Genetic analyses and microsatellite characterization for formal recognition of new species of host-generalist species of dinoflagellate (Cladocopium, Symbiodiniaceae) mutualistic with Indo-Pacific reef corals collected from 2003 through 2015

Website: https://www.bco-dmo.org/dataset/957700
Data Type: Other Field Results, Synthesis
Version: 1
Version Date: 2025-04-02

Project
» Collaborative Research: Stability, flexibility, and functionality of thermally tolerant coral symbioses (Thermally tolerant coral)

Contributors	Affiliation	Role
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Abstract
These genetic data were used to justify the formal characterization of new species of 'ecological generalists' zooxanthellae, the dinoflagellate symbionts living in the tissues of numerous marine animals including corals. Reef-building corals are dependent on endosymbiotic dinoflagellates (Family: Symbiodiniaceae) for their survival and growth but there is almost no taxonomy available for coral symbionts thus limiting research on them. These data include the sequences of genes originating from the nuclear genome, the mitochondrial genome as well as from the chloroplast genome. These sequences were analyzed independently and the resulting phylogenies from these analyses used to test for reciprocal monophyly, a way to verify the evolutionary divergence of two populations. Additionally, microsatellites were analyzed to test boundaries to genetic recombination indicated by the phylogenetic data.

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Coverage

Location: Indo-Pacific reefs (Palau, Japan, Australia, Zanzibar, and Thailand)
Spatial Extent: **N**:26.22354 **E**:-171.704 **S**:-23.4357 **W**:39.13373
Temporal Extent: 2002 - 2018

Dataset Description

These genetic data were used to justify the formal characterization of new species of 'ecological generalists' zooxanthellae, the dinoflagellate symbionts living in the tissues of numerous marine animals including corals.

There are 4 data files: one table of microsatellite data plus three sequence files.

Original paper is Butler, C. C., Turnham, K. E., Lewis, A. M., Nitschke, M. R., Warner, M. E., Kemp, D. W., Hoegh-Guldberg, O., Fitt, W. K., van Oppen, M. J. H. & LaJeunesse, T. C. 2023. Formal recognition of host-generalist species of dinoflagellate (Cladocopium, Symbiodiniaceae) mutualistic with Indo-Pacific reef corals. *J Phycol,* 59**:**698–711.

Methods & Sampling

Field collections
Samples of Scleractinia from various families were sampled by SCUBA with a hammer and chisel throughout various sites across the Indo-Pacific including the reefs of Palau, the Andaman Sea of western Thailand, Zanzibar of Tanzania, the Phoenix Islands, and the southern Great Barrier Reef (GBR) of Australia, New Caledonia, and Brazil. The samples used were collected from 2002–2018, including some from previously published diversity surveys (Butler et al., 2023). Coral fragments of approximately 1-3 cm2 were preserved in 20% DMSO buffer or 100% ethanol and stored at -20°C.

Genetic analyses
A modified Promega Wizard protocol was used to extract genomic DNA from 0.5 cm2 of each fragment (LaJeunesse et al., 2003). All samples were identified using the PCR-DGGE profiling of the internal transcribed space region 2 (ITS2) rDNA of the ribosomal array (LaJeunesse, 2002; T. C. LaJeunesse et al., 2004). Amplified products were examined with gel electrophoresis with a CB Scientific system with 45–80% denaturing gradient gels. Fingerprints of each sample were visualized using SYBR Green stain and compared to known standards and/or sequenced to confirm the identity of the ITS2 'type' designation. *Cladocopium* samples typed as C1, C3, C3u, and C40 were investigated by using additional genetic markers, including the large ribosomal subunit (*LSU*) (Zardoya, Costas, Lopez-Rodas, Garrido-Pertierra, & Bautista, 1995), mitochondrial cytochrome b (*cob*), mitochondrial cytochrome oxidase 1 (*cox 1*) (H. Zhang, Bhattacharya, Maranda, & Lin, 2008), partial chloroplast *cp23S* (Z. Zhang, Green, & Cavalier-Smith, 2000), and the non-coding region of the *psbA* minicircle (*psbA*ncr) (Moore, Ferguson, Loh, Hoegh-Guldberg, & Carter, 2003). These conventional phylogenetic markers (i.e., *LSU*, *cob*, *cox 1, cp23S*) were used to resolve lineages within *Cladocopium*, and then concatenated together to further differentiate putative species lineages (LaJeunesse *et al.* 2015, Lee *et al.* 2020). For comparison, the *psbAncr* region is a more rapidly evolving locus that provides finer-scale inter-species and intra-species resolution (LaJeunesse & Thornhill, 2011; Moore et al., 2003).

Successful PCR amplicons of each genetic marker were Sanger sequenced using the BigDye Terminator 3.1 Cycle Sequencing Kit (ThermoFisher Scientific, Waltham, MA), with sequences analyzed at the Penn State University Genomics Core Facility on an Applied Biosciences Sequencer. The *psbA*ncr region was sequenced in both directions. Sequences were quality checked, assembled, and edited in Geneious v 11.0.5 (Biomatters Ltd, Auckland, NZ).

Microsatellite characterization
To test for reproductive isolation in the C3-radiation *Cladocopium* samples, a total of seven microsatellite loci were used to generate multilocus genotypes using the following primers: SgrSpl_22, SgrSpl_24, SgrSpl_25, SgrSpl_78, Spl_1a, and Spl_16 were developed in Drew C. Wham, Carmichael, and LaJeunesse (2014), while the remaining locus (C1.05) was described by Bay, Howells, and van Oppen (2009). These loci are unlinked and have sufficient allelic diversity for population genetic analyses (Drew C. Wham et al., 2014). Loci were amplified in reaction volumes of 10 µL according to their respective development studies. Fragment analysis was performed on an ABI 3730 Genetic Analyzer (Applied Biosystems) using a 500-bp standard (LIZ-labeled) at the Penn State University Nucleic Acid Facility. Fragment sizes were scored visually using Geneious (v.2020.2.4). While *Cladocopium* spp. are haploid organisms, two alleles are often observed in each sample (D. C. Wham & LaJeunesse, 2016). This observation is most likely the result of *Cladocopium* having a partial or extensively duplicated genome (Chen et al., 2022; Liu et al., 2018). Previous work has shown that *psbA*ncr sequences are consistent with microsatellite data when differentiating lineages in the C1-radiation (Turnham *et al.* 2021). Microsatellite analyses were only conducted on symbionts from the C3-radiation, which, relative to the C1-radiation, were less differentiated by *psbA*ncr sequence divergence (Table S2 in Butler et al., 2023).

Data Processing Description

Sequences were quality checked, assembled, and edited in Geneious v 11.0.5 (Biomatters Ltd, Auckland, NZ).

Phylogenetic Analysis

LSU, cob, cox 1, and *cp23S* sequences were aligned using ClustalOmega. PAUP v4.0a169 was used to create an unrooted maximum parsimony phylogeny based on a heuristic search (Swofford, 2014). Independent phylogenies for each marker were compared (not shown). Because each phylogeny was congruent they were concatenated and a phylogenetic tree created with gaps (and insertions) treated as a 5th character and scored as one change. Bootstrap values based on 1,000 iterations were evaluated to statistically assess branch support, which was also assessed using Bayesian Inference using MrBayes v.3.2.1 implementing General Time Reversible (where gaps were treated as missing data). Each Markov chain Monte Carlo (MCMC) analysis were run for 106 generations total and sampled every 100 generations. The first quarter of trees was discarded as burn-in corresponding with the convergence of chains.

Sequences of the *psbA*ncr were then used for finer inter- and intra-species analysis. Sequences were aligned using ClustalOmega followed by manual editing. PAUP v4.0a169 was again used to create an unrooted maximum parsimony phylogeny based on a heuristic search. Gaps and insertions were also treated as a 5th character and scored as one change. Bootstrap values based on 1,000 iterations were evaluated to statistically assess node support, which was also assessed using Bayesian Inference using MrBayes v.3.2.1 implementing General Time Reversible. Each MCMC analysis was run for 106 generations and sampled every 100 generations. The first quarter of trees was discarded as burn-in corresponding with the convergence of chains.

Microsatellite Analysis

Duplicate (i.e. clone) genotypes and low-quality DNA samples with two or more microsatellites that did not amplify were removed from dataset prior to subsequent analyses. Clonality and basic summary statistics were assessed in GenAlEX v.6.5 (Peakall and Smouse 2012). A genotype accumulation curve was created to assess the resolution of the microsatellites used, and if the microsatellites used are an accurate representation of the population (Arnaud-Haond, Duarte, Alberto, & Serrao, 2007). Next, the hypothesis of reproductive isolation (i.e., the biological species concept) for members of the C3-radiation was tested via an unsupervised Bayesian clustering algorithm employed by Structure with no location prior, assuming no admixture, and uncorrelated allele frequencies (Pritchard et al. 2000). This analysis was conducted using K-values from K=1 to K=5 with ten runs per K-value. The optimum number of clusters was then estimated based on the ΔK statistic procedure through Structure Harvester v0.6.94 (Earl and von Holdt 2012). The average of the ten runs was obtained by CLUMPP to eliminate stochastic differences between Structure runs (Jakobsson & Rosenberg, 2007). Furthermore, a discriminate analysis of principal components (DAPC) was also used to classify samples into clusters of genetically related individuals through the adegenet R package (Jombart et al. 2010).

BCO-DMO Curation Notes

- Imported data from source file "SupplementaryTable_S3_Microsat_Data.xlsx" into the BCO-DMO data system.
- Renamed columns/modified parameter names to conform with BCO-DMO naming conventions. The only allowed characters are A-Z,a-z,0-9, and underscores. No spaces, hyphens, commas, parentheses, or Greek letters.

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Data Files

File	
957700_v1_microsatellite_multilocus_genotypes.csv	(Comma Separated Values (.csv), 5.65 KB) MD5:c745a4f4391f5e91ada8fb9072366691
Primary data file for dataset ID 957700, version 1. Microsatellite multi-locus genotypes for Cladocopium madreporum, C. patulum, and C. sodalum collected from Palau, Thailand, Zanzibar, Phoenix Islands, and Australia. Missing loci are designated “0”.	

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Supplemental Files

File
indopacific_sampling_site_locations.csv (Comma Separated Values (.csv), 672 bytes) MD5:222884a97a35523766fe0ba585f0cf9a Indo-Pacific coral sampling site locations Four column table with Country, Site Location, Latitude, and Longitude
Nexus file for concatenated sequences of conserved phylogenetic dna markers filename: Cladocopium_generalists_IndoPacific.nex (Plain Text, 34.35 KB) MD5:d4280bbbae4529b233ce0bfa3d569a53 This is a sequence alignment of the conserved genes: partial LSU RNA, full mitochondrial cob, full mitochondrial cox 1, and partial Chloroplast 23S.
Nexus file for the psbA non-coding region DNA sequences for Cladocopium symbionts filename: Cladocopium_C1_radiation_v3_Pacific.nex (Plain Text, 85.67 KB) MD5:be8cf9e4b24527d1baa2439381405d2e Sequences of the non-coding region of the psbA mini-circle found in dinoflagellates. These are from species associated with one of three adaptive radiations in the genus Cladocopium; the C1-radiation
Nexus file for the psbA non-coding region DNA sequences for Cladocopium symbionts filename: C3_radiation_Fig3.nex (Plain Text, 81.30 KB) MD5:e3736814eb9f7b5cf2cb3a54ea75aa71 Sequences of the non-coding region of the psbA mini-circle found in dinoflagellates. These are from species associated with one of three adaptive radiations in the genus Cladocopium; the C3-radiation

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Related Publications

Arnaud-Haond, S., Duarte, C.M., Alberto, F., & Serrao, E.A. (2007). Standardizing methods to address clonality in population studies. Molecular Ecology, 16(24), 5115–5139. Portico. https://doi.org/10.1111/j.1365-294x.2007.03535.x https://doi.org/10.1111/j.1365-294X.2007.03535.x
Software

Bay, L. K., Howells, E. J., & van Oppen, M. J. H. (2009). Isolation, characterisation and cross amplification of thirteen microsatellite loci for coral endo-symbiotic dinoflagellates (Symbiodinium clade C). Conservation Genetics Resources, 1(1), 199–203. https://doi.org/10.1007/s12686-009-9048-1
Related Research

Butler, C. C., Turnham, K. E., Lewis, A. M., Nitschke, M. R., Warner, M. E., Kemp, D. W., Hoegh-Guldberg, O., Fitt, W. K., van Oppen, M. J. H., & LaJeunesse, T. C. (2023). Formal recognition of host-generalist species of dinoflagellate (Cladocopium, Symbiodiniaceae) mutualistic with Indo-Pacific reef corals. Journal of Phycology, 59(4), 698–711. Portico. https://doi.org/10.1111/jpy.13340
Results
,
Methods

Chen, Y., Shah, S., Dougan, K. E., van Oppen, M. J. H., Bhattacharya, D., & Chan, C. X. (2022). Improved Cladocopium goreaui Genome Assembly Reveals Features of a Facultative Coral Symbiont and the Complex Evolutionary History of Dinoflagellate Genes. Microorganisms, 10(8), 1662. https://doi.org/10.3390/microorganisms10081662
Related Research

Earl, D. A., & vonHoldt, B. M. (2011). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetics Resources, 4(2), 359–361. https://doi.org/10.1007/s12686-011-9548-7
Software

Geneious | Bioinformatics Software for sequence data analysis. (n.d.). Retrieved from https://www.geneious.com/
Software

Jakobsson, M., & Rosenberg, N. A. (2007). CLUMPP: a cluster matching and permutation program for dealing

with label switching and multimodality in analysis of population structure. Bioinformatics, 23(14), 1801–1806. https://doi.org/10.1093/bioinformatics/btm233
Software

Jombart, T., & Kamvar, Z. N. (2007). adegenet: Exploratory Analysis of Genetic and Genomic Data [dataset]. In CRAN: Contributed Packages. The R Foundation. https://doi.org/10.32614/cran.package.adegenet https://doi.org/10.32614/CRAN.package.adegenet
Software

Jombart, T., Devillard, S., & Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics, 11(1), 94. https://doi.org/10.1186/1471-2156-11-94
Software

LaJeunesse, T. C.(2002). Diversity and community structure of symbiotic dinoflagellates from Caribbean coral reefs. Marine Biology, 141(2), 387–400. https://doi.org/10.1007/s00227-002-0829-2
Methods

LaJeunesse, T. C., & Thornhill, D. J. (2011). Improved Resolution of Reef-Coral Endosymbiont (Symbiodinium) Species Diversity, Ecology, and Evolution through psbA Non-Coding Region Genotyping. PLoS ONE, 6(12), e29013. https://doi.org/10.1371/journal.pone.0029013
Related Research

LaJeunesse, T. C., Bhagooli, R., Hidaka, M., DeVantier, L., Done, T., Schmidt, G. W., Fitt, W.K., and Hoegh-Guldberg, O. (2004). Closely related Symbiodinium spp. differ in relative dominance in coral reef host communities across environmental, latitudinal and biogeographic gradients. Marine Ecology Progress Series, 284, 147-161. https://www.int-res.com/articles/meps2004/284/m284p147.pdf
Methods

LaJeunesse, T. C., Lee, S. Y., Gil-Agudelo, D. L., Knowlton, N., & Jeong, H. J. (2015). Symbiodinium necroappetenssp. nov. (Dinophyceae): an opportunist 'zooxanthella' found in bleached and diseased tissues of Caribbean reef corals. European Journal of Phycology, 50(2), 223–238. https://doi.org/10.1080/09670262.2015.1025857
Methods

LaJeunesse, T. C., Loh, W. K. W., van Woesik, R., Hoegh-Guldberg, O., Schmidt, G. W., & Fitt, W. K. (2003). Low symbiont diversity in southern Great Barrier Reef corals, relative to those of the Caribbean. Limnology and Oceanography, 48(5), 2046–2054. Portico. https://doi.org/10.4319/lo.2003.48.5.2046
Methods

Lee, S. Y., Jeong, H. J., & Lajeunesse, T. C. (2020). Cladocopium infistulum sp. nov. (Dinophyceae), a thermally tolerant dinoflagellate symbiotic with giant clams from the western Pacific Ocean. Phycologia, 59(6), 515–526. https://doi.org/10.1080/00318884.2020.1807741
Methods

Liu, H., Stephens, T. G., González-Pech, R. A., Beltran, V. H., Lapeyre, B., Bongaerts, P., Cooke, I., Aranda, M., Bourne, D. G., Forêt, S., Miller, D. J., van Oppen, M. J. H., Voolstra, C. R., Ragan, M. A., & Chan, C. X. (2018). Symbiodinium genomes reveal adaptive evolution of functions related to coral-dinoflagellate symbiosis. Communications Biology, 1(1). https://doi.org/10.1038/s42003-018-0098-3
Related Research

Moore, R. B. (2003). Highly organized structure in the non-coding region of the psbA minicircle from clade C Symbiodinium. INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY, 53(6), 1725–1734. https://doi.org/10.1099/ijs.0.02594-0
Related Research

National Bioinformatics Infrastructure Sweden. (n.d.). MrBayes: Bayesian Inference of Phylogeny. https://nbisweden.github.io/MrBayes/
Software

Peakall, R., & Smouse, P. E. (2006). genalex 6: genetic analysis in Excel. Population genetic software for teaching and research. Molecular Ecology Notes, 6(1), 288–295. https://doi.org/10.1111/j.1471-8286.2005.01155.x
Software

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of Population Structure Using Multilocus Genotype Data. Genetics, 155(2), 945–959. https://doi.org/10.1093/genetics/155.2.945
Software

Ronquist, F., & Huelsenbeck, J. P. (2003). MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics, 19(12), 1572–1574. https://doi.org/10.1093/bioinformatics/btg180
Software

Swofford, D. L. 2014. PAUP* Phylogenetic analysis using parsimony (*and other methods). 4.0d146 ed. Sinauer Associates, Sunderland, MA
Software

Turnham, K. E., Wham, D. C., Sampayo, E., & LaJeunesse, T. C. (2021). Mutualistic microalgae co-diversify with reef corals that acquire symbionts during egg development. The ISME Journal, 15(11), 3271–3285. https://doi.org/10.1038/s41396-021-01007-8
Related Research

Wham, D. C., & LaJeunesse, T. C. (2016). Symbiodinium population genetics: testing for species boundaries and analysing samples with mixed genotypes. Molecular Ecology, 25(12), 2699–2712. Portico. https://doi.org/10.1111/mec.13623
Related Research

Wham, D., Carmichael, M., & LaJeunesse, T. (2014). Microsatellite loci for Symbiodinium goreaui and other Clade C Symbiodinium. Conservation Genetics Resources, 6, 127–129.
Related Research

Zardoya, R., Costas, E., Lopez-Rodas, V., Garrido-Pertierra, A., & Bautista, J. (1995). Revised dinoflagellate phylogeny inferred from molecular analysis of large-subunit ribosomal RNA gene sequences. Journal of Molecular Evolution, 41(5). https://doi.org/10.1007/bf00175822 https://doi.org/10.1007/BF00175822
Methods

Zhang, H., Bhattacharya, D., Maranda, L., & Lin, S. (2008). Mitochondrial cob and cox1 Genes and Editing of the Corresponding mRNAs in Dinophysis acuminata from Narragansett Bay, with Special Reference to the Phylogenetic Position of the Genus Dinophysis. In Applied and Environmental Microbiology (Vol. 74, Issue 5, pp. 1546–1554). American Society for Microbiology. https://doi.org/10.1128/aem.02103-07 https://doi.org/10.1128/AEM.02103-07
Methods

Zhang, Z., Green, B. R., & Cavalier-Smith, T. (2000). Phylogeny of Ultra-Rapidly Evolving Dinoflagellate Chloroplast Genes: A Possible Common Origin for Sporozoan and Dinoflagellate Plastids. Journal of Molecular Evolution, 51(1), 26–40. https://doi.org/10.1007/s002390010064
Methods

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Parameters

Parameter	Description	Units
Sample_ID	Unique identifier for individual samples.	unitless
Species	The identified evolutionary lineage of the Cladocopium sample.	unitless
Aphia_ID	AphiaID identifier for species ID	unitless
Sample_Origin	Location of sample origin	unitless
C1_22	Microsatellite peak for SgrSpl_22 developed by Wham, Carmichael, and LaJeunesse (2014)	base pairs
C1_22_2	Second variant microsatellite peak for C1_22	base pairs

C1_25	Microsatellite peak for SgrSpl_25 developed by Wham, Carmichael, and LaJeunesse (2014)	base pairs
C1_25_2	Second variant microsatellite peak for C1_25	base pairs
C1_24	Microsatellite peak for SgrSpl_24 developed by Wham, Carmichael, and LaJeunesse (2014)	base pairs
C1_24_2	Second variant microsatellite peak for C1_24	base pairs
C1_05	Microsatellite peak for C1.05 developed by Bay, Howells, and van Oppen (2009).	base pairs
C1_05_2	Second variant microsatellite peak for C1_05	base pairs
C1_16	Microsatellite peak for SgrSpl_16 developed by Wham, Carmichael, and LaJeunesse (2014)	base pairs
C1_16_2	The second variant microsatellite peak for C1_16	base pairs
Z1_Spl_1	Microsatellite peak for Spl_1a developed by Wham, Carmichael, and LaJeunesse (2014)	base pairs
Z1_Spl_1_2	The second variant microsatellite peak for Z1_Spl_1	base pairs
z78	Microsatellite peak for SgrSpl_78 developed by Wham, Carmichael, and LaJeunesse (2014)	base pairs
z78_2	The second variant microsatellite peak for z78	base pairs

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Instruments

Dataset-specific Instrument Name	CB Scientific gel electrophoresis system
Generic Instrument Name	Agarose Gel Electrophoresis System
Dataset-specific Description	Amplified products were examined with gel electrophoresis with a CB Scientific system with 45–80% denaturing gradient gels.
Generic Instrument Description	A gel electrophoresis system that is used to separate DNA or RNA molecules by size, achieved by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field.

Dataset-specific Instrument Name	Applied Biosciences Sequencer at Penn State University Genomics Core Facility
Generic Instrument Name	Automated DNA Sequencer
Dataset-specific Description	Successful PCR amplicons of each genetic marker were Sanger sequenced with sequences analyzed at the Penn State University Genomics Core Facility on an Applied Biosciences Sequencer. (Possibly the Applied Biosystems 3730XL)
Generic Instrument Description	A DNA sequencer is an instrument that determines the order of deoxynucleotides in deoxyribonucleic acid sequences.

Dataset-specific Instrument Name	ABI 3730 Genetic Analyzer (Applied Biosystems)
Generic Instrument Name	Gene Analyzer
Dataset-specific Description	Fragment analysis was performed on an ABI 3730 Genetic Analyzer (Applied Biosystems) using a 500-bp standard (LIZ-labeled) at the Penn State University Nucleic Acid Facility.
Generic Instrument Description	An automated analyzer designed for a wide range of sequencing and fragment analysis applications with the ability to perform comparative sequencing, linkage analysis, STR analysis, SNP detection, discovery and validation, mutation detection, and many other applications.

Dataset-specific Instrument Name	hammer and chisel
Generic Instrument Name	Manual Biota Sampler
Dataset-specific Description	Samples of Scleractinia from various families were sampled with a hammer and chisel throughout various sites across the Indo-Pacific
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.

Dataset-specific Instrument Name	SCUBA
Generic Instrument Name	Self-Contained Underwater Breathing Apparatus
Dataset-specific Description	Samples of Scleractinia from various families were sampled by SCUBA with a hammer and chisel throughout various sites across the Indo-Pacific
Generic Instrument Description	The self-contained underwater breathing apparatus or scuba diving system is the result of technological developments and innovations that began almost 300 years ago. Scuba diving is the most extensively used system for breathing underwater by recreational divers throughout the world and in various forms is also widely used to perform underwater work for military, scientific, and commercial purposes. Reference: https://oceanexplorer.noaa.gov/technology/technical/technical.html

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Project Information

Collaborative Research: Stability, flexibility, and functionality of thermally tolerant coral symbioses (Thermally tolerant coral)

Coverage: Coral Reefs of Palau, Micronesia

NSF abstract:

All reef-building corals require large numbers of internal symbiotic microalgae (called Symbiodinium) for their survival and growth. These mutualisms have shown considerable sensitivity to changes in the environment in recent decades, especially due to global increases in ocean temperatures. When exposed to severe thermal stress, corals loose their symbionts and often die. However, recent experiments show that some symbionts may be more stress-tolerant. Corals with these heat-resistant symbionts continue to receive high amounts of algal derived nutrients and grow under elevated temperatures. If the global trend in seawater warming continues to increase, these heat-resistant symbioses may become more ecologically prevalent on reef systems around the world and could play a critical role in maintaining healthy and productive coral communities. This project will examine the ecological and physiological attributes of stress-tolerant symbioses from the Indo Pacific where coral communities are the largest, most diverse, and productive in the world. The researchers will conduct a series of experiments to (1) evaluate host and symbiont attributes that contribute to thermal tolerance and (2) characterize the relative flexibility and functionality of various corals and symbionts exposed to typical ambient and stressful temperatures. Broader impacts of the project include the training of several Ph.D. students, undergraduates, and high school students in the disciplines of physiology and ecology. The researchers will partner with Global Ocean Exploration, Inc. to communicate this research to the general public through short documentary videos, editorials, and podcasts. An interactive K-5 program, "Invertebrates on the Road," will introduce elementary students in Pennsylvania to marine invertebrate diversity. Research results will also be disseminated to the public at the University of Delaware via educational seminars, as well as through hands-on research displays and demonstrations presented at the annual open house "Coast Day" festival in each year of the project.

This project will examine several attributes important to the functional ecology of coral-dinoflagellate symbioses. Specifically, the research team seeks to understand the interplay between coral and symbiont physiologies under different environmental conditions and determine the relative influence of biotic factors crucial to the performance of stress tolerant symbioses. Results from recent experiments on Indo-west Pacific corals found that Clade D (S. trenchii) symbionts are stress-tolerant. These symbionts are able to maintain function and provide nutrients to their hosts under high temperatures that typically elicit the breakdown of symbioses involving many other species of symbiont. A number of questions arise about how enhanced thermal tolerance symbioses may be aided by a combination of factors; for example: Are symbionts physiologically hardier in corals that are routinely feeding? Do host genotypes that are adapted to high

temperatures affect the physiology of their symbionts in ways that make the partnership more stress-tolerant? A series of experiments over three years will examine the functionality of different coral-symbiont pairings exposed to ambient and high temperatures. Reciprocal transplants between inshore (stress-tolerant) and offshore (stress-susceptible) reef sites will be used to produce specific host-symbiont parings. Controlled experiments will test the relative importance of coral trophic status (nutrient content) while holding symbiont type constant and how changes in both coral trophic status and symbiont species identity of the resident affect thermal tolerance. Tank experiments on shore will track rates of photosynthesis as well as carbon translocation and assimilation from symbiont to host tissues and skeletons. Long-term growth rates via skeletal density, linear extension, and biomass gain will also be measured. This project will help elucidate how biochemical, physiological and ecological differences among host-symbiont pairings may respond to rising ocean temperatures and enhance the future viability of coral reefs.

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Funding

Funding Source	Award
NSF Division of Ocean Sciences (NSF OCE)	OCE-1636022
NSF Division of Integrative Organismal Systems (NSF IOS)	IOS-1258058

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