

MARINE TUBE-FORMING DIATOMS AND THEIR COHABITANTS: A FLORISTIC SURVEY OF CANADIAN WATERS USING DNA BARCODING



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TUBE-FORMING DIATOMS

Lobban (1984, 1985, 1990) recorded 18 species of tube-dwelling diatoms along the intertidal in Canada. These species include representatives of *Berkeleya*, *Haslea*, *Parlibellus*, and *Navicula* (Fig. 1). Some of these diatoms grow their own tubes while others migrate to tubes that have already formed. Tube formation begins with a single cell. However, diatoms have been found to move between tubes so single tubes may contain multiple genetically distinct individuals in addition to any cohabitants that do not form tubes on their own.



Figure 1. Examples of tube-forming diatoms from eastern Canada. A) *Parlibellus delognei*; B) *P. berkeleyi*; D-E) *Navicula smithii*; F) *N. mollis*; G) *N. rusticensis*; H) *Berkeleya rutilans*. Scale bar = 10 microns.

DIATOM IDENTIFICATION USING THE DNA BARCODE

Identification of diatoms is routinely based on the morphology of their silica valves. Frustule features such as valve outline and number of striae in 10 microns often overlap (Fig. 2). These features lead to identification errors and different researchers may identify the same individual as different species (Mann 1999).

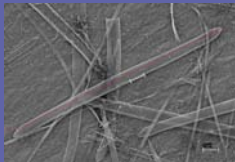


Figure 2. *Nitzschia* species. Red line = length. Blue line = width. White line = 10 microns.

To resolve these issues, we will utilize the DNA barcoding technique by sequencing part of the cytochrome oxidase I mitochondrial gene and assigning species to 'genetic groups' (Saunders 2005). Differences within a genetic group are often < 1% while differences between genetic groups are often > 4%. These genetic groups can then be examined for morphological characters that may be used to more easily identify these diatoms objectively.

STUDY OBJECTIVES

1. Develop the DNA barcode for tube-forming diatom colonies and single cells
2. Record the floristics, biogeography and taxonomy, of tube-forming diatoms in Canada
3. Establish a method to rapidly identify tube-forming diatoms from environmental samples
4. Explore systematics and taxonomy of tube-forming diatoms

1. DEVELOPING THE DNA BARCODE FOR DIATOM COLONIES AND SINGLE CELLS

Diatoms are unicellular and present some unique challenges for molecular research. Although tube-dwelling diatoms appear 'macroscopic', material collected in the field may contain multiple species and could be covered with epiphytes (other diatoms). Our approach to a DNA extraction protocol (leading to primer development) is two tiered; we will focus on growing tube-forming diatoms in clonal cultures while reducing the amount of material necessary for DNA extraction so that ultimately single cell extractions will be possible.

METHODS

- Collect many individual colonies and grow clonal cultures from this field material
- Develop primers for multiple species from these clonal cultures and culture collection material
- Assess the DNA barcode as a tool to delimit these diatom species
- Develop techniques to minimize the DNA extraction and allow us to barcode single cells
- Barcode single cells from colonies in culture and field material

PRELIMINARY RESULTS

- To date, clonal cultures are being established from 28 individual field-collected colonies (Fig. 3)
- A DNA extraction protocol for silica dried and ethanol preserved material has been developed; we are currently trying to adapt this procedure to single cells
- Primers for barcoding diatoms are being developed

2. FLORISTICS: BIOGEOGRAPHY AND TAXONOMY

Microscopic eukaryotes, including diatoms, are often considered cosmopolitan with their distribution described by environmental characteristics rather than historical distribution. However, as researchers have begun to focus on floras of specific areas, reports of endemic species have become increasingly prevalent. Our project will further explore the distributional patterns of these 'macroscopic' diatoms along the Atlantic and Pacific coasts of Canada using the DNA barcode to aid in the identification of these unique diatoms.

METHODS

- Approximately 20 sites along the Atlantic coast, 10-15 sites along the Pacific coast, and 5 sites in the Sub-Arctic will be sampled
- Sampling will occur throughout the year with a focus on spring and autumn blooms
- Individual colonies will be sampled and their substrate type and location within the intertidal (upper, mid, lower) recorded. A voucher will be preserved in ethanol when possible, or pressed on herbarium paper. A subsample for DNA analyses will be stored in silica gel.



Figure 4. Beaver Harbour in New Brunswick

PRELIMINARY RESULTS

- 41 individual colonies have been collected
- 4 sites along the Bay of Fundy (Fig. 4) and 2 sites along the coast of Grand Manan (Fig. 5) have been sampled
- *Berkeleya rutilans* is the most prevalent tube-forming diatom with individual colonies collected from all sites sampled to date
- A site near Lepreau, New Brunswick has the highest diversity of those sampled to date with 5 of the 13 species found in eastern Canada collected here

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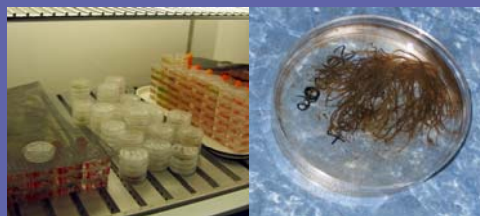


Figure 3. Growth chamber at UNB with diatom cultures (petri dishes) and an example of a macroscopic tube-forming diatom.

3. RAPID IDENTIFICATION OF TUBE-FORMING DIATOMS IN ENVIRONMENTAL SAMPLES

Tube-forming diatom colonies may be mixed assemblages with different species living in a single tube. The DNA barcode will allow us to screen these colonies for the presence/absence of particular species faster and more accurately than traditional visual counting methods. This technique will be applied to environmental samples from a variety of benthic habitats to look for single cells of these diatoms (not in colonies) as well. Often the presence of a single cell within a sample is not enough information and abundance values would be useful. To determine abundance of tube-forming diatoms in individual colonies or environmental samples, a protocol for real-time PCR will be established.

METHODS

- Specific primers for each species of tube-forming diatom will be developed from the DNA barcoding sequences previously generated
- Bulk DNA will be extracted from individual colonies or environmental samples and amplified in PCR using specific primers developed
- PCR products will be run on an electrophoresis gel and the presence/absence of a band will determine the presence/absence of specific tube-forming diatom species
- A standard curve will be generated during the real-time PCR run based on serial dilutions of the DNA barcode region that will give us a standard by which to compare our sample. A negative control without template and a positive control with non-target DNA will also be run in real-time PCR. This technique will allow us to determine the abundance of these diatoms in an individual colony or a benthic environmental sample.

4. SYSTEMATICS AND TAXONOMY OF BERKELEYA GREVILLE, PARLIBELLUS E. J. COX, AND NAVICULA BORY DE SAINT-VINCENT

The taxonomy of this group of diatoms is very interesting because, although their tube-forming growth habit is very similar, they are not assigned to a single genus. However, it is unknown how the tube-forming species are evolutionarily related relative to one another and other non-tube-dwelling species in their respective genera. While the DNA barcode is an effective identification tool, the sequenced region is generally too short (~660 bp) to evaluate phylogenetic relationships (Graybeal 1998). We will thus focus on the SSU rDNA region because it has been used successfully to infer phylogeny of diatoms at various taxonomic levels (Medlin and Kaczmarek 2004).

METHODS

- The SSU rDNA region will be used to investigate the evolutionary relationships among tube-forming diatoms relative to sequences of other Bacillariophyceae in GenBank
- The collection of sequence data will follow a similar protocol to those previously described. Modeltest will be used to find the best model of nucleotide substitution and PAUP will be used to conduct Maximum Likelihood analyses.

SUMMARY

In summary, this project will assess the use of the DNA barcode to delimit tube-forming diatom species. Protocols for barcoding diatoms from clonal cultures and single cells will be developed. A floristic survey of tube-forming diatoms throughout Canada will be conducted with biogeographical distributions documented for each species. A DNA barcode protocol for rapid identification will be created and the utility of real-time PCR for assessing the presence/abundance of benthic tube-forming diatoms in environmental samples will be established. In addition, the systematics of this unique group of diatoms will be explored.



Figure 5. Long Eddy Point site on Grand Manan

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