GenBank accessions for 18S rRNA gene amplicons from swab specimens collected in the Caribbean and Réunion (France) from May 2022 to Dec 2024

Website: https://www.bco-dmo.org/dataset/985574
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
Investigating the cause of mass mortality of Diadema antillarum (urn:lsid:marinespecies.org:taxname:124332) identified the etiological agent as the Diadema antillarum Scuticociliatosis Philaster Clade (DaScPc). We sought to investigate the prevalence of this ciliate in sympatric reservoirs in the coral reef environment by targeted PCR using primers designed around this 18S rRNA sequence. We used swab sampling of corals, macroalgae, and other surfaces, followed by phylogenetic analyses, to identify the presence and diversity of this ciliate. We found that DaScPc could be found primarily in association with the coral Siderastrea siderea, and that changes in its prevalence could be explained by disease state of urchins, macroalgal density, and proximity to disease sites. This dataset contains the GenBank accession, sequencing, and collection metadata for the study.

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Coverage

Location: St. Thomas, St. John, and St. Croix (USVI); Aruba; Grenada; Marathon, Florida (USA); Bayahibe, Dominican Republic; Antigua; Turks and Caicos Islands; St. Eustatius; Saba; Réunion (France); Caribbean Netherlands
Spatial Extent: **N**:24.9521 **E**:55.3294 **S**:-21.2705 **W**:-80.9669
Temporal Extent: 2022-05 - 2024-12

Methods & Sampling

Surface swab specimens were collected between May 2022 and December 2024. Samples were collected from sites affected (i.e. sites which bore DaSc-affected urchins) at the time of sampling or those which were previously affected; sites that were never affected (i.e. reference sites); and ports/marinas. Swab specimens were collected by snorkelers at depths of ~1–4 m. Polyester swabs (Dry Transport Systems, Puritan Medical Products) were transported to the survey site sealed in an air-filled transport tube and withdrawn into the water immediately adjacent to the swabbed surface. The swab was gently rubbed for 10 s across a ~2 cm2 surface before retrieval into the air-filled transport tube. Live swabbed surfaces, chosen haphazardly, were photographed to confirm the identity of invertebrate or plant species (seagrass and macroalgae). At some sites, identity of swabbed surfaces could not be determined (i.e. unidentifiable surface swabs), and in some cases, the taxonomy of swabbed specimens was unclear (e.g. macroalgae; n = 11), so they were assigned to higher taxonomy. Swabs were transported at ambient temperature to shore, where the swab tips were cut off with clean scissors into cryovials containing RNAlater Solution (Invitrogen).

DNA was extracted from swab tips were extracted using the Quick-DNA Insect/Tissue kit (Zymo Research). The manufacturer’s extraction protocol (v.2.21) was followed with these exceptions: we did not add beta-mercaptoethanol to the lysis buffer, bead bashing was shortened to 2 min, and DNA was eluted into nuclease-free sterile water instead of elution buffer. Specimens were subjected to PCR employing pan-Ciliophora 18S rRNA gene primers 384F (5’-YTB GAT GGT AGT GTA TTG GA-3’) and 1147R (5’-GAC GGT ATC TRA TCG TCT TT-3’) (Dopheide et al. 2008). PCR was performed in 50 µl reactions comprising 1× PCR buffer, 2.5 mM MgCl2, 0.2 mM deoxynucleoside triphosphates (Promega PCR Nucleotide Mix), 200 pmol forward and reverse primers, 1 µl of 2 ng ml–1 BSA (Sigma), 5U Taq DNA polymerase (Invitrogen), and 2 µl of pooled DNA extracts. Thermal cycling was preceded by an initial heating step for 3 min at 95°C, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min in a BioRad MyCycler.

The PCR amplicons (5 µl) were then visualized on a 1 % agarose gel in 1× Tris-borate-EDTA after electrophoresis at 85 V for 45 min and staining with SYBR Gold. PCR products were cleaned up using the Zymo Clean & Concentrator-5 kit and subject to dye terminator (Sanger) sequencing at the Biotechnology Resource Center at Cornell University.

BCO-DMO Processing Description

- Imported "Metadata_GenBankAccessions.txt" into the BCO-DMO system
- Adjusted values in "Latitude" and "Longitude" fields to BCO-DMO style, removing cardinal indicators and making West and South values negative
- Converted "Sample_Month" to ISO 8601 format YYYY-MM
- Removed "object_status" from dataset, upon consultation with submitter
- Removed all rows where "Sample_Type" contains "Tissue", upon request from the submitter
- Exported file as "985574_v1_18s_r_rna_gene_amplicons.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
985574_v1_18s_r_rna_gene_amplicons.csv (Comma Separated Values (.csv), 8.93 KB) MD5:a0960aed9971ee368108225d76617b70 Primary data file for dataset ID 985574, version 1

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Related Publications

Dopheide, A., Lear, G., Stott, R., & Lewis, G. (2008). Molecular Characterization of Ciliate Diversity in Stream Biofilms. Applied and Environmental Microbiology, 74(6), 1740–1747. https://doi.org/10.1128/AEM.01438-07
Methods

Vilanova-Cuevas, B. Y., Reyes-Chavez, B., Breitbart, M., & Hewson, I. (2023). Design and validation of a PCR protocol to specifically detect the clade ofPhilastersp. associated withDiadema antillarumscuticociliatosis. https://doi.org/10.1101/2023.09.11.557215
Results

Vilanova-Cuevas, B., Philipp, K., Altera, A., Apprill, A., Becker, C., Behringer, D., Brandt, M., Breitbart, M., Budd, K., DeRito, C., Duermit-Moreau, E., Evans, J., Hopson-Fernandes, M., Fleischer, J., Gittens, S., Henson, M., Hylkema, A., Kellogg, C., Maritan, A., … Hewson, I. (2025). Detection of the Diadema antillarum scuticociliatosis Philaster clade on sympatric metazoa, plankton, and abiotic surfaces and assessment for its potential reemergence. Marine Ecology Progress Series, 753, 19–35. https://doi.org/10.3354/meps14763
Results

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Parameters

Parameter	Description	Units
Genbank_Accession	Genbank Accession Number	unitless
Sequence_ID	Sequence identification code	unitless
Sample_Location	Geographic location of specimen collection	unitless
Sample_Type	Sample type: Swab	unitless
Sample_Month	Month of Swab Collection	unitless
Latitude	Latitude of specimen collection, positive is North, negative is South	decimal degrees
Longitude	Longitude of specimen collection, positive is East, negative is West	decimal degrees

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Instruments

Dataset-specific Instrument Name	ABI 3730xl genetic analyzer
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	PCR products were cleaned up using the Zymo Clean & Concentrator-5 kit and subject to dye terminator (Sanger) sequencing at the Biotechnology Resource Center at Cornell University.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	Photographed
Generic Instrument Name	Camera
Dataset-specific Description	Live swabbed surfaces, chosen haphazardly, were photographed to confirm the identity of invertebrate or plant species (seagrass and macroalgae).
Generic Instrument Description	All types of photographic equipment including stills, video, film and digital systems.

Dataset-specific Instrument Name	Snorkeler
Generic Instrument Name	Diving Mask and Snorkel
Dataset-specific Description	Swab specimens were collected by snorkelers at depths of ~1–4 m.
Generic Instrument Description	A diving mask (also half mask, dive mask or scuba mask) is an item of diving equipment that allows underwater divers, including, scuba divers, free-divers, and snorkelers to see clearly underwater. Snorkel: A breathing apparatus for swimmers and surface divers that allows swimming or continuous use of a face mask without lifting the head to breathe, consisting of a tube that curves out of the mouth and extends above the surface of the water.

Dataset-specific Instrument Name	BioRad MyCycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	Thermal cycling was preceded by an initial heating step for 3 min at 95°C, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 54°C for 30 s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 5 min in a BioRad MyCycler.
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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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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