Survey of Mosquito Habitats for Mosquito Predatory Ciliate Lambornella for Potential Biological Control

Radina Porashka, Mary Parsley, Sauleen Shamdeen, Nathan Smith, Emerniece Cooper, Anthony Farone, and Sharon Berk

Department of Biology, Middle Tennessee State University

INTRODUCTION

Protozoa are single cell animal-like organisms that are prevalent in water and moist soils. They move through these environments typically engulfing bacteria and other microbes as their food source. Ciliated protozoa are surrounded by hair-like structures that help to propel them through their environments and to gather food. Some ciliated protozoa will parasitize the exoskeletons of invertebrates including insects.

The ciliated protozoan, *Lambornella clarki*, and other *Lambornella* species are known to target specifically mosquito larvae. They parasitize the larvae by attaching to the exterior of the larvae cuticle, and eventually invading the tissue and killing them, which led to the suggestion that the organism could be used for the biological control of mosquito populations (Egerter et al., 1986).

To the best of our knowledge, *Lambornella clarki* is not available from culture collections. Therefore, the goal of this study was to find, isolate, and culture *Lambornella* species (clarki) in pure culture or with native bacteria as a food source by collecting samples from areas of standing water, some of which contained mosquito larvae.

To identify an isolate as *Lambornella*, DNA sequences recovered from mixed protozoa in samples can be compared to existing sequences found in DNA databases. However, current public genomic databases have limited sequences for *Lambornella* species.

To accomplish this objective, techniques that were utilized were field sampling, inverted phase-contrast microscopy, enrichment of protozoa, isolation techniques, and culturing. The samples that were collected ranged from the campus of Middle Tennessee State University to the Bahamas.

MATERIALS AND METHODS

- A variety of laboratory skills and knowledge were obtained throughout the project period. Attempts at physical isolation of *Lambornella clarki* involved an array of methods including capillary tube aspiration and serial dilutions in a 96-well cell culture plate in cereal grass medium (Cerophyll).
- Cultures were enriched with a *K. aerogenes* and *E. coli* mixture in cerophyll and Tris buffer solution. Protoslow™ was utilized during capillary picking to slow the motility of the ciliates.
- Cultured samples that appeared to have populations of ciliates matching descriptions of *Lambornella* spp. were pelleted by centrifugation. DNA was extracted from pellets using the Qiagen DNEasy kit following the protocol for cultured mammalian cells.
- *Lambornella* sequences from NCBI were aligned using the BLAST program with the most similar sequences in NCBI GenBank to identify potential sequences for the design of unique primers. The available sequences have high similarity to other Tetrahymenid sequences in GenBank.

MATERIALS AND METHODS

- Out of the primers we designed, the most successful were the Euk1A and Cil1174R. The samples were amplified using 25µL PCR consisting of 3µL of each primer, 10µL of NF water, and 9µL of DNA. Alongside our 7 samples, our control was Tetrahymena. Following amplification, a 1% TAE agarose gel was prepared and run at 100V for 30 minutes.
- The gel allowed for confirmation of the extracted DNA sequence from the species. Before sending the samples to be sequenced, nanodrop analysis was performed to obtain DNA concentration of the purified PCR. Once sequences were obtained, a phylogenetic tree was created comparing the ciliates in the samples to *Lambornella*.

RESULTS

Sample	Location	Temperature
17	Hickory Tree (tree hole), Unionville, TN	83°F
19	Cookeville, TN - larvae	85°F
26A	Redwood Treehole Felton, CA	n/d
28B	Oak Tree (treehole) Glenellen, CA	n/d
30A	Bay Tree (treehole) Kenwood, CA	n/d
30B	Bay Tree (treehole) 2 Kenwood, CA	n/d
32B	Oak Tree (treehole) Cloverdale, CA	n/d

Table 1: A table listing the samples collected with their indicated number, location and temperature

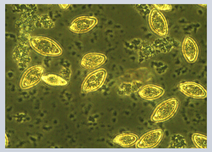

Figure 1: This image shows sample 28B ciliates feeding on bacteria.

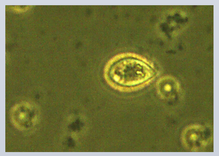

Figure 2: Image of sample 30B ciliates feeding on bacteria.

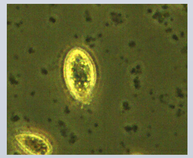

Figure 3: Picture of an Oak Tree ciliate from sample 32B.

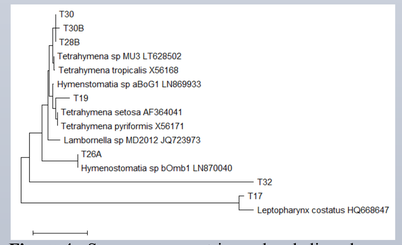

Figure 4: Sequences were trimmed and aligned using ClustalW. The phylogenetic tree was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site (Tamura et al., 2004). This analysis involved 15 nucleotide sequences. There were a total of 870 positions in the final dataset. The bar represents 0.05 substitutions per nucleotide position. All evolutionary analyses were conducted in MEGA X (Kumar et al., 2018).

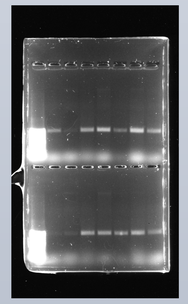

Figure 5: 1% TAE agarose gel. A double gel electrophoresis was run. From top to bottom samples 32B, 30B, 30A, 28B, 19, 17, Tet, and Ladder are displayed.

CONCLUSIONS

- A wide variety of ciliates and other species (flagellates, rotifers, amoeba) were found in water samples from tree holes and other environments. The techniques that were utilized were partially successful in isolating protozoa. However, we were not able to obtain a pure (clonal) isolate of protozoa during the short project period.
- Of the 37 samples screened for *Lambornella Clarki*, 7 samples appeared to have the greatest potential for containing *L. clarki*, based on photos and diagrams of *Lambornella* within the literature. Based on the sequencing results and phylogenetic tree, samples 28B and 30B were more closely related to *Lambornella clarki.* There is no known sequence for *L. clarki* in gene databases.
- The future steps that are to be taken are to increase the DNA sequence concentration through another round of PCR and sequencing reactions.
- As there is no known sequence for *L. clarki* in gene databases, we will also compare more of the morphology of these isolates to images of *L. clarki.*
- Because species related to *Lambornella* also infect mosquito larvae, prior to obtaining new samples, these ciliates will be introduced to mosquito larvae to test for parasitic behavior. If parasitic behavior is observed in the current ciliate samples, steps will be taken to utilize them as a biological control agent.

REFERENCES

Soldo AT, Brickson AS (1980) A simple method for plating and cloning ciliates and other protozoa. J Protozool 27:328–331

Egerter, D. E., Anderson, J. R., & Washburn, J. O. (1986). Dispersal of the parasitic ciliate Lambornella clarki: implications for ciliates in the biological control of mosquitoes. *Proceedings of the National Academy of Sciences of the United States of America*, *83*(19), 7335–7339. doi:10.1073/pnas.83.19.7335

Saitou N. and Nei M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution* 4:406-425.

Tamura K., Nei M., and Kumar S. (2004). Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proceedings of the National Academy of Sciences* (*USA*) 101:11030-11035.

Kumar S., Stecher G., Li M., Knyaz C., and Tamura K. (2018). MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. *Molecular Biology and Evolution* 35:1547-1549.

ACKNOWLEDGEMENTS

We would like to acknowledge those for the samples acquired that were utilized for this project. Dorna and Bailey Brent for the samples taken from the Redwood forest in Redwood National and State Parks, CA. The Mosquito and Vector Control Association of California (MVCAC) for the samples acquired from them. Tiana Purkey for the samples taken from National Deer Refuge in Big Pine Key, FL. Tracy Loutch for the samples taken from Glenellen, CA.

We would also like to acknowledge those for the guidance and input needed going forward with this research. Dr. Mary Farone for the guidance given on the techniques learned throughout this project. Dr. John Gunderson for the sequencing of sample 26A. To all the teammates on this research team for the input and effort incorporated through this research project. Finally, we would like to acknowledge URECA and SOAR for the opportunity that we have received through this program.