Gnathiid blood meal identification results

Website: https://www.bco-dmo.org/dataset/887465
Data Type: experimental
Version: 1
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Project
» Beyond Cleaning and Symbiosis: Ecology of 'Ticks of the Sea' on Coral Reefs (Gnathiid isopod ecology)

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Abstract
These data were used to identify the blood meal sources of free-living stages of Caribbean gnathiid isopods. Gnathiids with visible blood meals were collected between May and August of 2015 and 2016 from five study sites surrounding the U.S. Virgin Islands and Puerto Rico. Using fish-specific COI (cox1) primers, sequencing individual gnathiid blood meals led to the identification of 70 host species from 27 fish families. Gnathiids are the only marine blood-feeding specialists within Arthropoda and are among the most common ectoparasite of coral reef fishes. Considering the profound effects blood-feeding arthropods have on terrestrial communities, investigating the dynamics of gnathiid-host interactions is essential to our understanding of ecological processes in marine environments.

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Coverage

Spatial Extent: **N**:18.34 **E**:-64.723 **S**:17.943 **W**:-67.078

Temporal Extent: 2015-04 - 2016-09

Methods & Sampling

Fieldwork was conducted between May and August of 2015 and 2016. The coordinates for each site are as follows: 17°57’17.59” N, 67°3’9.96” W (Site 1: Cayo Enrique, La Parguera, PR), 17°56’33.60” N, 67°4’41.02” W (Site 2: San Cristobal, La Parguera, PR, 18°19’3.54” N, 65°19’5.06” W (Site 3: Tamarindo Bay, Culebra, PR), 18°20’25.08” N, 64°58’39.77” W (Site 4: Brewer’s Bay, St. Thomas, USVI), and 18°19’2.17” N, 64°43’21.09” W (Site 5: Great Lameshur Bay, St. John, USVI).

Sites off the coast of La Parguera, PR were accessed using powerboats (approximately 14 – 16 feet) at the Isla Magueyes Marine Laboratories of the University of Puerto Rico at Mayagüez. Our site in Tamarindo Bay, Culebra, PR was accessed from shore and did not require boat use. Sites in St. Thomas and St. John, USVI were accessed from shore and using small powerboats supplied by the University of the Virgin Islands, as necessary.

Gnathiids were collected using lighted plankton traps following the protocol of Artim and Sikkel, 2016. Briefly, light traps were deployed on shallow reefs overnight and sorted to remove visibly fed juvenile gnathiids (pranizae). Specimens were preserved in 100 percent molecular grade ethanol and stored at less than -20 degrees C until being shipped to the Arkansas Biosciences Institute (Arkansas State University, Jonesboro, AR) where they were kept at -80 degrees C.

DNA was extracted from individually fed gnathiids using the PureLink® Genomic DNA extraction kit (Invitrogen, Carlsbad, CA), following the manufacturer’s ‘Mammalian Tissue and Mouse/Rat Tail Lysate’ protocol. Purified DNA was concentrated from 50 microliters (µL) to 15 µL using the ThermoSavant ISS110 SpeedVac® System (Thermo Fisher Scientific, Wilmington, DE).

PCR reactions were carried out following the protocol of Hendrick et al., 2019. Primers of the mitochondrial gene cytochrome c oxidase subunit 1 (cox1, COI, or MT-CO1) (5’-TCAACYAATCAYAAAGATATYGGCAC-3’; 5’-ACTTCYGGGTGRCCRAARAATCA-3’). PCR reactions included 10 µL of concentrated template DNA and 10 µL of master mix solution containing forward and reverse COI primers, 1.25 units GoTaq Hot Start Polymerase, 1x buffer with 1.5 mM MgCl2 (Promega, Madison, WI), and 0.2 mM dNTP Mix (Thermo Fisher Scientific, Wilmington, DE). A Veriti 96 Well Thermal Cycler (Applied Biosystem, Foster City, CA) was used to carry out PCR reactions with the following thermocycling conditions: initial denaturation step of 94 degrees C for 2 minutes, 30 cycles of 96 degrees C for 20 seconds, 55 degrees C for 20 seconds, and 72 degrees C for 45 seconds, and a final extension step of 72 degrees C for 7 minutes.

ExoSAP-IT (Applied Biosystem, Foster City, CA) was used for enzymatic digestion of excess PCR reagents prior to Sanger sequencing. All samples were sent to the University of Chicago Comprehensive Cancer Center, DNA Sequencing & Genotyping Facility for sequencing. For samples that did not result in successful host identification after one PCR reaction, a second PCR was performed using 10 µL of PCR product from the initial PCR reaction as template DNA.

Known Issues:
The DNA sequences within this dataset originated from partially digested blood meals of gnathiid isopods and should not be used as a reference for the comparison of sequence mutations.

Data Processing Description

The DNA sequences within this dataset had the primer sequences removed and ends trimmed using Geneious R10 (Biomatters Limited, Auckland, New Zealand). DNA sequences were identified using the Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website. Sequence similarity of greater than or equal to 98 percent was considered species-level identification. For sequence similarity of less than 98 percent, host families were identified using neighbor-joining trees of the BLAST results as outlined by Jones et al., 2007.

BCO-DMO Curation Notes

- Import "
- Adjusted field/parameter names to comply with BCO-DMO naming conventions
- Added a conventional header with dataset name, PI names, version date
- Added columns for “Latitude” and “Longitude”
- Rounded columns: “Latitude” and “Longitude” to 3 decimal places (or to the thousandth place)

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Supplemental Files

File
Host ID sequencing results filename: 887465_Dolan_Sikkel_HostIDSupplement.pdf (Portable Document Format (.pdf), 1.01 MB) MD5:e9dec63c47b7813889b876f8b564f990 Sequencing results for individual gnathiid blood meals that could not be identified using BLAST; one sequence where the host family could not be identified with confidence; sequences from individual gnathiid blood meals with homologies to other gnathiid species; BLAST query results matching to bacteria; and results from poor quality sequencing reads (less than 80%)

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Related Publications

Artim, J. M., Nicholson, M. D., Hendrick, G. C., Brandt, M., Smith, T. B., & Sikkel, P. C. (2020). Abundance of a cryptic generalist parasite reflects degradation of an ecosystem. Ecosphere, 11(10). Portico. https://doi.org/10.1002/ecs2.3268
Methods

Hendrick, G. C., Dolan, M. C., McKay, T., & Sikkel, P. C. (2019). Host DNA integrity within blood meals of hematophagous larval gnathiid isopods (Crustacea, Isopoda, Gnathiidae). Parasites & Vectors, 12(1). https://doi.org/10.1186/s13071-019-3567-8
Methods
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Results

Hendrick, G. C., Nicholson, M. D., Pagan, J. A., Artim, J. M., Dolan, M. C., & Sikkel, P. C. (2023). Blood meal identification reveals extremely broad host range and host-bias in a temporary ectoparasite of coral reef fishes. Oecologia, 203(3–4), 349–360. https://doi.org/10.1007/s00442-023-05468-w
Results

Jones, C. M., Nagel, L., Hughes, G. L., Cribb, T. H., & Grutter, A. S. (2007). Host specificity of two species of Gnathia (Isopoda) determined by DNA sequencing blood meals. International Journal for Parasitology, 37(8–9), 927–935. https://doi.org/10.1016/j.ijpara.2007.01.011
Methods

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Parameters

Parameter	Description	Units
Site_Number	The number given to the site as listed in our results publication currently in review	unitless
Site_Location	Regional location of the site	unitless
Latitude	Latitude of sampling site	decimal degrees
Longitude	Longitude of sampling site (West is negative)	decimal degrees
Site_Name	Name of reef or bay where site is located	unitless
Sample_ID	Unique sample identifier based on site name (L = Site 1, ESG = Site 1 Seagrass, B = Site 2, CSG = Site 2 Seagrass, C = Site 3, TSG = Site 3 Seagrass, T = Site 4, R = Site 4 Seagrass, J = Site 5) and number of the specimen processed at each site	unitless
Family	Family of the closest matching reference sequence in BLAST results	unitless
Host_ID	Scientific name of the closest matching reference sequence in BLAST results	unitless
Ident_Percent	The percent identity according to the results of BLAST queries for each sequence	percent (%)
BLAST_sequence	The mtDNA sequence entered into BLAST	unitless

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Instruments

Dataset-specific Instrument Name	None
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	All samples were sent to the University of Chicago Comprehensive Cancer Center, DNA Sequencing & Genotyping Facility for sequencing. For samples that did not result in successful host identification after one PCR reaction, a second PCR was performed using 10 µL of PCR product from the initial PCR reaction as template DNA.
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	ThermoSavant ISS110 SpeedVac® System
Generic Instrument Name	Concentrator Device
Generic Instrument Description	A concentrator is a device designed to increase the weight per unit volume of a substance. This category includes vacuum centrifuge concentrator, which include a vacuum chamber within which a centrifuge rotor is mounted for spinning a plurality of vials containing a solution at high speed while subjecting the solution to a vacuum condition for concentration and evaporation. Alternative names: sample concentrator; speed vacuum; speed vac.

Dataset-specific Instrument Name	Lighted plankton trap
Generic Instrument Name	Plankton Net
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	Veriti 96 Well Thermal Cycler
Generic Instrument Name	Thermal Cycler
Dataset-specific Description	A Veriti 96 Well Thermal Cycler (Applied Biosystem, Foster City, CA) was used to carry out PCR reactions with the following thermocycling conditions: initial denaturation step of 94 °C for 2 min, 30 cycles of 96 °C for 20 s, 55 °C for 20 s, and 72 °C for 45 s, and a final extension step of 72 °C for 7 min.
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

Beyond Cleaning and Symbiosis: Ecology of 'Ticks of the Sea' on Coral Reefs (Gnathiid isopod ecology)

Coverage: Eastern Caribbean, Philippines, Australia

NSF Award Abstract:
Most research on the complex biological interactions that inhabit coral reefs has focused on larger organisms that are easily observed by divers. However, marine scientists are increasingly aware of the importance of the tiny organisms that make up the "smaller majority." This includes parasites, organisms that feed on other organisms without killing them, which may make up as many as 80% of the species on coral reefs. Among the

most important parasitic organisms on coral reefs are gnathiid isopods, so-called 'ticks of the sea', that share many similarities with blood-feeding ticks and other arthropods on land. Like ticks and mosquitoes, gnathiids transmit malaria-like blood parasites. In high numbers, they can remove enough blood to kill adult fish, but even a single gnathiid can kill a juvenile fish. Thus, gnathiids may have a significant effect on coral reef communities through their effects on coral reef fishes. This project will use an integrative interdisciplinary approach involving field and laboratory observations and experiments, and molecular tools. In addition to contributing to our understanding of life in our oceans, this research will provide continued support for U.S. Doctoral and Masters students and will create valuable research opportunities for undergraduates from multiple institutions. The project will further build on the investigators existing relationships with resource managers, local divers, fishers, and boat operators, as well as K-12 schools and environmental education programs, and will contribute to local economies. A major goal of our outreach efforts will include an exhibit featuring our research at Coral World Ocean Park on St. Thomas, participation in Virgin Islands radio programs, and hosting high school students from South Carolina Governor's School.

The overall goal this investigation is to understand the ecology of fish-parasite interactions on coral reef and associated ecosystems. This project focuses on fish-parasitic gnathiid isopods, the most common ectoparasites of coral reef fishes that are best known for their role in cleaning symbiosis, as the major food item of cleaner fishes. However, their abundance, host range, role as micropredator, disease vector, and potential prey item for other species, as well as their strong association with the benthos suggests the potential for much stronger community impacts. The goals for this project are to: 1) characterize the factors influencing local gnathiid isopod density by examining the role of fish-hosts, benthic cover, gnathiid predators including cleaners, and gnathiid conspecific attraction; 2) determine and quantify variation in host exploitation and the effects of gnathiid density on larval fish-host recruitment. To accomplish the first objective, the investigators will trap gnathiids from the substrate at sites in the Caribbean, Australia, and the Philippines. Variables associated with benthic habitat as well as local fish communities will be quantified and compared with local gnathiid abundance. Laboratory experiments will be conducted to determine the effects of different host species on gnathiid growth and reproduction and to determine the role of conspecific attraction in the formation of aggregations. Predators of gnathiids will be identified through examination of gut contents and through laboratory feeding studies. To accomplish the second objective, patterns of host-exploitation will be determined by DNA barcoding of blood meals from wild-caught gnathiids and results compared with the availability of different host species. To determine the effects of gnathiids on early life history stages of coral reef fishes, gnathiid abundance will be manipulated on small artificial patch reefs onto which newly-settled reef fishes will be transplanted.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1536794

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