

Planktonic ciliate DNA sequence GenBank accession numbers for samples collected on the R/V Cape Hatteras (CH0112) cruise in the NW Atlantic Continental Shelf during 2015 (CiliateSequencing project)

Website: <https://www.bco-dmo.org/dataset/626566>

Version: 2015-11-18

Project

» [Diversity and dynamics of planktonic ciliates - what can next-generation sequencing technologies tell us?](#)
(CiliateSequencing)

Contributors	Affiliation	Role
McManus, George	University of Connecticut (UConn - Avery Point)	Principal Investigator
Katz, Laura A.	Smith College	Co-Principal Investigator
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Dataset Description

This dataset includes 4 sets of NCBI accession numbers for planktonic ciliates. Some of the links to the NCBI pages are not yet live.

Contact for the single cells from Long Island Sound: Luciana Santoferrara, post-doc: luciana.santoferrara_at_uconn.edu. Contact for the other data: George McManus.

Methods & Sampling

DGGE_Weiker: Surface samples were collected while following a surface drifter (crosse window shade type). DNA was extracted and amplified with PCR, and amplicons were separated with denaturant gradient gel electrophoresis. Sequencing use the Sanger method. Details can be found in Grattepanche, et al, 2014.

454 Long Island Sound: Surface and deep samples were taken from the Sound on several occasions. DNA was extracted and a ciliate clade-specific portion of the small subunit ribosomal gene was amplified using the polymerase chain reaction. Amplified fragments were sequenced using the Roche 454 platform. Details are reported in Santoferrara, et al, 2014.

454 and DGGE Hatteras: Vertically-resolved samples were collected along a section due south from Naragansett RI to the shelf break. DNA was extracted and amplified with PCR, and amplicons were separated with denaturant gradient gel electrophoresis or sequenced with Roche 454. Methodological details can be found in Grattepanche, et al, 2014 and Santoferrara, et al, 2014.

Single cells, Long Island Sound: Individual cells of tintinnid microzooplankton were picked with a micropipette, photo documented and placed in buffer for extraction and sequencing. Sequences of the internal transcribed spacer regions (ITS1-5.8S-ITS2), the small sub unit ribosomal gene (SSU), and the large subunit ribosomal gene (LSU) were obtained by Sanger sequencing.

References:

Grattepanche, J-D, L Santoferrara, J Andrade, AM Oliverio, GB McManus, and LA Katz. 2014. Distribution and diversity of oligotrich and choreotrich ciliates assessed by morphology and by DGGE in temperate coastal waters. *Aquat. Microb. Ecol.* 71:211-221. DOI:10.3354/ame01675.

Santoferrara, L, J-D Grattepanche, LA Katz, and GB McManus. 2014. Pyrosequencing for assessing diversity of eukaryotic microbes: analysis of data on marine planktonic ciliates and comparison with traditional methods. *Environ. Microbiol.* DOI: 10.1111/1462-2920.12380.

Santoferrara, LF, GB McManus, and VA Alder. 2012. Utility of Genetic Markers and Morphology for Species Discrimination within the Order Tintinnida (Ciliophora, Spirotrichea). *Protist*, doi:10.1016/j.protis.2011.12.002

Data Processing Description

DGGE_Weiker: Raw chromatograms were checked by eye and fragments were assembled into contains using LaserGene.

454 Long Island Sound: Sequences were processed in QIIME using a quality score of 37 without clustering.

454 and DGGE Hatteras: For DGGE, raw chromatograms were checked by eye and fragments were assembled into contains using LaserGene. For 454 data, sequences were processed in QIIME using a quality score of 37 and without clustering.

Single cells, Long Island Sound: Raw chromatograms were edited by eye and contains were assembled using MEGA. Details can be found in Santoferrara, et al, 2012.

BCO-DMO Processing:

- Created a table with columns: dataset_id, location, lat, lon, cruise_id, date, description, comment, accession_number.
- Created links to the accessions.

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Data Files

File
ciliate_accessions.csv (Comma Separated Values (.csv), 54.41 KB) MD5:e3ab49e00513599156edc0a222adf7db
Primary data file for dataset ID 626566

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Parameters

Parameter	Description	Units
dataset_id	name of dataset	unitless
location	location of sample collection	unitless
lat	latitude; north is positive	decimal degrees
lon	longitude; east is positive	decimal degrees
cruise_id	cruise identification	unitless
date	date of collection	yyyy-mm-dd
description	DGGE: Denaturing Gradient Gel Electrophoresis; ITS: Internal Transcribed Spacer region of the ribosomal gene (ITS1-5.8S-ITS2); SSU: Small Sub-Unit of the ribosomal gene; LSU: Large Sub-Unit of the ribosomal gene	unitless
comment	comment	unitless
NCBI_accession	NCBI accession number	unitless

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Instruments

Dataset-specific Instrument Name	
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Roche 454 platform and others
Generic Instrument Description	General term for a laboratory instrument used for deciphering the order of bases in a strand of DNA. Sanger sequencers detect fluorescence from different dyes that are used to identify the A, C, G, and T extension reactions. Contemporary or Pyrosequencer methods are based on detecting the activity of DNA polymerase (a DNA synthesizing enzyme) with another chemoluminescent enzyme. Essentially, the method allows sequencing of a single strand of DNA by synthesizing the complementary strand along it, one base pair at a time, and detecting which base was actually added at each step.

Dataset-specific Instrument Name	
Generic Instrument Name	Thermal Cycler
Generic Instrument Description	A thermal cycler or "thermocycler" is a general term for a type of laboratory apparatus, commonly used for performing polymerase chain reaction (PCR), that is capable of repeatedly altering and maintaining specific temperatures for defined periods of time. The device has a thermal block with holes where tubes with the PCR reaction mixtures can be inserted. The cycler then raises and lowers the temperature of the block in discrete, pre-programmed steps. They can also be used to facilitate other temperature-sensitive reactions, including restriction enzyme digestion or rapid diagnostics. (adapted from http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html)

Deployments

UConn_McManus_2015

Website	https://www.bco-dmo.org/deployment/626637
Platform	Univ_Connecticut
Start Date	2015-11-17
End Date	2015-11-17
Description	genomic analyses of ciliates

CH0112

Website	https://www.bco-dmo.org/deployment/59041
Platform	R/V Cape Hatteras
Start Date	2012-07-06
End Date	2012-07-09
Description	Cruise departed from and returned to Narragansett, RI. 39 stations were completed in 3 days. Each station included a CTD cast, water sampling, and a plankton net tow. Part of the project "Diversity and dynamics of planktonic ciliates - what can next-generation sequencing technologies tell us?" Sampling activity included: CTDFO Zooplankton (vertical tows 150 um mesh) Plankton DNA (3-5 depths); 2 L sample Preserved (lugols) for microzooplankton (3-5 depths) Cruise information and original data are available from NSF R2R data catalog.

Project Information

Diversity and dynamics of planktonic ciliates - what can next-generation sequencing technologies tell us? (CiliateSequencing)

Website: <http://microzooplankton.uconn.edu>

Coverage: NW Atlantic Continental Shelf

The Ocean's biomass and diversity are predominantly microbial, yet this aspect of diversity remains underexplored. Efforts in recent years have begun to document microbial diversity in marine systems, and to elucidate the processes that structure assemblages across space and time. This project focuses on two important sister clades of microbial eukaryotes, the oligotrich and choreotrich ciliates. These organisms comprise a major component of planktonic food webs as they graze on phytoplankton, and are in turn eaten by zooplankton and larval fish.

Earlier molecular work on ciliate diversity relied on light microscopy, construction of clone libraries and Sanger sequencing. This revealed a high degree of cryptic diversity (similar species that are genetically distinct), which is surprising, given the long-held idea that all microbes are globally distributed and that few species exist, at least as compared to animals and plants. This past work also showed that ciliate assemblages contain a few highly abundant forms and many rare ones, consistent with the concept of a "rare biosphere". However, these methods are limited by high costs of both labor and materials, so that efforts to sample any local assemblage comprehensively usually resulted in undersaturation (repeated sampling continued to uncover new species). Next generation approaches are needed to truly assess the depths of biodiversity in planktonic ciliates.

This project brings together investigators with strengths in ecology, taxonomy and oceanography (PI McManus) and in molecular evolution, systematics and bioinformatics (PI Katz). Pyrosequencing will be used to sample the oligotrich and choreotrich ciliates 'to exhaustion' in coastal environments. Denaturing gradient gel electrophoresis (DGGE), a technique that generates a fingerprint of the diversity in a sample, will be used to pre-select samples for pyrosequencing based on where strong gradients are observed in the composition of assemblages in relation to environmental factors (density fronts, thermoclines, etc.). Using these approaches, combined with the informatics pipeline already in place, this project will address three specific objectives:

Objective 1. Determine the spatial scale of variability in ciliate diversity by measuring how ciliate assemblages change over meter, kilometer, 100 km, and basin scales.

Objective 2. Assess the contributions of different size classes of ciliates to overall assemblage diversity.

Objective 3. Experimentally evaluate factors that control the temporal shift of individual species from rarity to commonness in a natural assemblage, and vice versa.

Note: See the related collaborative project, "[Patterns of diversity in planktonic ciliates: spatio-temporal scales and community assembly in the coastal ocean](#)", funded by awards OCE-1435515 and OCE-1436003.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1130033

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