

Testing the effectiveness of cox-1 barcoding as a taxonomic tool to identify *Tetrahymena* species and to elucidate their evolutionary history

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INTRODUCTION

- Ciliated protozoa are a diverse group of organisms that all possess three major defining characteristics:
 - complex network of infraciliature
 - both a macronucleus and a micronucleus
 - periodically undergo conjugation
- Ciliates are a crucial component of all aquatic ecosystems.
- Tetrahymena* spp. are easily cultured ciliates that are used as model organisms in many biological disciplines.
- Advanced microscopy must be used to differentiate between *Tetrahymena* species, but this is not without problems:
 - some species exhibit polymorphic life cycles
 - all *Tetrahymena* are generally asexual
 - several cryptic/sibling species exist



Figure 1: *Tetrahymena thermophila* has recently had its entire genome sequenced. A study of its close phylogenetic relatives could shed light on the functioning of genes in a comparative sense.

OBJECTIVE

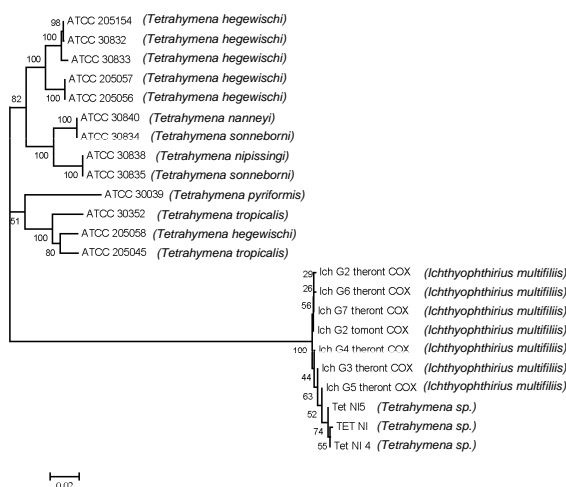
- DNA barcoding via the cytochrome c oxidase subunit I (cox-1) gene can be used to complement traditional taxonomic approaches in several animal phyla.
- We aim to determine whether cox-1 barcoding is a good tool for species identification in *Tetrahymena* by confirming that intraspecific sequence divergence is <1%.
- We will also determine whether cox-1 barcoding can further elucidate the evolutionary history of *Tetrahymena* by using cox-1 and nuclear SSUrRNA sequences to create a hierarchical tree that will increase the support value of nodes.
- Barcoding may also be able to resolve whether or not *Tetrahymena* exhibit geographic restriction based on the species compositions and genotype clades of different lakes.
- Additionally, the historical origin of several *Tetrahymena* species will be estimated using coalescent theory.

METHODS

- Isolates of *Tetrahymena* species have been donated primarily by the American Type Culture Collection and Dr. F. Paul Doerder or Cleveland State University.
- Cultures are maintained at the University of Guelph in a proteose peptone-based media containing a mixture of penicillin, erythromycin, and streptomycin.
- DNA is extracted using either the Chelex® protocol or the MasterPure™ Total Nucleic Acid Purification kit.
- The ~980 bp barcoding region of the cox-1 gene is amplified by polymerase chain reaction via primers 288 (FW) and Fol-B (REV).
- If necessary, DNA is purified using the QIAGEN MinElute Gel Extraction kit prior to sequencing at the University of Guelph DNA Sequencing Facility.
- Consensus sequences are created using Sequencher v3.1 and aligned using MEGA v3.1 before being uploaded to the Barcode of Life Data Systems (BOLD).
- Phylogenetic analyses are conducted using PHYLIP, PAUP v4.0, and MrBayes v3.1.

RESULTS

- The sequence divergence value for 6 isolates of *Tetrahymena hegewischi* is 0.08%.
- The sequence divergence value for 7 isolates of *Ichthyophthirius multifiliis* (a fish parasite closely related to the genus *Tetrahymena*) is 0.005%.
- A neighbour-joining tree (see Fig. 2) suggests that the 3 unknown "Tet-NI" isolates are more closely related to *I. multifiliis* than the genus *Tetrahymena*. Interestingly enough, these isolates are also fish parasites.
- T. hegewischi* (ATCC 205058) is the only isolate not grouping with the rest of its species. It is possible that this isolate is not a true sample of *T. hegewischi* and may have been misidentified at some point in time.



SIGNIFICANCE

- cox-1 barcoding appears to be effective as *Tetrahymena* species can be classified quickly and efficiently with a high degree of accuracy.
- This is particularly useful as *Tetrahymena* spp. are commonly used as model biological organisms.
- This technique can then be extended to other protist species, including lethal parasites such as *Plasmodium* spp.
- Over time, barcoding could prove to be an invaluable tool for protistologists when used in conjunction with classical microscopy techniques.
- Barcoding can improve our knowledge of protist biodiversity by helping to discover new species and further understand their phylogenetic history.

ACKNOWLEDGEMENTS

Cultures were donated by the American Type Culture Collection with the help of J. Cole, and J. Cooper, and P. Ikononi. Additional cultures were provided by F. P. Doerder. Other isolates were donated by H. Dickerson and D. Zilberg.

The authors wish to thank C. Chantangsi, T. Crease, E. Gentekaki, P. Hebert, M. Noyes, and M. Strüder-Kypke.