# COI mtDNA sequences for trematodes from fish collections across the Northern Line Islands and French Polynesia archipelagos collected between 2009 and 2023

Website: https://www.bco-dmo.org/dataset/953401

Data Type: Other Field Results

Version: 1

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#### **Project**

» Collaborative Research: Decomposing the effects of diversity on the abundance of marine parasites (Diversity-disease)

Contributors	Affiliation	Role
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#### **Abstract**

The loss of biological diversity is considered one of the principal environmental challenges of the 21st century, and there are hints that this massive reorganization of food webs could affect how parasites are transmitted among hosts. Parasites are often hidden and can be easy to overlook, but they are ecologically important and ubiquitous - so it is important to understand whether we should expect more or fewer of them as biodiversity disappears. Does biodiversity loss increase the abundance of parasites by eroding natural "checks and balances" on transmission? Or does it decrease parasite abundance by removing the free-living biodiversity on which parasites depend? Answers to these questions are urgently needed if we are to mitigate or prevent an uptick in parasite transmission for ecosystems experiencing biodiversity loss. In a joint collaborative research project among the University of Washington, Scripps Institution of Oceanography at UC San Diego, and California State University Monterey Bay, we created a parasite dataset of unprecedented size and taxonomic resolution. We sampled parasites of coral reef fishes from 19 replicate islands in the central equatorial Pacific to study how biodiversity and parasite burden covary. This data set contains COI mtDNA sequence accession numbers, collection locations, and life stages for trematodes from the family Microscaphidiidae and Paramphistomatidae from fish collections across the Northern Line Islands and French Polynesia archipelagos collected between 2009 and 2023. Specifically this data set represents 87 Microscaphidiidae samples from the Northern Line Islands and 132 Paramphistomatidae from French Polynesia.

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# Coverage

Location: Sampling was conducted across three archipelagos of the central equatorial Pacific,

encompassing 19 islands

**Spatial Extent: N**:6.46 **E**:-145.33 **S**:-17.95 **W**:-162.32

Temporal Extent: 2009 - 2023

#### Methods & Sampling

#### Field collections and sample processing

Coral reef fish host species Acanthurus nigricans, Stegastes aureus, and Stegastes fasciolatus were caught at islands from Northern Line Islands (NLI) and French Polynesia (FP) during expeditions in 2010, 2019, 2020, and 2021. Sampling was conducted across three archipelagos of the central equatorial Pacific, encompassing 19 islands (Jarvis, Kingman, Kiritimati, Palmyra, Tabuaeran, and Teraina in the Northern Line Islands; Flint, Malden, Millennium, Starbuck, and Vostok in the Southern Line Islands; Huahine, Moorea, Raiatea, Rangiroa, Tahiti, Takapoto, Tetiaroa, and Tikehau in French Polynesia).

Acanthurus nigricans are a type of surgeonfish belonging to the Family Acanthuridae. Stegastes aureus and Stegastes fasciolatus are damselfish of Family Pomacentridae. Reef fish were collected from the forereef of each island from depths between 8m and 18m using three-pronged spears and hand nets. Fish were euthanized humanely according to UC San Diego IACUC protocol #S09392 and frozen for parasitological assessment.

Parasites were identified and counted by the Wood Lab at the University of Washington using standard dissection methods [see appendix E in (Wood et al. 2014)]. Parasites were identified to the lowest possible taxonomic level [see appendix F in (Wood et al. 2014)] and stored in 70% ethanol. All of the adult trematodes used in this study were sampled from host species A. nigricans, while the NLI larval samples came from S. aureus, and the FP larval samples came from S. fasciolatus. Following morphological assessment conducted by the Wood Lab, it was determined that all of the adult trematodes sampled from the NLI were of the Family Microscaphididae (NLI adults), and all of the adult trematodes sampled from FP were of the Family Paramphistomatidae (FP adults). Parasite vouchers were sent to the Haupt Lab at California State University, Monterey Bay for further genetic analysis in 2022.

# DNA Extraction, PCR, and Sequencing

Each voucher was emptied into a petri dish containing 70% ethanol, one at a time. The petri dish was then examined under a dissecting microscope at the lowest magnification. A disposable glass pipette was used to move the parasite to a microcentrifuge tube containing autoclaved DI water in order to rinse the parasite of residual fish tissue. Individuals were not soaked for any longer than two hours as this could lead to degradation of the specimen. After 30 minutes to an hour, the parasite was then transferred into another microcentrifuge tube containing tissue lysis buffer and proteinase K and incubated at 56°C for at least one hour. Larger individuals were incubated for 2 or more hours. Extractions were then conducted using either the QIAGEN QIAamp DNA Micro Kit (Cat. #51304) or the QIAGEN DNeasy Blood & Tissue Kit (Cat. #69504). The DNeasy kit was used for those parasite individuals that were larger and had more tissue present. DNA yield was estimated after extraction using a Nanodrop spectrophotometer. Due to the small amount of tissue present, DNA yields were often very small. Therefore, there were no restrictions on which samples moved forward to polymerase chain reaction (PCR).

A 743 bp segment of the mtDNA cytochrome oxidase 1 gene (CO1) was amplified using three primers designed by Brant and Loker (2009) for trematodes. PCR was performed in two consecutive 50 uL reactions. The first reaction was conducted using the DNA extraction and the forward and reverse primers. The PCR product of the first run was then used in the second reaction with the forward and internal primers. Both reactions were run on thermocyclers with the following reaction conditions: Initial denaturation at 94°C for 2 min, followed by 35 cycles of 94°C for 30 s, 55°C for 40 s, 72°C for 1 min, with a final step of 72°C for 10 min. Final PCR products were run on a 1.5% agarose gel using SYBR™ Safe DNA Gel Stain (Cat. #S33102) and the GeneRuler 1 kb DNA Ladder (Cat. #SM0314). The gels were run at 120 V for 30-45 minutes. However, we discovered that even if a band could not be seen, the sample was often still able to be sequenced. Therefore, we decided to send all amplified DNA samples off for sequencing. Amplified DNA was purified using the QIAGEN QIAquick PCR Purification Kit (Cat. #28104). Samples were sent for Sanger sequencing (MCLAB, San Francisco, CA) and were sequenced in the forward and internal directions.

## **Data Processing Description**

The consensus sequences were trimmed and assembled from the forward and reverse sequences using the

Geneious assembler in Geneious Prime® ver 2023.2.1, and visually examined for accuracy and polymorphisms. All analyses were conducted from multiple sequence alignments created using the Clustal Omega 1.2.2 alignment in Geneious Prime® ver 2023.2.1. Alignments were visually examined for accuracy and excess ends were manually trimmed.

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### **Related Publications**

Brant, S. V., & Loker, E. S. (2009). Molecular Systematics of the Avian Schistosome Genus Trichobilharzia (Trematoda: Schistosomatidae) in North America. Journal of Parasitology, 95(4), 941–963. https://doi.org/10.1645/ge-1870.1 <a href="https://doi.org/10.1645/GE-1870.1">https://doi.org/10.1645/GE-1870.1</a> Methods

Wood, C. L., Sandin, S. A., Zgliczynski, B., Guerra, A. S., & Micheli, F. (2014). Fishing drives declines in fish parasite diversity and has variable effects on parasite abundance. Ecology, 95(7), 1929–1946. Portico. https://doi.org/10.1890/13-1270.1 Methods

Wood, C. L., Zgliczynski, B. J., Haupt, A. J., Guerra, A. S., Micheli, F., & Sandin, S. A. (2018). Human impacts decouple a fundamental ecological relationship—The positive association between host diversity and parasite diversity. Global Change Biology, 24(8), 3666–3679. Portico. https://doi.org/10.1111/gcb.14159

Results

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#### **Related Datasets**

#### References

Wood, C. L., Sandin, S., Haupt, A. (2025) **Parasite abundance data collected from coral reef fishes across 19 islands in the central equatorial Pacific from 2009 to 2021.** Biological and Chemical Oceanography Data Management Office (BCO-DMO). (Version 1) Version Date 2025-01-22 doi:10.26008/1912/bco-dmo.945218.1 [view at BCO-DMO]

Relationship Description: Dataset is created based on samples collected in this related dataset. See methods for more information on sample provenance.

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#### **Parameters**

Parameters for this dataset have not yet been identified

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#### Instruments

Dataset-specific Instrument Name	Eppendorf tabletop centrifuge
Generic Instrument Name	Centrifuge
Dataset-specific Description	Eppendorf tabletop centrifuge
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	Fisherbrand water bath incubator
Generic Instrument Name	Incubator
Dataset- specific Description	Fisherbrand water bath incubator
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 ( <a href="https://www.bco-dmo.org/instrument/629001">https://www.bco-dmo.org/instrument/629001</a> ) and in-situ incubators ( <a href="https://www.bco-dmo.org/instrument/494">https://www.bco-dmo.org/instrument/494</a> ).

Dataset- specific Instrument Name	Fisherbrand thermocyclers
Generic Instrument Name	Thermal Cycler
Dataset- specific Description	Fisherbrand thermocyclers
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 <a href="http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html">http://serc.carleton.edu/microbelife/research_methods/genomics/pcr.html</a> )

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# **Project Information**

Collaborative Research: Decomposing the effects of diversity on the abundance of marine parasites (Diversity-disease)

**Coverage**: Central equatorial Pacific (Line Islands and French Polynesia)

#### NSF Award Abstract:

Nontechnical explanation of the project's broader significance and importance

As Earth's ecosystems experience rapid biodiversity change, disease ecologists have turned to an urgent question; how might reductions in biodiversity affect the transmission of parasites? In other words, does biodiversity loss increase the abundance of parasites by eroding natural checks and balances on transmission? Alternatively, does it decrease parasite abundance by removing the free-living biodiversity on which parasites depend? This study will constitute the first comprehensive test of these questions in any ecosystem. It will evaluate the relationship between fish biodiversity and parasite abundance across 18 replicate coral reef ecosystems. Not only will the work explore whether reductions in fish biodiversity are associated with increases or decreases in parasite burdens, it will also assess whether parasite and host traits or geographical distance influence the direction and strength of this relationship. The theories that are tested are among the most important and controversial in the rapidly growing field of disease ecology and our work represents a novel, creative approach to a long standing, but unresolved research guestion. The work will yield transformative insights into the nature of parasite transmission in a changing world. Furthermore, the project will intimately intermingle education with research by launching the Research Internship in Molecular Ecology at California State Monterey Bay, which will place a group of underrepresented undergraduates in a central research role, and by developing and disseminating quality educational tools for teaching about parasite biodiversity through collaboration with the Network of Conservation Educators and Practitioners at the American Museum of Natural History. Parasites are often hidden and can be easy to overlook, but they are ecologically important and affect every population of marine animals.

#### Technical description of the project

The field of disease ecology is plagued by uncertainty and disagreement over whether biodiversity loss exacerbates parasite transmission, because it lacks the comprehensive, multi-host, multi-parasite, broadspatial-scale dataset needed to formulate a convincing empirical test.

This project will answer this recalcitrant question, using a dataset of unprecedented replication and taxonomic and spatial resolution, by exploiting the advantages of a marine model system. The project is centered on a natural experiment in which the abundance of parasites across a highly resolved gradient of host biodiversity, for more than 77 parasite species and 18 replicate coral reef ecosystems will be quantified. Dataset will critically test hypotheses for the biodiversity-parasite abundance relationship, revealing how the direction, shape, and scale-dependence of this relationship vary across a diverse array of parasite taxa, and resolving questions of burning interest in the disease ecology literature - and of vital importance to marine conservation. This project will address the following questions: (Q1) For each parasite species detected, what is the direction and shape of the relationship between biodiversity and parasite abundance? (Q2) What factors (e.g., parasite traits like transmission strategy and host specificity, host traits like body size) determine the direction and shape of the relationship between biodiversity and parasite abundance? (Q3) How does spatial scale interact with parasite dispersal capacity to moderate the effects of biodiversity on parasite abundance? The work will integrate an existing dataset on fish biodiversity and abundance of coral reef fish parasites from six equatorial Pacific islands (the Northern Line Islands) with new sampling from 12 additional islands (the Southern Line Islands and French Polynesia). The resulting dataset will reflect the burden of >77 metazoan parasite taxa for seven species of coral reef fishes across 18 islands. The work will provide the world's first data on the direction, magnitude, and shape of the biodiversity-disease relationship across a diversity of parasite taxa, host taxa, and spatial scales, and will comprehensively identify conditions under which biodiversity is likely to be important in determining the abundance of parasites - a fundamental contribution to ecology and to biological oceanography. The project will intimately integrate education with research by placing a group of underrepresented minority undergraduates in a central research role; performing the molecular analyses required to estimate parasite dispersal distance. A summer Research Internship in Molecular Ecology will be established at California State University Monterey Bay, a Hispanic-Serving Institution. The project will also underwrite the development of a peer-reviewed learning module on parasite biodiversity, to be developed and disseminated in collaboration with the American Museum of Natural History, and will support the training of two graduate students, one postdoctoral scholar, and several undergraduates.

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.

# **Funding**

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1829509

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