DNA sequences of viruses associated with copepods collected from the shoreside from Tampa Bay, Florida from 2004-2011 (Viruses in Copepods project)

Website: https://www.bco-dmo.org/dataset/4075
Version: 2013-11-5
Version Date: 2013-11-05

Project
» Discovery of viruses infecting marine Copepoda (Viruses in Copepods)

Contributors	Affiliation	Role
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Dataset Description

DNA sequence information of viruses associated with copepods (*Acartia tonsa* and *Labidocera aestiva*)

All sequence information associated with this study has been deposited into NCBI Genbank. The accession numbers for the two circoviruses detected are JF912805 and JQ837277. Additional metagenomic sequences have been deposited under accession nos. JY253391–JY253440).

All Quantitative PCR data obtained as part of this study has been published in peer-reviewed journals.

No environmental metadata was collected as part of this study.

Dunlap, D.S., T.F.F. Ng, K. Rosario, J.G. Barbosa, A.M. Greco, M. Breitbart, I. Hewson (2013). Molecular and microscopic evidence of viruses in marine copepods. *Proceedings of the National Academy of Sciences of the USA*, 110 1375.

Eaglesham JB, Hewson I (2013). Widespread detection of eukaryotic circular ssDNA viral genotypes in estuarine, coastal and open ocean net plankton. *Marine Ecology Progress Series*, in press (doi: 10.3354/meps10575).

Methods & Sampling

Zooplankton were collected using a 335-um plankton net, and copepods were individually picked from the bulk zooplankton samples, washed three times in 100-kDa filtered seawater, and incubated overnight in a fecatron to allow for gut clearing. After gut clearing, the copepods were rinsed three times in 100-kDa filtered seawater and then frozen at -80 deg C until further processing. For viral metagenomic analysis, *L. aestiva* and *A. tonsa* were collected from Bayboro Harbor in April 2009 and May 2010, respectively. For qPCR detection, *L. aestiva* were collected from multiple locations, including Bayboro Harbor, Eckerd Pier, the mouth of the Alafia River,

and Fort Desoto Beach. To determine the temporal dynamics of viral infection in A. tonsa, samples were collected from Bayboro Harbor once a month throughout 2011.

Virus particles were purified from each copepod species based on size, density, and nuclease resistance, and viral metagenomes were sequenced according to standard protocols The copepods were homogenized in sterile SM buffer (50 mM TrisCl, 10 mM MgSO4, 0.1 M NaCl; pH 7.5), centrifuged at 10,000 x g for 10 min at 4 deg C to pellet animal tissues, and passed through a 0.22-um filter to remove bacteria and animal cells. The *A. tonsa* filtrate was also loaded onto a cesium chloride step gradient with 1 mL each of 1.2, 1.5, and 1.7 g mL-1 in SM buffer. After ultracentrifugation at 61,000 x g for 3 h at 4 deg C, the viral fraction (between the 1.2- and 1.5-g mL-1 density layers) was collected, then concentrated and washed twice on a Microcon YM-30 column (Millipore). Both the *A. tonsa* and *L. aestiva* viral fractions were treated with 0.2 volumes of chloroform for 10 min, and then incubated with 2.5 U DNase I per uL of sample for 3 h to eliminate free nucleic acids. After the reaction was stopped by incubation at 65 deg C for 10 min, viral DNA was extracted with the QIAmp MinElute Virus Spin Kit (Qiagen) and amplified with the strand-displacement method of the Genomiphi V2 DNA Amplification Kit (GE Healthcare). The GenomePlex Whole Genome Amplification Kit (Sigma-Aldrich) was used to fragment and amplify the DNA, which was then cloned into the pCR4 vector using TOPO TA cloning (Invitrogen). A total of 38 transformants were sequenced for each copepod species using dideoxynucleotide sequencing, and the resulting metagenomic sequences were analyzed using tBLASTx against the GenBank nonredundant database. In this study, the main purpose of sequencing these small viral metagenomes was to identify putative viral targets for further quantitative ecological study.

Several sequences from the viral metagenomes of both *A. tonsa* and *L. aestiva* had tBLASTx similarities to viruses in the Circoviridae family. Given that known circoviruses have small circular genomes, back-to-back PCR primers (L. aestiva primers: 5'-CACCAGCAACTACAGCATCAA-3' and 5'-GTGACTATGATCCGCTTGGG-3'; A. tonsa primers: 5'-ACGAAGTAGCGCTCGAACTG-3' and 5'-CGTGAACTACGCTGGTCGTA-3') were designed from the metagenomic sequences using Primer 3 to amplify the complete circular genome of the copepod circo-like viruses directly from unamplified copepod DNA extracts through inverse PCR. The PCR reactions [containing 1 uM of each primer, 200 uM dNTPs, 1 U RedTaq DNA Polymerase (Sigma-Aldrich), 1 x Red Taq Reaction Buffer, and 5 uL of target DNA in a 50-uL reaction] were amplified as follows: 95 deg C for 5 min; 45 cycles of 94 deg C for 1 min, 58 deg C minus 0.2 deg C per cycle for 1 min, and 72 deg C for 3 min; and a final extension at 72 deg C for 10 min. The resulting whole genome PCR products were cloned into the pCR4 vector using TOPO TA cloning and sequenced to 3 x coverage. ORFs were predicted and annotated using SeqBuilder (DNASTAR), and stem- loop structures were manually annotated by locating complementary sections.

Data Processing Description

Genomes were analyzed for the presence of a nuclear localization signal using NLStradamus. Alignments of the copepod circo-like virus replication initiator protein (Rep) amino acid sequences with viral and associated satellite members of the Pfam viral Rep family PF02407 and other circular Rep-encoding ssDNA viruses from various environmental sources were performed using the PRALINE server. A maximum likelihood phylogenetic tree was constructed using the PhyML server, with the (LG+I+G) model chosen as the best-fit substitution model according to ProtTest. Branch support was assessed with the approximate likelihood ratio test, and values >60 percent are reported.

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Data Files

File
circovirus_sequence.csv (Comma Separated Values (.csv), 8.98 KB) MD5:b240e65452838e37261342bcab5b2c26 Primary data file for dataset ID 4075

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Parameters

Parameter	Description	Units
data_type	Sample analysis type.	unitless
location	Sampling location name.	unitless
lat	Latitude component of geographic location where samples were obtained.	decimal degrees
lon	Longitude component of geographic location where samples were obtained.	decimal degrees
date	Date, reported as YYYYMMDD.	unitless
taxon	Taxonomic name reported as genus species.	unitless
accession_no	Accession number corresponding to the GenBank respository.	unitless

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Instruments

Dataset-specific Instrument Name	Electrophoresis Chamber
Generic Instrument Name	Electrophoresis Chamber
Dataset-specific Description	USA Scientific electrophoresis chamber was used in conjunction with a Bio Rad power supply (no model numbers were available, for more information see: http://www.usascientific.com/electrophoresis.aspx).
Generic Instrument Description	General term for an apparatus used in clinical and research laboratories to separate charged colloidal particles (or molecules) of varying size through a medium by applying an electric field.

Dataset-specific Instrument Name	Plankton Net
Generic Instrument Name	Plankton Net
Dataset-specific Description	General Oceanics plankton net (335 um mesh size).
Generic Instrument Description	A Plankton Net is a generic term for a sampling net that is used to collect plankton. It is used only when detailed instrument documentation is not available.

Dataset-specific Instrument Name	Thermal Cycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	DNA amplification was conducted using a Bio Rad Thermal Cycler (no model number available, for more information see http://www.bio-rad.com/en-us/category/thermal-cyclers).
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)

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Deployments

Tampa_Bay_Seawall_Breitbart

Website	https://www.bco-dmo.org/deployment/59112
Platform	shoreside Tampa_Bay_Seawalls
Start Date	2004-04-11
End Date	2011-10-06
Description	Sampling locations for the project 'Circovirus DNA Sequence Information' (PIs: Breitbart, and Hewson). Samples wer collected by hand from a seawall, using a 335 micron plankton net.

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Project Information

Discovery of viruses infecting marine Copepoda (Viruses in Copepods)

Coverage: Tampa Bay, Florida

(Adapted from the NSF Award Abstract)

Mesozooplankton are critical components of marine food webs as the primary consumers of phytoplankton, as prey for upper trophic level predators including commercially important fish, and by mediating carbon transfer from the atmosphere to the ocean interior through fecal matter deposition. Thus, the factors that control population dynamics of zooplankton are critical to understanding the structure and function of marine food webs. Two decades of research on marine viruses have demonstrated their profound effects on all levels of marine life from bacteria to whales. The majority of marine virus studies have focused on infection of microorganisms, particularly heterotrophic prokaryotes and eukaryotic phytoplankton. Despite the ecological importance of zooplankton, virtually nothing is known about the impact of viruses on the most abundant zooplankton group - the copepods.

This project will prospect for novel viruses in Acartia tonsa, a dominant calanoid copepod in Tampa Bay, Florida using metagenomic sequencing approaches. The viral load, prevalence, and environmental reservoirs for

selected newly identified viruses will then be examined using quantitative PCR. This study will present the biological oceanography community with novel information on the diversity and prevalence of viruses in copepods. An increased understanding of the types of viruses infecting mesozooplankton will increase knowledge of viral diversity in the oceans and provide sequence information on a poorly-constrained genetic pool. The long-term goal of this project is to understand the impact of viruses on zooplankton population dynamics as agents of mortality and, therefore, ultimately how viral infections affect food web interactions and biogeochemical cycling in the oceans. Determining the type of viruses present in copepods and the prevalence of these viruses are critical first steps in determining the role of viruses in zooplankton ecology. Understanding the role of viruses in mesozooplankton ecology will fill a major gap in knowledge of marine ecosystem dynamics, and has the potential to be transformative for oceanography.

No environmental metadata was collected as part of this study.

All sequence information associated with this study has been deposited into NCBI Genbank. The accession numbers for the two circoviruses detected are JF912805 and JQ837277. Additional metagenomic sequences have been deposited under accession nos. JY253391–JY253440).

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1049670

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