







**Public Symposium**  
Thursday, April 30, 2009

- 9:00 am **Welcome – Laurence P. Madin** (Director of Research, Woods Hole Oceanographic Institution)
- 9:15 am **Keith Crandall** (Brigham Young University, USA)  
*DNA barcoding: triumphs and tribulations*
- 9:45 am **Rob DeSalle** (American Museum of Natural History, USA)  
*What a DNA barcode is, is unclear*
- 10:15 am **Allen Collins** (Smithsonian Institution, USA)  
*Barcoding and phylogenetics of early diverging metazoans*
- 10:45 am Coffee Break
- 11:15 am **Christoffer Schander** (University of Bergen, Norway)  
*From barcodes to ecosystems functioning – a peek into the near future*
- 11:45 am WHOI Biology Seminar preparation
- 12:00 pm **Nancy Knowlton** (Smithsonian Institution, USA) – Keynote and Biology Seminar  
*Coral reefs: canaries in the environmental coal mine*
- 1:00 pm Lunch
- 2:30 pm **Chris Meyer** (Smithsonian Institution, USA)  
*Barcoding all marine species in a tropical ecosystem*
- 2:50 pm **Ryuji Machida** (Ocean Research Institute, University of Tokyo, Japan)  
*Zooplankton metagenomics and its comparison with Chaetognatha barcode data*
- 3:10 pm **Simon Creer** (University of Wales, Bangor, UK)  
*Assessing benthic meiofaunal diversity with 454 ultrasequencing*
- 3:30 pm Coffee Break and Poster Viewing
- 4:00 pm **Jon Geller** (San Francisco State University, USA)  
*Marine biomics: exploring diversity in marine microhabitats with whole-community DNA and sequence databases*
- 4:20 pm **Ann Bucklin** (University of Connecticut, USA)  
*DNA barcodes for North Atlantic zooplankton: Current status and applications for ecosystem monitoring*
- 4:40 pm **Dirk Steinke** (University of Guelph, Canada)  
*Overview of the MarBOL Project*
- 5:00 pm Wrap-up: Nancy Knowlton and Jon Geller
- 5:30 pm Adjourn



# SYMPOSIUM | THURSDAY, APRIL 30

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## DNA BARCODING: TRIUMPHS AND TRIBULATIONS

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**Keith A. Crandall**

*Department of Biology  
Brigham Young University  
Provo, UT USA*

*Keith Crandall is a Professor and Chair of the Department of Biology at Brigham Young University. Professor Crandall studies the molecular systematics, population genetics, and bioinformatics of a variety of organisms, from crustaceans to agents of infectious diseases. His lab also focuses on the development and testing of bioinformatic methods for studying sequence evolution. Professor Crandall earned his BA degree from Kalamazoo College in Biology and Mathematics, an MA degree from Washington University in Statistics, and a PhD from Washington University in Biology and Biomedical Sciences. He also served as a Peace Corps Volunteer in Puyo, Ecuador.*

DNA BARCODING approaches have a wide variety of applications with many examples of success. However, there are some general concerns, especially with respect to pseudogenes, bioinformatics infra-structure, and use in phylogenetic inference. Here I outline some recent research in decapod crustaceans using DNA barcoding. I provide examples from conservation biology of the application of DNA barcoding and phylogenetics to questions of species boundaries, species diagnoses, and defining conservation areas. I outline a number of bioinformatic questions currently being pursued with respect to DNA barcoding. I then explore some potential pitfalls of the problems of single gene phylogenies and pseudogenes. I then conclude with an overview of the benefits of DNA barcoding and the status of barcoding the decapod crustaceans.

## WHAT A DNA BARCODE IS, IS UNCLEAR

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**Rob DeSalle**

*Division of Invertebrates and  
Molecular Systematics Laboratory  
American Museum of Natural  
History  
New York, USA*

*Rob DeSalle is a Curator of Entomology at the American Museum of Natural History. He is affiliated with the AMNH Division of Invertebrate Zoology and works at the Sackler Institute for Comparative Genomics, where he leads a group of researchers working on molecular systematics, molecular evolution, population and conservation genetics, and evolutionary genomics of a wide array of life forms. He is also Adjunct Professor at Columbia University (Department of Ecology, Evolution and Environmental Biology), Distinguished Professor in Residence at New York University (Department of Biology), Adjunct Professor at City University of New York (Subprogram in Ecology, Evolutionary Biology, and Behavior), Resource Faculty at the New York Consortium in Evolutionary Primatology, and Professor at the AMNH Richard Gilder Graduate School.*

USING examples from *Drosophila* the limits of DNA barcoding using distance and character based approaches are examined. Specifically four groups of *Drosophila* will serve as general examples of taxonomic breadth in an insect group - *simulans*, *pseudoobscura*, *bipunctinata* and *quinaria*. The results of this analysis suggest that distance based approaches have severe limitations in identifying species in these well worked out systems. In addition, the impact of DNA barcoding on taxonomic thinking will be discussed.



## BARCODING AND PHYLOGENETICS OF EARLY DIVERGING METAZOANS

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### **Allen Collins**

*Department of Invertebrate Zoology  
National Museum of Natural History  
Smithsonian Institution  
Washington DC, USA*

*Allen is an Invertebrate Zoologist with the National Systematics Laboratory of NOAA's Fisheries Service in the Smithsonian's National Museum of Natural History. He is an affiliated scientist with the Department of Invertebrate Zoology and curator of National Museum's collections of Medusozoa and Hexactinellida. He received his PhD in Integrative Biology at the University of California Berkeley. He is co-PI on two NSF-funded Assembling the Tree of Life projects for Cnidaria and Porifera. Much of his research focuses on elucidating the evolution of these and other poorly known metazoan groups through collaborative efforts emphasizing the integration of molecular and morphological data, collection data, and bioinformatics tools. He is broadly interested in marine natural history stories and how marine biodiversity can be used to spark interest and move the public toward actions that preserve marine resources.*

THE TASKS of systematics are identification, naming, description, classification, and phylogenetics. In a strict sense, barcoding aims to accomplish identification. However, genetic barcodes contain phylogenetic information, particularly near the level of species. In addition, barcodes have the potential to be used as descriptors and clusters of them could be provided with names through bioinformatics tools. Thus, barcoding has the potential to greatly broaden the impact of studies aimed at creating phylogenetic backbones for large clades. They provide a pathway for accomplishing

more comprehensive taxon sampling, which informs systematists about clade compositions and sharpens evolutionary inferences about ancestral character states, modes of speciation, etc. The early diverging metazoan groups, Cnidaria (probably including Myxozoa), Ctenophora, Placozoa, and Porifera are amenable to barcoding efforts, but they pose a number challenges. First and foremost is a relative lack of study of their biodiversity. The most diverse groups, Cnidaria and Porifera, each on the order to 10,000 accepted species, have received the most attention, but community standards for barcode generation have been slow to develop. Further, it is still widely perceived that unknown diversity well exceeds known diversity. In fact, the genetic diversity within Porifera appears to be comparable to that within the rest of Metazoa. As a result, many examples exist where commonly used barcode markers have not been obtained for subclades (often major ones) within these groups, or they simply fail due to slow rates of evolution. Another problem for barcoding efforts is an inability to take or create long-term archival vouchers for some groups, particularly ctenophores and placozoans, but also some medusae. Many taxa are too small to both barcode and voucher, necessitating the need for para-vouchers. Finally, extreme simplicity and morphological plasticity confound our ability to simply know what we are looking at. Given these difficulties, phylogenetics and barcoding must be integrated in order to make significant strides in our understanding of the biodiversity of early diverging metazoans. We need bioinformatics tools that work for both types of efforts simultaneously.



# FROM BARCODES TO ECOSYSTEMS FUNCTIONING – A PEEK INTO THE NEAR FUTURE

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**Christoffer Schander**  
*Department of Biology*  
*University of Bergen*  
*Bergen, Norway*

*Christoffer Schander is a professor in Marine Biodiversity at the University of Bergen, Norway. He took his PhD at the University of Gothenburg, Sweden in 1997. He is thematic leader at the centre of excellence in Geobiology funded by the Norwegian Research Council. The goal of his research is to understand the role that evolutionary forces and phylogeny have played in creating organismal diversity, focusing mainly on the Mollusca. He has also worked on the problems of using formalin fixed material for molecular studies. Recently he has become involved in the development of automated methods for species identification.*

BARCODING for species identification has gained an impressive momentum in the past few years with successful annotations of many of the major organism groups. The data entered into databases has an enormous potential not only for species identifications, but also for phylogeography, community structure and state. Also, the use of metagenomic and metatranscriptomic studies opens up the possibility of coupling biodiversity assessments with the functional state of the ecosystem. Up until now these studies have mainly focused on microbial communities, but through the barcoding initiative studies of the metazoan communities are becoming particularly attractive. The explosion within the development of high throughput and resolution “-omic” technologies brings great potential for large scale, spatial and

temporal, ecosystems analyses. Similar to the field of human medicine, we are soon assessing a diagnosis of ecosystem state in a changing environment.

# CORAL REEFS: CANARIES IN THE ENVIRONMENTAL COAL MINE

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**Nancy Knowlton**  
*Department of Invertebrate Zoology*  
*National Museum of Natural History*  
*Smithsonian Institution*  
*Washington DC, USA*

*Dr. Nancy Knowlton holds the Sant Chair in Marine Science at the Smithsonian's National Museum of Natural History. Her research focuses on the ecology, evolution and conservation of coral reef organisms. Her analyses have led to the now widespread recognition that estimates of marine diversity are probably too low by a factor of ten. Dr. Knowlton received her undergraduate degree at Harvard University and her PhD at the University of California at Berkeley, and was a professor at Yale University prior to moving to the Smithsonian Tropical Research Institute in Panama. Later, she joined the Scripps Institution of Oceanography at the University of California at San Diego, where she was the founding Director of the Center for Marine Biodiversity and Conservation. She is an elected fellow and member of the Board of Directors of the American Association for the Advancement of Science and an Aldo Leopold Leadership Fellow. She currently serves on the National Geographic Society's Committee on Research and Exploration, chairs the World Bank's Targeted Research Program for Coral Reefs, is principle investigator of the Census of Marine Life's Coral Reef Initiative, and is a member of the editorial board of the Annual Review of Marine Science. In 2009 she received the Peter Benchley Award for science in the service of marine conservation.*



CORAL reefs are the most diverse and threatened of all marine ecosystems. On the one hand, overfishing, poor water quality and greenhouse gas emissions have already decimated reefs around the world. On the other hand, we have no clear idea of how many species live on coral reefs or how many we might be losing. A global assessment of reef diversity, using standardized sampling enabled by bar coding and ultrasequencing, could provide both the scientific answers and the public enthusiasm needed to protect these ecosystems.

## BARCODING ALL MARINE SPECIES IN A TROPICAL ECOSYSTEM

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**Christopher Meyer**

*Department of Invertebrate Zoology  
National Museum of Natural History  
Smithsonian Institution  
Washington DC, USA*

*Chris Meyer is a Research Zoologist in the Department of Invertebrate Zoology at the Smithsonian's Museum of Natural History and also the director of the Moorea Biocode Project. He received his MA and PhD at UC Berkeley. Much of his research focuses on the systematics and phylogeography of marine gastropod groups. He is interested in the patterns and processes associated with marine biodiversity and how to accelerate our understanding of these communities using emergent technologies, including both genetic data and informatics tools.*

DNA BARCODING of marine taxa faces a number of logistical challenges, yet also provides novel opportunities for significant improvement in our understanding of biodiversity. In an attempt to genetically characterize an entire tropical ecosystem's macrobiota, including marine communities, the Moorea Biocode Project

is committed to these challenges and provides a proving ground for discovery. With all but one metazoan phylum, the phyletic diversity of marine species is unprecedented and poses significant challenges to the goal of universality in locus recovery at all steps in the barcoding pipeline. Effective sampling, databasing, tissue collection, vouchering, extraction and sequencing of marine species are being determined. Because the island of Moorea is geographically constrained, we have developed multiple methods for sampling biodiversity that expand efforts beyond traditional, adult, voucher-based approaches. Semi-quantitative, standardized approaches are being adopted to address temporal and spatial variability and project effectiveness and stopping rules. After the first year of this three-year project, three big unknowns remain as to our understanding of marine diversity. (1) Where is all the recorded, but non-vouchered, diversity? Are these taxa just rare as adults, living hidden within the reef matrix, or found only on the deep reef? (2) What is the scale of diversity in parasitic taxa? Is it true that there are at least three parasitic species for every macrospices? And (3) What is the scale of structuring in marine complexes across vast marine realms? Is there a multiplier we can adopt based on better known groups?



# ZOOPLANKTON METAGENOMICS AND ITS COMPARISON WITH CHAETOGNATHA BARCODE DATA

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**Ryuji J. Machida**  
*Plankton Laboratory*  
*Ocean Research Institute*  
*University of Tokyo*  
*Tokyo, Japan*

*Dr. Ryuji Machida is a postdoctoral fellow at the Ocean Research Institute of the University of Tokyo, Japan. He has served as the Census of Marine Zooplankton (CMarZ) – Asia Office Manager since 2005. He received a Research Fellowship for Young Scientist (Japan Promotion of Science) 2004-2005, and was awarded the Okada prize for 2007 from the Oceanographic Society of Japan (OSJ). Commemorating the late Professor Takematsu Okada, the Okada Prize is awarded to a young member of the Society who has made outstanding contributions to the progress of oceanography. Dr. Machida was recognized for his research accomplishments "molecular genetics and evolution of marine zooplankton". He received the Ph.D. degree from the University of Tokyo.*

**Other authors: Hiroomi Miyamoto, and Shuhei Nishida**

COMBINED application of zooplankton metagenomics and DNA barcoding will be a powerful tool for the census of marine animals. We have established a methodology for zooplankton metagenomics with an intent to estimate the species richness and identify all the species in bulk zooplankton samples. However, only a limited number of zooplankton metagenomics DNA sequences have been identified through comparisons

with the DDBJ/GenBank/EMBL BLAST database. In this context, we have barcoded Chaetognatha, one of the most important carnivorous zooplankton, and compared their sequences with the zooplankton metagenomics data. As a result, most of the zooplankton metagenomics DNA sequences belonging to the Chaetognatha clade were identified to the species. It is expected that the species of the zooplankton metagenomics analysis will be further clarified as more gene sequences of marine animals accumulate by the barcoding project.

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## ASSESSING BENTHIC MEIOFAUNAL DIVERSITY WITH 454 ULTRASEQUENCING

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**Simon Creer**  
*School of Biological Sciences*  
*Bangor University*  
*Bangor, United Kingdom*

*Simon Creer research interests focus on the use of molecular tools in addressing diverse hypotheses focusing on animal ecology and evolution. By using 454 ultrasequencing, he is currently investigating the magnitude and distribution of marine/estuarine benthic Eukaryotic biodiversity, transcriptomic responses to environmental perturbation and performing mitogenomic analyses within the Araneae. These projects (alongside ongoing interests in population genomics and phylogeography) facilitate the investigation of links between biodiversity and ecosystem processes, understanding environmental genomic responses to change/adaptive evolution and the advancement of the field of phylogenetics.*



*Other authors: Vera G Fonseca, Gary Carvalho, Deborah M Power, Kelly Thomas, Way Sung, Mark Blaxter and John Lamshead*

THE MARINE meiobenthos is an ecologically important, ubiquitous and diverse community assemblage comprised of between 50-90% nematodes. Despite their abundance and ecological importance, a current estimate of global nematode diversity remains speculative, and there are considerable knowledge gaps pertaining to the magnitude of the remaining meio- and micro-eukaryotic diversity fraction. This knowledge gap results from a range of issues, such as the small size of taxa, difficulties in sampling, availability of taxonomists, and convergent evolution. In order to solve the biodiversity identification issue, molecular identification strategies are now being used to assist species identification and molecular appraisals of accepted marine morphospecies routinely uncover cryptic species. Here, we test a 454 metagenetic approach to (a.) estimate 18S rDNA molecular operational taxonomic unit (MOTU) diversity from a subsample of the European marine benthos (b.) test the efficacy of 454 ultrasequencing for eukaryotic biodiversity appraisal and (c.) explore comparative levels of MOTU richness and patterns of amplicon abundances throughout phyla. The results are discussed with respect to the disparity between molecular and morphological datasets, the possibility of using ultrasequencing for biodiversity appraisals and overarching patterns of organismal diversity across wide taxonomic ranges.

## MARINE BIOMICS: EXPLORING DIVERSITY IN MARINE MICROHABITATS WITH WHOLE- COMMUNITY DNA AND SEQUENCE DATABASES

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**Jon Geller**

*Moss Landing Marine Laboratories  
California State University  
Moss Landing, USA*

*Jonathan Geller is a Professor of Invertebrate Zoology and Molecular Ecology at the Moss Landing Marine Laboratories (MLML) of the California State University, located in Monterey Bay, California. He received BA, MS, and PhD degrees at the UC Davis and Berkeley. Longstanding research focuses on marine biological invasions and employs population genetics to detail sources, pathways, and consequences of invasions. Other published research reflects broad interests in invertebrate evolution and ecology, and include descriptions of new species, phylogenetic analysis, phylogeography, population extinction, genetics of exploited populations, larval recruitment, and symbiosis. The description of biodiversity in discrete habitats using molecular inventories is a current emphasis, as well as environmental sequencing to monitor and detect marine invasions.*

THE MAGNITUDE and spatial patterns of marine biodiversity remain largely unknown. At the same time, biodiversity is threatened by multiple and rapidly acting stressors. The analysis in whole-community nucleic acids provides one approach to quickly interrogate the diversity found in discrete marine habitats, a practice we term "biomics." This approach has been widely adopted in microbial ecology but scarcely so for multicellular eukaryotes because of various conceptual and technical



impediments. Here, we present data from sequencing of biomic DNA from endolithic organisms inhabiting molluscan shells. Sequences of cloned 18S rRNA gene fragments from heavily encrusted abalone shells (*Haliotis rufescens*) included sponges, annelids, fungi, chlorophytes, rhodophytes, and phaeophytes. The presence of kelp DNA suggests that abalone may assist the propagation of the plants it consumes. Sequencing from endoliths from other molluscan shells has yielded sequences most similar to tardigrades and myxozoans, widely expanding the known ecological range of both taxa. Obstacles to the biomic approach include intrinsic biological biases and the potential loss of representativeness with each molecular manipulation, as well as lack of desired specificity in PCR. We illustrate some of these effects with results from marine plankton from Moorea and settling plates from Hawaii. Continued research is needed to develop methods to control bias in biomic samples. Combined with conventional biodiversity inventories, systematics and taxonomy, marine biomics can accelerate the discovery and understanding of marine biodiversity.

## DNA BARCODES FOR NORTH ATLANTIC ZOOPLANKTON: CURRENT STATUS AND APPLICATIONS FOR ECOSYSTEM MONITORING

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**Ann Bucklin**

*Professor and Head, Marine Sciences  
University of Connecticut,  
Groton, Connecticut, USA*

*Ann Bucklin is a professor and head of the Department of Marine Sciences and Director of the Marine Sciences and Technology Center at the University of Connecticut. She was a Fulbright Senior Scholar in Norway (1992-1993) and was elected Fellow of the American Association for the Advancement for Science in 1995. Since 2004, Dr. Bucklin has served as the principal investigator and lead scientist for a Census of Marine Life ocean realm field project, the Census of Marine Zooplankton (CMarZ). Dr. Bucklin received her B.A. in biology from Oberlin College, and her Ph.D. in zoology from the University of California, Berkeley. Her research focuses on molecular systematics, phylogeography, and phylogenetics of marine crustacean holozooplankton.*

**Other authors: Leocadio Blanco Bercial, Brian D. Ortman and Lisa M. Nigro**

AS PART of the Census of Marine Zooplankton (CMarZ), a global survey of marine holozooplankton biodiversity, we are working toward a taxonomically-comprehensive DNA barcode database for North Atlantic holozooplankton. The DNA barcode database will be useful to: identify individual specimens, reveal cryptic species, describe biogeographical distribution, discover new species, and characterize species diversity through environmental sequencing. In the future, DNA barcodes may be used for rapid and automated taxonomic analysis of zooplankton samples, including identification and quantification of known species, as well as estimation of species not found in the database and/or undescribed. DNA-based analysis of zooplankton samples may be particularly useful for ecosystem health assessment, and fisheries and environmental monitoring.



## OVERVIEW OF THE MARBOL PROJECT

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### **Dr. Dirk Steinke**

*University of Guelph Canadian Centre  
for DNA Barcoding Guelph, Canada*

*Dirk Steinke completed his Diploma in 2002 at the Johann-Wolfgang Goethe University in Frankfurt (Germany) under the supervision of Bruno Streit and Markus Pfenninger. He received his PhD in Zoology in early 2006 at the University of Konstanz (Germany) working with Axel Meyer. Dirk's doctoral research employed phylogenomic methods and computational biology to address questions on genome evolution of vertebrates especially fishes. He also developed software for distance based species identification using DNA barcodes and was involved in early efforts of DNA barcoding in Germany. Dirk Steinke leads the global marine barcoding campaign (MarBOL) and is affiliated with FishBOL and work in the Canadian Arctic (through PolarBOLI and CHONe).*

MARBOL is joint effort of the Consortium for the Barcode of Life (CBOL) and the Census of Marine Life (CoML) to enhance our capacity to identify marine life by utilizing DNA Barcoding. The goal of this project is a huge acceleration in the rate of marine barcoding, so 2010 barcodes are available for members of all key marine groups and reasonably thorough coverage has been achieved for groups of highest scientific or societal importance. The breadth of coverage by barcoding, together with other molecular information as well as other new information in systematic biology, will allow the Census in 2010 to offer an updated “tree” covering the evolution of ocean life. This talk will summarize the activities of MarBOL and the methods used. It will highlight achievements to date and showcase first insights and results.

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## POSTER SESSION

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### **Advances in molecular taxonomy of Oithona spp. in the Argentine Sea**

*Georgina Cepeda. Instituto Nacional de Investigación y Desarrollo Pesquero, (INIDEP) Mar del Plata, Argentina.*

### **Status of Puget Sound Zooplankton Biodiversity Sampling**

*Adelaide Rhodes. University of Florida. Florida, USA.*

### **DNA Barcoding of Shrimps: An Indian Scenario**

*Rajesh Giridhar. National Institute of Oceanography (CSIR), Regional Center. Cochin. India.*

### **Identification of Chaetognaths Using mtCOI and 16s genes**

*Francis Kidangan. National Institute of Oceanography (CSIR), Regional Center. Cochin. India.*

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