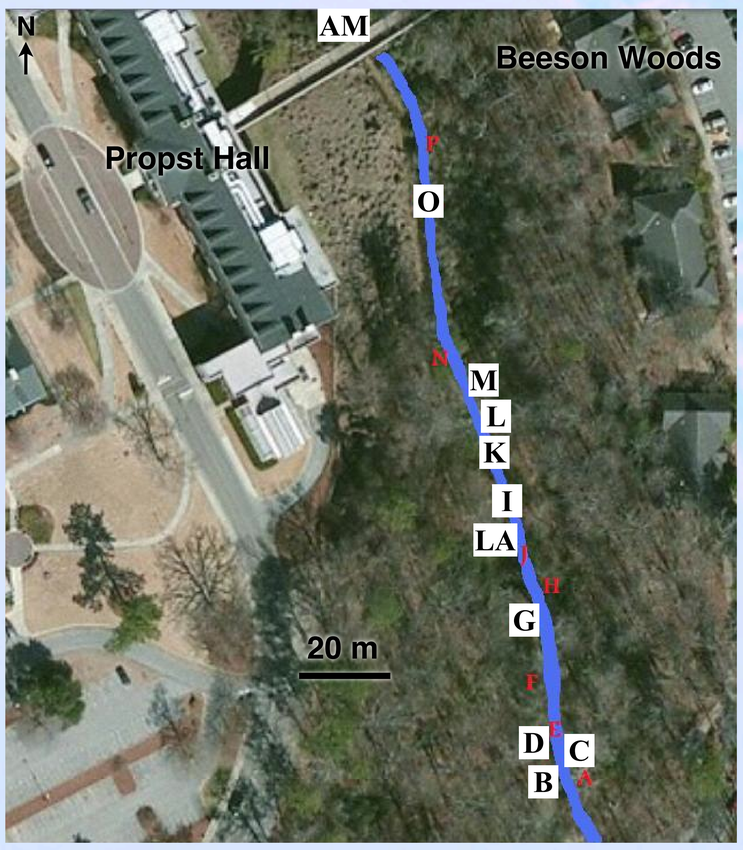

Figure 1. Sampling sites. Sampling sites are indicated in black letters. (Unsuccessful sampling sites are in red.) Propst Hall is on the eastern edge of the Samford campus separated from Beeson Woods dormitories by the creek in this study.

Results

SSU rDNA amplified from water samples showed amplicons of the expected size (Figure 2). Upon cloning and sequencing, the 61 unique ciliate sequences discovered and the outgroup sequence (*Eutreptia viridis*) were aligned with Geneious Alignment (cost matrix 65%, open gap penalty 12, gap extension penalty 3, global alignment with free end gaps, 2 iterations)(data not shown). A neighbor-joining consensus tree was constructed using Geneious (Tamura-Nei, bootstrap resampling 100 replicates, 50% threshold support)(Figure 4). DGGE revealed distinct subpopulations of ciliates within single amplicons (Figure 3).

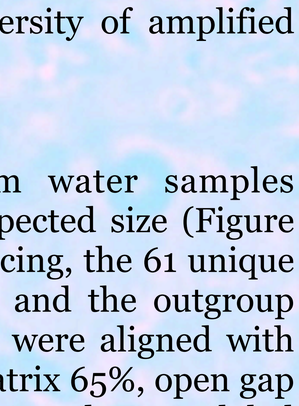

Figure 2. PCR-amplification of ciliate SSU rDNA. Ciliate-specific primers 384-F and 1147-R were used to amplify ciliate DNA (lane 1). Lane 2 is a control with no template DNA. Lane 3 is Hyperladder II marker (Bioline USA).

Discussion

The 61 unique sequences amplified from the stream near Samford's Propst Hall represented approximately 22 families of ciliates, based on a consensus of published classifications found on the National Center for Biotechnology Information website (NCBI), which Geneious software searches to find similar sequence data. DGGE proved to be helpful in estimating the diversity of amplified samples, but we need to refine the parameters of the procedure to guarantee reliable repeatability. Our future goals are to further analyze the sequence data, investigating the relationships between the organisms present and parameters such as 1) habitat and 2) the season of collection. We also plan to further develop DGGE as a method for preliminary classification (preceding sequencing) and using next generation sequencing methodology to expand the survey of ciliates present in this stream.

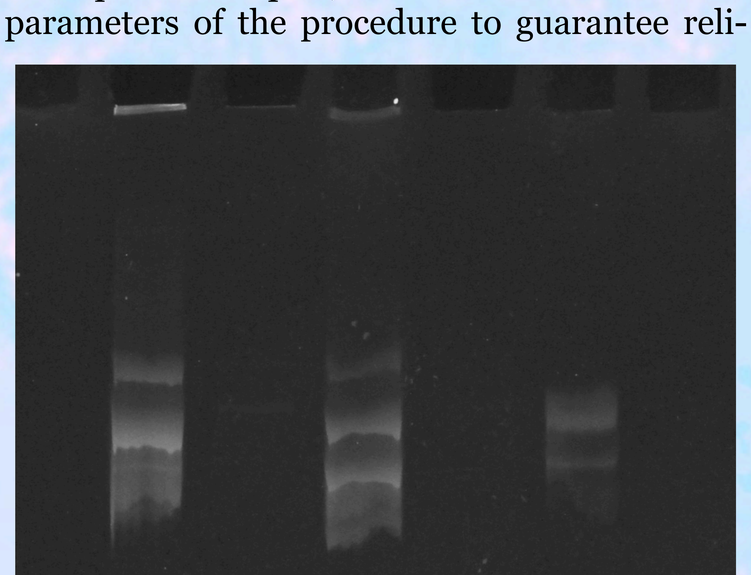

Figure 3. DGGE. Single amplicon bands, like that shown in Figure 2, revealed subpopulations of DNA molecules upon DGGE, as with these three samples.

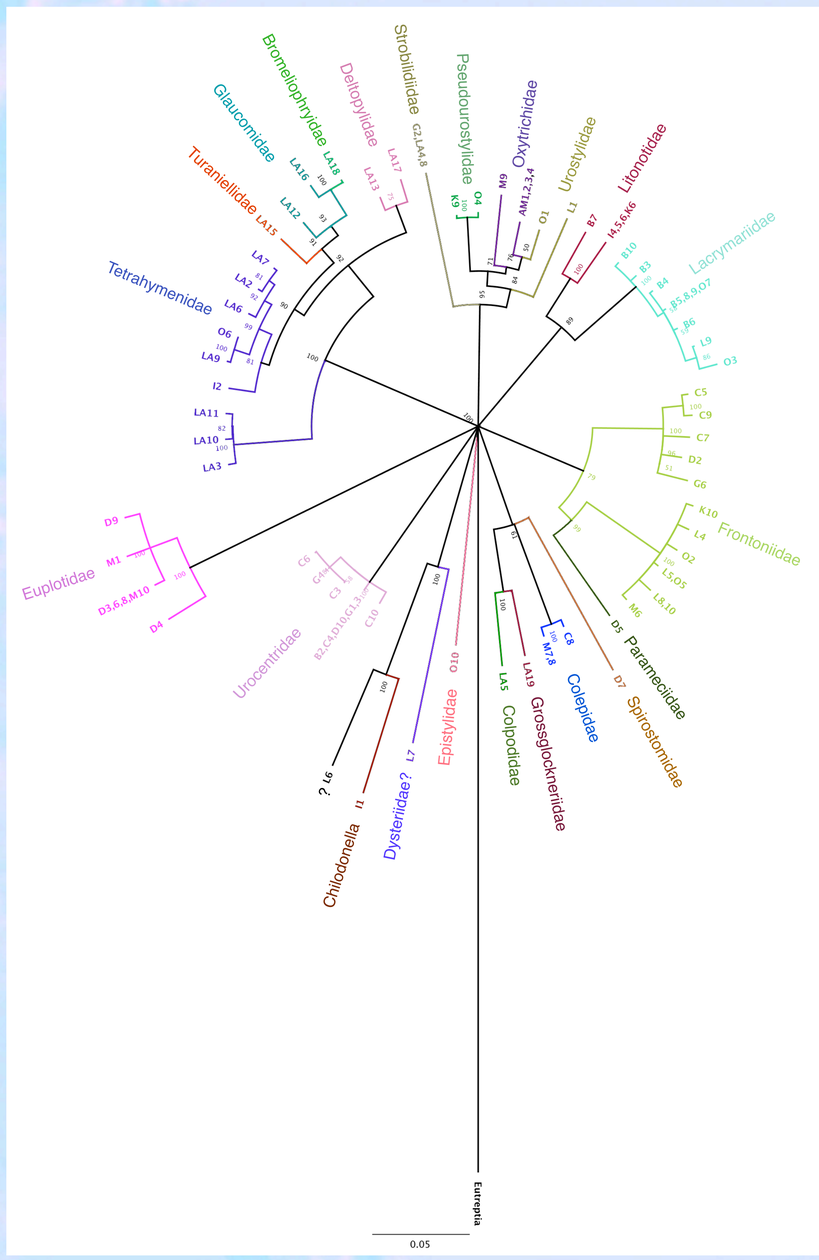

Figure 4. Neighbor-joining consensus tree of sequences. A neighbor-joining consensus tree was constructed using Geneious (Tamura-Nei, bootstrap resampling 100 replicates, 50% threshold support--shown at nodes). Family names are shown peripherally and are based on consensus published classifications found on NCBI.

Literature Cited

Dopheide *et al.*, 2008. *Applied & Environ. Microbiology*, 74: 1740–1747.

Drummond *et al.*, 2011. Geneious v5.5, Available from http://www.geneious.com

NCBI: http://www.ncbi.nlm.nih.gov