Viral metagenomic investigation of the Caribbean echinoderm Holothuria floridana collected in Marathon, Florida on 25 Aug 2023

Website: https://www.bco-dmo.org/dataset/985655
Data Type: Other Field Results
Version: 1
Version Date: 2025-10-07

Project
» Exploring the role of boundary layer microbial remineralization in flavivirus-host dynamics (Holothurian Flaviviruses)

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Abstract
Echinoderms play crucial roles in coral reef ecosystems, where they are significant detritivores and herbivores. The phylum is widely known for its boom and bust cycles, driven by food availability, predation pressure and mass mortalities. Hence, surveillance of potential pathogens and associates of grossly normal specimens is important to understanding their roles in ecology and mass mortality. We performed viral surveillance of the coral reef echinoderm Holothuria floridana (urn:lsid:marinespecies.org:taxname:210900), using metagenomics. Grossly normal H. floridana specimens were collected from a reef in Florida. Viral metagenomes were assembled and aligned against viral genomes and protein encoding regions. Metagenomic reads and previously sequenced transcriptomes were further investigated for putative viral elements by Kraken2. H. floridana yielded viral taxa similar to those found in other sea cucumbers, including Pisoniviricetes (Picornaviruses), Ellioviricetes (Bunyaviruses), and Magsaviricetes (Nodaviruses). This dataset includes the accession information for the sequences performed and archived at the National Center for Biotechnology Information (NCBI) Sequence Read Archive.

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Coverage

Location: Florida; Marathon; depth 1m
Spatial Extent: **Lat**:24.7592 **Lon**:-81.1076
Temporal Extent: 2023-08-25

Methods & Sampling

Specimens (n = 4) of grossly normal *Holothuria floridana* (urn:lsid:marinespecies.org:taxname:210900) were collected offshore of Marathon, Florida (24.7592N, 81.1076W) on 2023-08-25 and immediately subsampled by five mm biopsy punches, which were transferred to sterile cryovials per Crandell et al. (2023). Punches were frozen at −80 °C on arrival to the lab at the Florida Fish and Wildlife Commission (within an hour of collection) before shipping to Cornell University. Small subsamples of each specimen were prepared for viral metagenomic sequencing broadly following the approach of Ng et al. (2011). Briefly, tissues were homogenized in sterile, 0.02 µm filtered phosphate buffered saline by beating using Zymo ZR Basher Beads (part number S6012-50) in a Biospec Instruments homogenizer for 1 min at maximum speed. The homogenates were centrifuged at 3,000 × g for 1 min to remove large cell debris, before filtering the supernatant through 0.2 µm pore size polyethersulfone syringe filters. Filtered homogenates were treated with 5U Turbo DNase (Invitrogen; part number AM2238), 5U Benzonase nuclease (Sigma Aldrich; part number E1014) and 20U RNase ONE (Promega; part number M4261) for 2 hours at 37 °C to digest extracellular and non-ribosome-bound nucleic acids. Following incubation, RNA was extracted from subsamples of purified virus-sized material using the Zymo Viral RNA kit (part number R1034) before storing RNA at −80 °C until further processing.

RNA in viral extracts was amplified using the SeqPlex RNA kit (Sigma Aldrich; part number SEQXE-10RXN, n = 11 libraries). Subsequent specimens were amplified using the TransPlex kit (WTA2; Sigma Aldrich; part number WTA2-10RXN, n = 3 libraries), which was used in prior RNA viral metagenomic work. Amplification products were purified using the Zymo DNA Clean and Concentrator -5 (part number D4004), quantified by Pico Green fluorescence (Invitrogen; part number P11496), and submitted for sequencing at the Cornell Biotechnology Resource Center. Libraries were prepared for sequencing using the Nextera Flex kit (Illumina; part number 20018704) and sequenced on the Illumina MiSeq platform using the 500 bp Nano kit. Sequences from all libraries were archived at NCBI under Bioproject PRJNA1117494 and SRA accessions SRR29258991-SRR29258993 and SRR29259000.

BCO-DMO Processing Description

- Imported "Virome_Accession_Metadata.txt" into the BCO-DMO system
- Removed empty fields (fasta_file, assembly, filename3, filename4, filename5, filename6, filename7, filename8)
- Added latitude, longitude, and date of collection from the methods information
- Renamed NCBI identifier fields to include more standard and descriptive titles
- Removed "object_status" upon consultation with the submitter
- Exported file as "985655_v1_virome_accession.csv"

Scientific names in the data were checked using World Register of Marine Species (WoRMS) Taxon Match. All scientific names in the data are valid and accepted names as of 2025-10-01.

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Data Files

File
985655_v1_virome_accession.csv (Comma Separated Values (.csv), 1.80 KB) MD5:cee6a3ba6b4d889b5ff6c5e9963f5343 Primary data file for dataset ID 985655, version 1

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Related Publications

Crandell, J. G., Altera, A. K., DeRito, C. M., Hebert, K. P., Lim, E. G., Markis, J., Philipp, K. H., Rede, J. E., Schwartz, M., Vilanova-Cuevas, B., Wang, E., & Hewson, I. (2023). Dynamics of the Apostichopus californicus-associated flavivirus under suboxic conditions and organic matter amendment. Frontiers in Marine Science, 10. https://doi.org/10.3389/fmars.2023.1295276
Methods

Hewson, I., Brandt, M., Budd, K., Breitbart, M., DeRito, C., Gittens Jr, S., Henson, M. W., Hylkema, A., Sevier, M., Souza, M., Vilanova-Cuevas, B., & Von Hoene, S. (2024). Viral metagenomic investigation of two Caribbean echinoderms, Diadema antillarum (Echinoidea) and Holothuria floridana (Holothuria). PeerJ, 12, e18321. Portico. https://doi.org/10.7717/peerj.18321
Results

Ng, T. F. F., Wheeler, E., Greig, D., Waltzek, T. B., Gulland, F., & Breitbart, M. (2011). Metagenomic identification of a novel anellovirus in Pacific harbor seal (Phoca vitulina richardsii) lung samples and its detection in samples from multiple years. Journal of General Virology, 92(6), 1318–1323. https://doi.org/10.1099/vir.0.029678-0
Methods

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Related Datasets

IsRelatedTo

Hewson, I., Brandt, M., Budd, K., Breitbart, M., DeRito, C., Gittens, S., Henson, M., Hylkema, A., Sevier, M., Souza, M., Vilanova-Cuevas, B., Von Hoene, S. (2025) **Transcriptome and viral metagenome data from Diadema antillarum collected at St. Thomas (USVI) and Saba (Caribbean Netherlands) in Apr 2022.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-10-07 doi:10.26008/1912/bco-dmo.985619.1 [view at BCO-DMO]
Relationship Description: Data are referenced in the same results publication.

References

Cornell University. Viral metagenomic investigation of two Caribbean echinoderms. 2024/05. In: BioProject [Internet]. Bethesda, MD: National Library of Medicine (US), National Center for Biotechnology Information; 2011-. Available from: http://www.ncbi.nlm.nih.gov/bioproject/PRJNA1117494. NCBI:BioProject: PRJNA1117494. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1117494

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Parameters

Parameter	Description	Units
SRA_accession	NCBI Run accession in the Sequence Read Archive (SRA)	unitless

SRA_study	NCBI study accession in the Sequence Read Archive (SRA)	unitless
bioproject_accession	NCBI BioProject identifier	unitless
biosample_accession	NCBI BioSample accession number	unitless
sample_name	Sample Name	unitless
library_ID	Sample Name	unitless
title	Title of library	unitless
library_strategy	Sequencing strategy: RNA-Seq	unitless
library_source	Source of sequencing library: Metagenomic DNA	unitless
library_selection	Size selection of DNA used	unitless
library_layout	Description of sequencing reads: paired	unitless
platform	Platform used for sequencing	unitless
instrument_model	Model of instrument used for sequencing	unitless
design_description	Brief description of how libraries were prepared from source material	unitless
filetype	Format of sequencing file	unitless
filename	First filename	unitless
filename2	Second filename	unitless
Latitude	Latitude the specimens were collected, North is positive	decimal degrees
Longitude	Longitude the specimens were collected, West is negative	decimal degrees
Date_specimen_collected	Date the specimens were collected	unitless

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Instruments

Dataset-specific Instrument Name	Illumina MiSeq
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Libraries were prepared for sequencing using the Nextera Flex kit (Illumina; part number 20018704) and sequenced on the Illumina MiSeq platform using the 500 bp Nano kit.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	The homogenates were centrifuged at 3,000 × g for 1 min to remove large cell debris, before filtering the supernatant through 0.2 µm pore size polyethersulfone syringe filters.
Generic Instrument Description	A machine with a rapidly rotating container that applies centrifugal force to its contents, typically to separate fluids of different densities (e.g., cream from milk) or liquids from solids.

Dataset-specific Instrument Name	Biospec Instruments homogenizer
Generic Instrument Name	Homogenizer
Dataset-specific Description	Briefly, tissues were homogenized in sterile, 0.02 µm filtered phosphate buffered saline by beating using Zymo ZR Basher Beads (part number S6012-50) in a Biospec Instruments homogenizer for 1 min at maximum speed.
Generic Instrument Description	A homogenizer is a piece of laboratory equipment used for the homogenization of various types of material, such as tissue, plant, food, soil, and many others.

Dataset-specific Instrument Name	Incubator
Generic Instrument Name	Incubator
Dataset-specific Description	Filtered homogenates were treated with 5U Turbo DNase (Invitrogen; part number AM2238), 5U Benzonase nuclease (Sigma Aldrich; part number E1014) and 20U RNase ONE (Promega; part number M4261) for 2 hours at 37 °C to digest extracellular and non-ribosome-bound nucleic acids. Following incubation, RNA was extracted from subsamples of purified virus-sized material using the Zymo Viral RNA kit (part number R1034) before storing RNA at −80 °C until further processing.
Generic Instrument Description	A device in which environmental conditions (light, photoperiod, temperature, humidity, etc.) can be controlled. Note: we have more specific terms for shipboard incubators (https://www.bco-dmo.org/instrument/629001) and in-situ incubators (https://www.bco-dmo.org/instrument/494).

Dataset-specific Instrument Name	
Generic Instrument Name	Manual Biota Sampler
Dataset-specific Description	Specimens (n = 4) of grossly normal H. floridana were collected offshore of Marathon, Florida (24.7592N, 81.1076W), on 2023-08-25 and immediately subsampled by five mm biopsy punches, which were transferred to sterile cryovials per Crandell et al. (2023).
Generic Instrument Description	"Manual Biota Sampler" indicates that a sample was collected in situ by a person, possibly using a hand-held collection device such as a jar, a net, or their hands. This term could also refer to a simple tool like a hammer, saw, or other hand-held tool.

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Project Information

Exploring the role of boundary layer microbial remineralization in flavivirus-host dynamics (Holothurian Flaviviruses)

Coverage: Northeastern Pacific Ocean

NSF Award Abstract:

Marine diseases pose considerable risks to invertebrates, such as sea cucumbers, in the face of changing ocean conditions. While many invertebrate diseases are driven by pathogens, the interplay between animal biology and environmental conditions often mediates the outcome of the pathogen-host relationship. Sea cucumbers are ecologically and economically important animals that occur in a wide range of marine habitats. This project aims to decipher how the interaction between the biology of sea cucumbers, environmental conditions, and a newly-discovered type of virus, seemingly innocuous under typical conditions, may lead to lethal disease in giant Pacific sea cucumbers in the U.S. West Coast. The study includes surveys in coastal regions in southeast Alaska, Washington, and California as well as laboratory experiments manipulating seawater oxygen concentrations, temperature, and simulated microalgal blooms. The project engages community scientists, fishers, high school students, and indigenous groups, and supports training of one graduate and several undergraduate students. A workshop that brings together scientists across marine ecology, disease, and veterinary disciplines is planned to prepare a handbook of best practices in marine disease investigation.

Metagenomic and community-level sequencing efforts have revealed an astonishing diversity of viruses associated with grossly normal marine invertebrates. The vast majority of detected viruses likely represents asymptomatic infections under typical conditions but may generate pathology in hosts under changing environmental conditions. This project investigates the ecology of a group of enveloped positive sense single-stranded RNA viruses (flaviviruses) that this research team has recently discovered in the giant California sea cucumber Apostichopus californicus by addressing three hypotheses: 1) Aquatic insect-only Flaviviruses (aiFVs) do not cause gross pathology under typical conditions; 2) aiFVs proliferate and generate clinical and gross pathology under suboxic stress; and 3) Periodic increases in primary production and mean temperature excursions cause aiFV proliferation and subsequently exacerbate holothurian disease process. The study comprises a restricted survey of aiFV diversity via amplicon sequencing and their prevalence within and between populations, development of an antibody-based approach for aiFV detection, and examination of aiFV behavior in concert with host transcription and veterinary pathology. The study includes field surveys and in laboratory manipulative experiments.

This award reflects NSF's statutory mission and has been deemed worthy of support through evaluation using the Foundation's intellectual merit and broader impacts review criteria.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-2049225

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