# V3-V4 16S sequence accessions from samples of whole Gigantidas childressi as well as seafloor water samples collected on R/V Thompson cruise TN391 in Mississippi Canyon 853 in the Gulf of Mexico during May 2021

**Website**: https://www.bco-dmo.org/dataset/940537
**Data Type**: Cruise Results
**Version**: 1
**Version Date**: 2024-10-15

**Project**
» Collaborative Research: dispersal depth and the transport of deep-sea, methane-seep larvae around a biogeographic barrier (SALT)



| Contributors | Affiliation | Role |
|---|---|---|
| Arellano, Shawn M. | Western Washington University - Shannon Point Marine Center (SPMC) | Principal Investigator |
| Eggleston, David B. | North Carolina State University - Center for Marine Science and Technology (NCSU CMAST) | Principal Investigator |
| Young, Craig M. | University of Oregon (OIMB) | Principal Investigator |
| He, Ruoying | North Carolina State University - Marine, Earth and Atmospheric Sciences (NCSU MEAS) | Co-Principal Investigator |
| Van Gaest, Ahna | Western Washington University (WWU) | Scientist |
| Beaver, Tessa Francisca | Western Washington University (WWU) | Student |
| Rauch, Shannon | Woods Hole Oceanographic Institution (WHOI BCO-DMO) | BCO-DMO Data Manager |



**Abstract**
This dataset includes V3-V4 16S sequence accessions from samples of whole Gigantidas childressi larvae and juveniles as well as seafloor water samples, collected from Mississippi Canyon 853 in the Gulf of Mexico during May 2021 and sequenced in August 2021, with links to the National Center for Biotechnology Information Sequence Read Archive (NCBI SRA) pages. Samples were collected on SALT cruise TN391 with AUV Sentry and ROV Jason and later sequenced to examine the microbiome of G. childressi across early developmental stages, compared with the water samples. Researchers interested in chemosymbiotic organisms and the acquisition of beneficial symbionts during early development may find this data useful. The collection of animals was carried out by the group of scientists aboard the R/V Thompson during the TN391 SALT cruise. The party responsible for the collection and interpretation of samples and sequences were Tessa Beaver, M.S.and Dr. Shawn Arellano (both of Western Washington University).

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## Coverage

**Location**: Mississippi Canyon 853, Gulf of Mexico
**Spatial Extent**: **Lat**:28.122833 **Lon**:-89.140333
**Temporal Extent**: 2021-06 - 2021-08

### Methods & Sampling

Veligers, pediveligers, juveniles, and adult mussels of *Gigantidas childressi* were collected using the remotely-operated vehicle (ROV) Jason II and automated underwater vehicle (AUV) Sentry (National Deep Submergence Facility, Woods Hole Oceanographic Institution) onboard the R/V Thomas G. Thompson (University of Washington) during cruise TN391. Samples were collected from Mississippi Canyon 853 (28° 7.37’ N, 89° 8.42’ W) in the Gulf of Mexico at a depth of 1070 meters on June 16th, 2021 (Jason dive J2-1337 and Sentry dive S595P). Adult *Gigantidas childressi* are morphologically distinguishable. *Gigantidas childressi* adults were sampled with ROV Jason and recovered to the surface in insulated bioboxes. Pediveligers (swimming larvae with a developed foot that are competent to undergo metamorphosis) and juveniles (metamorphosed) of *G. childressi* were collected from the interstices of mussel beds with the ROV Jason suction sampler and within samples of adults. Veligers (pre-competent swimming larvae without a developed foot) were collected in the water column at an altitude of 5 meters above bottom with AUV Sentry fitted with the SyPRID plankton sampler with 150-micron mesh nets (Billings et al. 2016). A paired 2-liter (L) water sample was also taken at an altitude of 1.5 meters above the bottom at the time of fauna collection with two 4L Niskin bottles equipped on ROV Jason.

Plankton samples recovered by the AUV Sentry SyPRID sampler were immediately rinsed from the collectors with cold 0.3-micrometer (um) filtered seawater (FSW) into canisters of chilled FSW. Larvae and juveniles recovered from ROV Jason suction and scoop samples were retained on a 253 um mesh sieve and resuspended in cold FSW. All live plankton and juveniles were maintained below 8 degrees Celsius (°C) (ambient temperature) during subsequent processing. Live *G. childressi* larvae were immediately sorted from the samples under a dissecting microscope, imaged on a compound light microscope, then preserved individually in 0.5 milliliters (mL) centrifuge tubes with 95% molecular grade ethanol. Snips of gill tissue from adult mussels were dissected aseptically on the ship and stored at -80°C in 2 mL cryovials. Background water samples were collected into sterilized plastic canisters, stored at ambient seafloor temperature (8°C), and processed immediately after recovery. Each sample was vacuum filtered onto a 0.2 um polycarbonate filter. Filters were placed into sterile 2-mL centrifuge tubes and stored at -80 °C until further processing.

All sample processing for DNA sequencing was conducted at Shannon Point Marine Center in Anacortes, Washington. DNA was extracted from individual larvae, juveniles, and samples of adult gill tissue (2.5 milligrams) using the Nucleospin Tissue XS kit (Machery-Nagel) following the manufacturer's instructions. DNA was extracted from filters using the Fast DNA Spin Kit for Soil (MP Bio) according to the manufacturer's instructions. The concentration of extracted DNA was quantified using a Qubit fluorometer (2.0).

For microbiome community composition, amplification, library preparation, and sequencing of the V3-V4 regions of the 16S rRNA gene was conducted by Exact Scientific (Ferndale, WA). Library preparation was performed following the Illumina 16S Metagenomic Sequencing Library Preparation protocol (15044223B). DNA was amplified using the following bacterial-specific primers: Forward: 5’ TACGGGNGGCWGCAG, Reverse: 5’ GACTACHVGGGTATCTAATCC (S-DBact-0341-b-S-17/S-D-Bact-0785-a-A-2; Klindworth et al. 2013). PCR conditions for 16S rRNA amplicons were 95 °C for 3 minutes, with 25 cycles at 95°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, followed by 72°C for 5 minutes and a holding temperature of 4°C. Multiplexing indices and Illumina overhang adapters were attached with a second limited-cycle PCR step using the NEXTERA® XT Index Kit. Resulting PCR products were purified after each step with Ampure XP Beads™, 80% EtOH, and 10 mM Tris pH 8.5. Libraries were then normalized, pooled, and sequenced on an Illumina MiSeq platform using 600 cycle v3 chemistry.

### Data Processing Description

Processing and analysis of Illumina sequences followed the QIIME2 computational pipeline for the V3/V4 region of the 16S rRNA gene as in Carrier et al. (2021). Illumina reads with quality information were processed using QIIME 2 (ver. 2021.8; Bolyen et al., 2019). Adapters and primers were removed and forward and reverse reads were paired with VSEARCH (Rognes et al. 2016). Paired sequences were filtered by a minimum quality

score of 25 and denoised with Deblur (Amir et al. 2017). Features were analyzed as amplicon sequence variants (ASVs) and were assigned taxonomy using the latest version of the SILVA (ver. 138.1) bacterial database.

### BCO-DMO Processing Description

- Imported original file "BeaverAndArellano2024_SRA.csv" into the BCO-DMO system.
- Added columns for collection latitude, longitude, date, and depth.
- Renamed fields to comply with BCO-DMO naming conventions.
- Saved final file as "940537_v1_v3-v4_16s_rrna_sequences_g_childressi.csv"

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## Related Publications

Amir, A., McDonald, D., Navas-Molina, J. A., Kopylova, E., Morton, J. T., Zech Xu, Z., Kightley, E. P., Thompson, L. R., Hyde, E. R., Gonzalez, A., & Knight, R. (2017). Deblur Rapidly Resolves Single-Nucleotide Community Sequence Patterns. MSystems, 2(2). https://doi.org/10.1128/msystems.00191-16
https://doi.org/10.1128/mSystems.00191-16
*Software*

Beaver, Tessa F. (2022). Microbial Community Dynamics During Key Life History Transitions in the Deep-Sea Chemosymbiotic Mussel, Gigantidas childressi. WWU Graduate School Collection. 1115.
https://cedar.wwu.edu/wwuet/1115
*Results*

Billings, A., Kaiser, C., Young, C. M., Hiebert, L. S., Cole, E., Wagner, J. K. S., & Van Dover, C. L. (2017). SyPRID sampler: A large-volume, high-resolution, autonomous, deep-ocean precision plankton sampling system. Deep Sea Research Part II: Topical Studies in Oceanography, 137, 297–306.
https://doi.org/10.1016/j.dsr2.2016.05.007
*Methods*

Bolyen, E., Rideout, J. R., Dillon, M. R., Bokulich, N. A., Abnet, C. C., Al-Ghalith, G. A., … Asnicar, F. (2019). Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nature Biotechnology, 37(8), 852–857. doi:10.1038/s41587-019-0209-9
*Software*

Carrier, T. J., Beaulieu, S. E., Mills, S. W., Mullineaux, L. S., & Reitzel, A. M. (2021). Larvae of Deep-Sea Invertebrates Harbor Low-Diversity Bacterial Communities. The Biological Bulletin, 241(1), 65–76.
doi:10.1086/715669
*Methods*

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## Related Datasets

### IsRelatedTo

Western Washington University. 16S microbiome of larvae and juveniles of Gigantidas childressi. 2023/12. 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/PRJNA1047647. NCBI:BioProject: PRJNA1047647.

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## Parameters

| Parameter | Description | Units |
|---|---|---|
| Cruise | Number identifier for UNOLS research vessel cruise | unitless |
| Site | Site name where samples were taken | unitless |
| Latitude | Latitude of collection site | unitless |
| Longitude | Longitude of collection site | unitless |
| Collection_Date | Date of sample collection | unitless |
| Depth | Depth of sample collection | meters (m) |
| Dive_Collected | ROV Jason (J2-) or AUV Sentry (S-) dive numbers on which the samples were collected | unitless |
| Collection_Method | Mussel scoopers and slurps were taken by Jason, SyPRID samples were take by Sentry | unitless |
| Sample_ID | Unique ID for each sample | unitless |
| Sample_Group | Developmental stage, tissue type, or type for each sample | unitless |
| Sequence_File_ID | unique ID for each sequence file | unitless |
| SRA_Accession | SRA Accession number | unitless |
| Raw_Reads | total unprocessed read count | counts |
| Final_Reads | number of reads after quality score filtering | counts |
| Chimeric_Reads | number of chimeric reads | counts |
| Pcnt_Reads_Retained | Proportion of raw reads retained in the dataset | proportion |
| Number_of_ASVs | total number of ASVs | counts |

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## Instruments

| | |
|---|---|
| **Dataset-specific Instrument Name** | automated underwater vehicle (AUV) Sentry |
| **Generic Instrument Name** | AUV Sentry |
| **Generic Instrument Description** | The autonomous underwater vehicle (AUV) Sentry is a fully autonomous underwater vehicle capable of exploring the ocean down to 6,000 meters (19,685 feet) depth. Sentry builds on the success of its predecessor the ABE, with improved speed, range, and maneuverability. Sentry's hydrodynamic shape also allows faster ascents and descents. Sentry carries a superior science sensor suite and an increased science payload enabling it to be used for both mid-water and near-seabed oceanographic investigations. Sentry produces bathymetric, sidescan, subbottom, and magnetic maps of the seafloor and is capable of taking digital bottom photographs in a variety of deep-sea terrains such as mid-ocean ridges, deep-sea vents, and cold seeps at ocean margins. Sentry is uniquely able to operate in extreme terrain, including volcano caldera and scarps. Sentry's navigation system uses a doppler velocity log and inertial navigation system, aided by acoustic navigation systems (USBL or LBL). The USBL system also provides acoustic communications, which can be used to obtain the vehicle state and sensor status as well as to retask the vehicle while on the bottom. In addition its standard sensors, Sentry has carried a variety of science-supplied sensors, including the Nakamura redox potential probe, ACFR 3-D imaging system, and the Tethys in-situ mass spectrometer. Sentry can be used to locate and quantify hydrothermal fluxes. Sentry is also capable of a much wider range of oceanographic applications due to its superior sensing suite, increased speed and endurance, improved navigation, and acoustic communications. Sentry can be used as a stand alone vehicle or in tandem with Alvin or an ROV to increase the efficiency of deep-submergence investigations. More information is available from the operator site at URL: http://www.whoi.edu/main/sentry |

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| **Dataset-specific Instrument Name** | Qubit fluorometer (2.0) |
| **Generic Instrument Name** | Qubit fluorometer |
| **Generic Instrument Description** | Benchtop fluorometer. The Invitrogen Qubit Fluorometer accurately and quickly measures the concentration of DNA, RNA, or protein in a single sample. It can also be used to assess RNA integrity and quality.  Manufactured by Invitrogen, Carlsbad, CA, USA (Invitrogen is one of several brands under the Thermo Fisher Scientific corporation.) |

| | |
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| **Dataset-specific Instrument Name** | remotely-operated vehicle (ROV) Jason II |
| **Generic Instrument Name** | ROV Jason |
| **Generic Instrument Description** | The Remotely Operated Vehicle (ROV) Jason is operated by the Deep Submergence Laboratory (DSL) at Woods Hole Oceanographic Institution (WHOI). WHOI engineers and scientists designed and built the ROV Jason to give scientists access to the seafloor that didn't require them leaving the deck of the ship. Jason is a two-body ROV system. A 10-kilometer (6-mile) fiber-optic cable delivers electrical power and commands from the ship through Medea and down to Jason, which then returns data and live video imagery. Medea serves as a shock absorber, buffering Jason from the movements of the ship, while providing lighting and a bird’s eye view of the ROV during seafloor operations. During each dive (deployment of the ROV), Jason pilots and scientists work from a control room on the ship to monitor Jason’s instruments and video while maneuvering the vehicle and optionally performing a variety of sampling activities. Jason is equipped with sonar imagers, water samplers, video and still cameras, and lighting gear. Jason’s manipulator arms collect samples of rock, sediment, or marine life and place them in the vehicle’s basket or on "elevator" platforms that float heavier loads to the surface. More information is available from the operator site at URL. |

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| **Dataset-specific Instrument Name** | SyPRID plankton sampler |
| **Generic Instrument Name** | Sentry Precision Robotic Impeller Driven Sampler |
| **Generic Instrument Description** | The SyPRID (Sentry Precision Robotic Impeller Driven) sampler is an innovative deep-rated (6000 m) plankton sampler that partners with the Sentry Autonomous Underwater Vehicle (AUV) to obtain paired, large-volume plankton samples at specified depths and survey lines to within 1.5 m of the seabed and with simultaneous collection of sensor data. SyPRID uses a perforated Ultra-High-Molecular-Weight (UHMW) plastic tube to support a fine mesh net within an outer carbon composite tube (tube-within-a-tube design), with an axial flow pump located aft of the capture filter. The pump facilitates flow through the system and minimizes the bow wave at the mouth opening. The cod end, a hollow truncated cone, is also made of UHMW plastic and is designed to 'soften' the landing of zooplankton on the capture surface. SyPRID attaches as a saddle-pack to the Sentry vehicle. Sentry itself is configured with a flight control system that enables autonomous survey paths to altitudes as low as 1.5 m. In its inaugural deployment at the Blake Ridge Seep (2160 m) on the US Atlantic Margin, SyPRID was operated for 6 h at an altitude of 5 m. It recovered plankton samples from that stratum in excellent condition and with greater larval numbers than recovered in a typical 'near-bottom' MOCNESS sample from comparable habitats and depths. The prototype SyPRID and its next generations will enable studies of plankton or other particulate distributions associated with patchy habitats, localized physico-chemical strata (e.g., above and below the thermocline), or discrete water masses at an unprecedented spatial resolution for a large volume system [1]. More information is available by contacting: Carl Kaiser Program Manager Applied Ocean Physics & Engineering NDSF AUV Operations Manager Office Phone: +1 508 289 3269 ckaiser@whoi.edu [1] Billings, A., Kaiser, C., Young, C. M., Hiebert, L. S., Cole, E., Wagner, J. K. S., & Van Dover, C. L. (2017). SyPRID sampler: A large-volume, high-resolution, autonomous, deep-ocean precision plankton sampling system. In Deep Sea Research Part II: Topical Studies in Oceanography (Vol. 137, pp. 297–306). Elsevier BV. https://doi.org/10.1016/j.dsr2.2016.05.007 |

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|---|---|
| **Dataset-specific Instrument Name** | PCR amplification |
| **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) |

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## Deployments

**TN391**

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| **Website** | https://www.bco-dmo.org/deployment/893731 |
| **Platform** | R/V Thomas G. Thompson |
| **Start Date** | 2021-05-25 |
| **End Date** | 2021-06-20 |
| **Description** | See more information at R2R: https://www.rvdata.us/search/cruise/TN391 During the TN391 cruise, we conducted 14 dives with the ROV Jason to collect animal specimens from the seafloor and to recover/redeploy Seep Larval Observatories (SLOs) from each sample site. We also had 12 dives with the AUV Sentry to use the SyPRID plankton sampler. Additionally, five CTD casts were conducted during the duration of the cruise. |

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## Project Information

**Collaborative Research: dispersal depth and the transport of deep-sea, methane-seep larvae around a biogeographic barrier (SALT)**

**Website**: https://wp.wwu.edu/arellanolab/category/salt/

**Coverage**: Methane seeps on the shelf and slope of Louisiana, Mississippi, Florida, North Carolina, Virginia and Maryland

*NSF Award Abstract:*
Ever since hydrothermal vents and methane seeps were first discovered in the deep ocean more than 40 years ago, scientists have wondered how these isolated communities, fully dependent on underwater "islands" of toxic chemicals, are first colonized by organisms, and how the populations of these specialized animals are exchanged and maintained. These fundamental processes depend on the transport of babies (larvae) by the ocean currents, yet because the larvae are microscopic and diluted in the vastness of the ocean, it is very difficult to determine where and how they drift. This project uses an autonomous underwater vehicle to collect larvae from precise regions of the water column. Larval traps on the bottom and chemical analyses of larval shells will also be used to determine the depths where larvae swim. These findings will provide realistic estimates for mathematical models that show how biology interacts with ocean currents to predict which

methane seeps will be colonized by larvae originating at different depths. A detailed knowledge of larval dispersal is needed for conservation and management of the deep sea. Without such information, we cannot know the best placement of marine protected areas, nor can we facilitate the reestablishment of communities impacted by deep-sea mining, drilling, or other human activities. This project will provide hands-on at-sea training for college students to learn the rapidly vanishing skills needed for studies of larvae and embryos in their natural habitats. Learning opportunities will also be available to individuals of all ages through new, interactive exhibits on deep-sea biology and larval ecology produced for small museums and aquaria on the coasts of Oregon, Washington and North Carolina.

Reliable estimates of connectivity among metapopulations are increasingly important in marine conservation biology, ecology and phylogeography, yet biological parameters for biophysical models in the deep sea remain largely unavailable. The movements of deep-sea vent and seep larvae among islands of habitat suitable for chemosynthesis have been inferred from current patterns using numerical modeling, but virtually all such models have used untested assumptions about biological parameters that should have large impacts on the predictions. This project seeks to fill in the missing biological parameters while developing better models for predicting the dispersal patterns of methane seep animals living in the Gulf of Mexico and on the Western Atlantic Margin. Despite the existence of similar seeps at similar depths on two sides of the Florida peninsula, the Western Atlantic seeps support only a subset of the species found in the Gulf of Mexico. It is hypothesized that the ability of larvae to disperse through the relatively shallow waters of the Florida Straits depends on an interaction between the adult spawning depth and the dispersal depth of the larvae. Dispersal depth, in turn, will be influenced by larval flotation rates, swimming behaviors, feeding requirements, and ontogenetic migration patterns during the planktonic period. The recently developed SyPRID sampler deployed on AUV Sentry will be used to collect larvae from precise depth strata in the water column, including layers very near the ocean floor. Larval traps deployed on the bottom at three depths in each region will be used in conjunction with the plankton collections to determine what proportion of larvae are demersal. Comparisons of stable oxygen isotopes between larval and juvenile mollusk shells will provide information on the temperatures (and therefore depths) that larvae develop, and geochemical analyses of larval and juvenile shells will determine whether larval cohorts mix among depth strata. Ocean circulation and particle transport modeling incorporating realistic biological parameters will be used to predict the movements of larvae around the Florida Peninsula for various spawning depths and seasons.

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-1851383 |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1851286 |
| NSF Division of Ocean Sciences (NSF OCE) | OCE-1851421 |


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