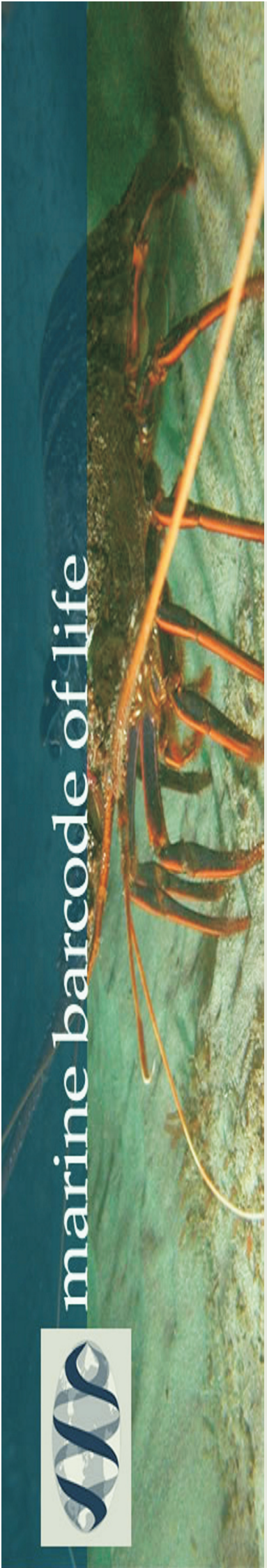

DNA Barcoding of Marine Biodiversity (MarBOL) Public Symposium and Workshop

Current Status of Barcoding Bioinformatics and Statistics

21-22 May 2009
Tokyo - Japan

Public Symposium

Thursday, May 21, 2009

9:15 am	Welcome – S. Nishida (ORI, University of Tokyo)
9:30 am	**S. Sun**, S. Wang, C. Li (Institute of Oceanography, Chinese Academy of Sciences) ***DNA barcodes of zooplankton from the Chinese coastal areas***
10:00 am	**S.A. Khan**, C.P. Kumar, P.S. Lyla, S. Murugan (Centre of Advanced Study in Marine Biology, Annamalai University, Tamil Nadu, India) ***DNA barcoding the marine fishes of Parangipettai waters (India)***
10:30 am	Coffee Break
11:00 am	**A. Bucklin**, L. Blanco Bercial, B.D. Ortman, L.M. Nigro (University of Connecticut, USA) ***DNA barcodes for North Atlantic zooplankton: Current status and future applications for ecosystem monitoring***
11:30 am	**N. Utsuki**, U. Jinbo, M. Ito (University of Tokyo; Japanese Barcode of Life Initiative) ***Activities and projects on DNA barcoding in Japan***
12:00 pm	Lunch
1:30 pm	**M. Miya** (Natural History Museum and Institute, Chiba) ***Deep-sea mystery solved: Astonishing larval transformations and extreme sexual dimorphism (plus DNA sequences) unite three fish families***
1:50 pm	**E. Goetze** (University of Hawaii at Manoa, USA) ***On species discovery using molecular data***
2:10 pm	**F. Pradillon** (Japan Agency for Marine-Earth Science and Technology) ***Whose larvae? In situ hybridization methods for the identification of marine early-life stages***
2:40 pm	Coffee Break
3:00 pm	**L. Amaral-Zettler**, P. Neal, S. Huse, M.L. Sogin (Marine Biological Laboratory, Woods Hole) ***The International Census of Marine Microbes: Unveiling the ocean's hidden majority through community 454 tag pyrosequencing***
3:20 pm	**K. Barott**, R.V. Thurber, F. Rohwer (San Diego State University) ***Understanding the role of viruses on coral reefs through metagenomics***
3:40 pm	**K. Hamasaki**, A. Taniguchi (University of Tokyo) ***Diversity of active microbial communities in the South Pacific Ocean revealed by bromodeoxyuridine labeling and 454 tag sequencing technology***
4:00 pm	**R. Machida**, H. Miyamoto, S. Nishida (University of Tokyo) ***Zooplankton metagenomics and its comparison with Chaetognatha barcode data***
4:20 pm	**K. Kogure** (ORI, University of Tokyo) – Comments and welcome to Poster Viewing
4:30 pm	Reception and Poster Session
5:30 pm	Adjourn

DNA BARCODES OF ZOOPLANKTON FROM THE CHINESE COASTAL AREAS

Professor Dr. SUN Song
Director
Institute of Oceanology
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Qingdao, China

Prof. SUN Song, director of the Institute of Oceanology, Chinese Academy of Sciences (IOCAS), which was established in 1950 and is the largest and most comprehensive multidisciplinary marine research institution in China. He earned his PhD in marine ecology from IOCAS in 1994. He was a visiting scholar at Australian Antarctic Division for cooperative research in Antarctic krill biology from 1992 and 1994. Since 1998, he has served as vice director of IOCAS, taking charge of the education school of the institute, research vessel fleet management, human resources management, marine ecology and environmental science research activity coordination and ecological station administration. As the team leader in marine science, he took part in the Chinese Antarctic Expedition three times. His research interest is mainly in marine ecology, especially in the zooplankton population dynamics and Antarctic krill biology. He is also chairman of the Marine Ecology Division of the China Oceanology & Limnology Society, and of the Chinese National Implementation Committee for CoML (Census of Marine Life), and is involved in a number of professional organizations, such as SSC of the CoML, Census of Marine Zooplankton (CMarZ), OBIS and the SCOR Technology Panel.

Other authors: Wang Shiwei, Li Chaolun

MARINE zooplankton biodiversity is very high in the coastal waters in China; more than 560 copepod species have been identified. It is very difficult to describe and identify all the species, especially their eggs and all the developmental stages, but this information is very important for zooplankton ecology. DNA barcoding, sequencing a small region of genes, offers a rapid and accurate means for the identification of zooplankton species at all life history stages. To promote the application of DNA barcoding of marine zooplankton in China, a comprehensive platform, from sample collection to data publication, has been established in the IOCAS. Hitherto, more than 1,000 samples have been collected, including 300 samples from an "Arctic Ocean-equator-Antarctic Ocean" meridian transect. Around 500 sequences were determined for 115 copepod species. It is shown that the 650-bp cytochrome oxidase subunit 1 (COX1) sequence was capable of discriminating 99% of the species. The utility of this molecular marker was also confirmed by the monophyletic status of the species in the NJ tree. Sibling species were revealed using DNA barcoding. The formerly-identified *Acartia pacifica* comprised 3 lineages: *Acartia pacifica*, *Acartia ohtsukai* and *Acartia spinicauda*. Potential cryptic species in the genus *Pleuromamma* were also found. In addition to the core marker COX1 recommended by CBOL and CoML, more loci were investigated. We sequenced almost the complete mitochondrial genome of *Calanus sinicus* to find molecular markers for intraspecific genetic diversity from different environments. Six loci from both mitochondrial and nuclear genes were compared in cnidarians to determine the proper markers for DNA barcoding.

DNA BARCODING THE MARINE FISHES OF PARANGIPETTAI WATERS (INDIA)

Prof. Dr. S. Ajmal Khan
Centre of Advanced Study in Marine Biology
Annamalai University
Tamil Nadu, India

Dr.Syed Ajmal Khan is a Professor in the Centre of Advanced Study in Marine Biology. After completing his B.Sc. in Zoology, he did his M.Sc. in Marine Biology. He was awarded the Ph.D. degree for his work on the diversity of hermit crabs. All along he has been concentrating on biodiversity investigations of decapod crustaceans and has published a number of monographs on this group of organisms. He is an specialist in methods for assessing biodiversity. In view of the difficulties he encountered with the analogue character arrays for identifying crustaceans, he has turned to digital DNA characters to identify the species accurately and to resolve the taxonomic disputes.

Other authors: C. Prasanna Kumar, P.S. Lyla and S. Murugan

PARANGIPETTAI, historically called Porto Novo, is a panchayat town in the Cuddalore district in the Indian state of Tamil Nadu. Parangipettai is situated on the north bank of the Vellar estuary which flows in to Bay of Bengal. Vellar estuary and its coastal waters are well known for extensive marine fish diversity. About 510 species of marine fishes have been documented so far in and around Parangipettai coastal waters. Here we show that cytochrome c oxidase I DNA barcodes have effectively discriminated species belonging to 5 orders of marine fishes occurring in Parangipettai waters of Bay of Bengal. About 44 marine fishes from Parangipettai waters were barcoded for 655 bp region of the cytochrome oxidase subunit I gene (COI). Most species were represented by a single specimen, and 47 sequences were generated. The GC content of 25 fishes from Perciformes ranged from 41.9% to 50.5%. The maximum GC content of 54% was noted in 3 species of the order Tetradontiformes. The GC content of 3 species of fishes belonging to Siluriformes ranged from 41.2% to 48%. In case of 6 species of fishes belonging to Clupeiformes the GC content ranged from 46.5% to 52.3%. The GC content of 6 species of Gasterosteiformes ranged from 38.4% to 49.1%. The Neighbor Joining tree was used for all the above 5 orders individually as aggregation of sequences of the above orders misplaced species in different clades. This might be due to the usage of sequences of different sizes for phylogram construction. Among Perciformes, perfect clustering of different families like Polynemidae, Mugilidae, Stromateidae, Leiognathidae and Trichiuridae was noted. Similarly the clustering of Clupeidae and Engraulidae was noted in order Clupeiformes. Bootstrap values of all clades in the phylogram were found to be more than 50% as expected, excerpt in the case of Perciformes. This may be due to usage of diversified sequence sizes (250 bp – 600 bp) employed in phylogram construction. Also species of the same genus invariably clustered in the same clade, which is evident in the phylogram of Perciformis where the members of family Polynemidae, Mugilidae, Stromateidae, Leiognathidae and Trichiuridae got clustered separately in the phylogram. Hereby we conclude that COI sequencing (barcoding) is helpful in the identification of marine fish species of Parangipettai waters.

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

Prof. Dr. 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. During 1992 – 2005, she was a professor in the Department of Zoology and a member of the Institute for the Study of Earth, Oceans, and Space at the University of New Hampshire. 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. The theme underlying her research interest – spatial and temporal patterns of molecular genetic variation in marine organisms – developed from her early studies of sea anemones. Her current focus is the molecular systematics, phylogeography, and phylogenetics of marine crustacean holozooplankton.

Other authors: Leocadio Blanco Bercial, Brian D. Ortman, 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.

ACTIVITIES AND PROJECTS ON DNA BARCODING IN JAPAN

Dr. Nozomu Utsuki
Department of General Systems Studies
University of Tokyo
Japan

Nozomu Utsuki is currently a researcher at the University of Tokyo, Japan and works on the Global Biodiversity Information Facility (GBIF) Japan National Node. He studied biology at the Tokyo Metropolitan University, Japan and his PhD is focused on taxonomy and phylogeny of Japanese Asilidae (Diptera). His recent interest is focused on Biodiversity Informatics.

Other authors: Utsugi Jinbo, Motomi Ito

DNA BARCODINg is currently recognized as an important technique in biodiversity researches, and is the focus of many large-scale projects such as the Marine Barcode of Life (MarBOL).In Japan, DNA barcoding has lately attracted considerable attention. At present, two DNA barcoding projects have been initiated; one is focused on woody plants and another is on parasite wasps. In addition, Japanese researchers participate in international DNA barcoding projects such as the Fish Barcode of Life (FishBOL), the All Birds Barcoding Initiative (ABBI), the Bee Barcode of Life Initiative (Bee-BOL) and MarBOL.The Japan Barcode of Life Initiative (JBOLI) is a working group, which acts as the center of DNA barcoding projects in Japan. It is associated with the GBIF Japan National Node, Union of Japanese Society for Systematic Biology and the National BioResource Project (NBRP) of the Ministry of Education, Culture, Sports, Science and Technology (MEXT). The goal of JBOLI is to support DNA barcoding activities in Japan and to establish a data-sharing framework with related organizations such as the DNA Databank of Japan (DDBJ) and the Barcode of Life Data Systems (BOLD). Currently, JBOLI holds meetings and symposiums, manages a portal website and is developing the JBOLI Data System (JBOLI-DS). JBOLI-DS is a BOLD-like system consisting of specimen and sequence repositories with DNA barcode identification system. In addition, JBOLI supports DNA barcoding of Japanese longicorn beetles (ALL-CERAM-JPN) as a pilot project. For the next step JBOLI is preparing a DNA barcode registration system in cooperation with DDBJ.In this presentation we introduce ongoing barcode projects and the current activities of JBOLI.

DEEP-SEA MYSTERY SOLVED: ASTONISHING LARVAL TRANSFORMATIONS AND EXTREME SEXUAL DIMORPHISM (PLUS DNA SEQUENCES) UNITE THREE FISH FAMILIES

Dr. Masaki Miya
Department of Zoology
Natural History Museum and Institute
Chiba, Japan

Dr. Masaki Miya is Curator of Fishes at Natural History Museum and Institute, Chiba, and Adjunct Associate Professor at the Graduate School of Chiba University. Dr. Miya has been trained as a marine biologist and took his Ph.D. degree from the University of Tokyo in 1987. One of his major contributions to the scientific community is mitochondrial phylogenomics of fishes, with >1250 sequences assembled from various fish groups. In total he published 91 papers on various topics on biology of fishes, which have been cited 1,753 times in various scientific journals as of last October.

THE OCEANIC bathypelagic realm (1000-4000 m) is a nutrient-poor habitat. Most fishes living there have pelagic larvae utilizing the rich waters of the upper 200 m. Morphological and behavioral specializations necessary to occupy such contrasting environments have resulted in remarkable developmental changes and life history strategies. We resolve a long-standing biological and taxonomic conundrum by documenting the most extreme example of ontogenetic metamorphoses and sexual dimorphism in vertebrates. Based on morphology and mitogenomic sequence data, we show that fishes currently assigned to three families with greatly differing morphologies, Mirapinnidae (tapetails), Megalomycteridae (bignose fishes), and Cetomimidae (whale fishes), are larvae, males, and females, respectively, of a single family Cetomimidae. Morphological transformations involve dramatic changes in the skeleton, most

spectacularly in the head, and are correlated with distinctly different feeding mechanisms. Larvae have small, upturned mouths and gorge on copepods. Females have huge gapes with long, horizontal jaws and specialized gill arches allowing them to capture larger prey. Males cease feeding, lose their stomach and esophagus, and apparently convert the energy from the bolus of copepods found in all transforming males to a massive liver that supports them throughout adult life.

ON SPECIES DISCOVERY USING MOLECULAR DATA

Dr. Erica Goetze
Department of Oceanography
University of Hawaii
Manoa, USA

Dr. Erica Goetze is a biological oceanographer whose work focuses on the evolution, ecology and genetics of marine planktonic invertebrates. Her research utilizes field, laboratory, and experimental approaches to examine biological processes occurring across a range of temporal and spatial scales. She is currently an Assistant Professor in the Department of Oceanography at the University of Hawaii at Manoa, and is an Instructor in the undergraduate Global Environmental Science Program as well as in the graduate program in Oceanography. She has a soft spot for calanoid copepods (Crustacea: Maxillopoda).

ONE primary goal of DNA Barcoding efforts has been the discovery of new species. This objective has proved contentious, with critics focusing on the weaknesses of using a single gene locus for species delineation, the accuracy of single-gene thresholds for specimen identification, and the potential pitfalls of PCR co-amplification of nuclear pseudogenes in barcoding studies. Despite these potential limitations, I argue that large-scale genetic screening is a powerful means of primary detection of new species, in particular for marine invertebrates. Using data from a global study of planktonic copepods (> 1300 specimens), I demonstrate that evolutionarily significant units (ESUs) discovered via DNA sequencing of short mitochondrial gene fragments are often supported as valid, reproductively isolated species following examination of additional morphological, biogeographic, or nuclear gene sequence data. DNA barcoding can be an effective tool to facilitate species discovery, but must be followed be careful taxonomic study for delineation and description of new species.

WHOSE LARVAE? IN SITU HYBRIDIZATION METHODS FOR THE IDENTIFICATION OF MARINE EARLY-LIFE STAGES

Dr. Florence Pradillon
Extremobiosphere Research center
JAMSTEC
Japan

As a PhD student at the University Pierre et Marie Curie in Paris, she investigated reproduction and development of the thermophile annelid from hydrothermal vent Alvinella pompejana, *using in situ and in vitro approaches, including newly designed pressure vessels. She developed in situ hybridization methods for the identification of embryonic and larval stages from the environment during a post-doc at the Max-Planck Institute for Marine Microbiology in Bremen in Germany. She is now working in JAMSTEC (Japanese Agency for Marine-Earth Science and Technology) on reproduction, development and dispersal strategies of polychaets from whale falls.*

THE LIFE cycle of many marine organisms includes embryonic and larval stages that disperse through the water column, before undergoing metamorphosis and beginning their adult life. The form of many of these early life stages has yet to be described and when they are collected it is almost impossible to match them to their adult counterparts, using morphology only. Therefore, larval distribution patterns remain unknown in many cases, preventing our full understanding of population biology and dynamics, of connectivity mechanisms, as well as of the planktonic diversity. Molecular methods based on the identification of diagnostic sequence have been widely used, but they involve destructive processing of the organisms. Yet, conserving morphological information may be desirable for numerous species for which development is unknown. More recently, we developed new methods that use whole-larvae *in situ* hybridization with oligonucleotide probes targeting ribosomal RNA diagnostic sequences. These methods are powerful as they may allow identification to the species level. They can reveal the morphology of species that have

not yet been related to their adult form, providing new and valuable information for evolutionary biology and systematics. In addition, tens to hundreds of larvae may be simultaneously processed, making real-time surveys during field trips possible. Here, I will give examples of probe development and use in the case of polychaets from different types of chemosynthesis-based ecosystems.

THE INTERNATIONAL CENSUS OF MARINE MICROBES (ICOMM): UNVEILING THE OCEAN'S HIDDEN MAJORITY THROUGH COMMUNITY 454 TAG PYROSEQUENCING

Dr. Linda Amaral-Zettler
The Josephine Bay Paul Center for Comparative Molecular Biology and Evolution
Marine Biological Laboratory
Woods Hole, USA

Dr. Amaral-Zettler holds an Sc.B. in Aquatic Biology from Brown University and a Ph.D. in Biological Oceanography from the MIT-Woods Hole Oceanographic Institution Joint Program. She has been at the Marine Biological Laboratory in Woods Hole, MA since 1996 where she began as a postdoctoral fellow in the laboratory of Dr. Mitchell Sogin. She now leads her own research group as a member of the research faculty in the Josephine Bay Paul Center for Comparative Molecular Biology and Evolution. She also holds an appointment as an Assistant Professor in the Ecology and Evolutionary Biology Department at Brown University as part of the Joint Brown-MBL Graduate Program. Since 2004, Dr. Amaral-Zettler has served as the Secretariat and education and outreach leader for the International Census of Marine Microbes - one of 14 ocean realm projects in the Census of Marine Life in an international effort to census microbial life in the ocean.ologies, including both genetic data and informatics tools.

ICoMM IS A PART of the Census of Marine Life Program that seeks to determine the diversity, distribution and abundance of microbes in the ocean. In collaboration with an international community of marine microbiologists, ICoMM has forged a large-scale effort to characterize microbial diversity in the sea through massively-parallel, 454-based sequencing of hypervariable regions of the SSU rRNA genes of bacteria, archaea and microbial eukaryotes. Sequencing is now complete on 52 separate projects from environments including deep and shallow hydrothermal vent systems, polar regions, coastal and estuarine environments, the open ocean, the deep biosphere, oxygen minimum zones, corals and 8 of the 13 aquatic US Long Term Ecological Research Sites. ICoMM is simultaneously collecting data on environmental parameters that characterize all sampling sites and making these available through its affiliated website VAMPS (http://vamps.mbl.edu) that provides the ICoMM community with tools for comparing similarities and differences in the composition of microbial populations. To date, ICoMM has generated over 18 million tags. Analyses underway integrating diversity data with contextual information should inform us about the interplay between microbial mediated activities and oceanic processes.

UNDERSTANDING THE ROLE OF VIRUSES ON CORAL REEFS THROUGH METAGENOMICS

Katie Barott
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San Diego State University
San Diego, USA

She currently works at San Diego State University in the lab of Dr. Forest Rohwer. Her research is focused on the interactions between microbes (bacteria and viruses) and corals. Coral reefs are declining worldwide, and coral disease has become a major problem. However, little is known about the role of microbes in these ecosystems. She is interested in the diversity and function of bacteria and viruses associated with corals, as well as understanding how perturbations of the system (overfishing, pollution, etc.) affect the interaction between corals and their microbial associates.

Other authors: R.V. Thurber, F. Rohwer

CORAL reefs are one of the most diverse ecosystems on the planet, but have been declining worldwide due to a wide variety of factors. Threats to coral reefs include local influences such as overfishing, habitat destruction, and pollution. Global factors threatening reefs include rising sea surface temperatures and ocean acidification due to increasing atmospheric carbon dioxide levels. Coral

disease has also become a severe problem, however little is known about the etiological agents. The healthy coral animal is associated with a multitude of other organisms, including algae, protists, fungi, and microbes, together termed the holobiont. Most research to date has focused on the role of the symbiotic algae living within the coral tissue, and to an increasing extent the bacteria living in association with the coral. One major potential player in the coral holobiont that has yet to be explored is viruses. Using a combination of viral metagenomics and real-time PCR, we show that *Porites compressa* corals contain a suite of eukaryotic viruses, many related to the Herpesviridae. This coral-associated viral consortium was found to shift in response to abiotic stressors. In particular, when exposed to reduced pH, elevated nutrients, and thermal stress, the abundance of herpes-like viral sequences rapidly increased in 2 separate experiments. Herpes-like viral sequences were rarely detected in apparently healthy corals, but were abundant in a majority of stressed samples. These data support the hypotheses that corals experience viral infections, which are exacerbated by stress, and that herpes-like viruses are common in Cnidarians.

DIVERSITY OF ACTIVE MICROBIAL COMMUNITIES IN THE SOUTH PACIFIC OCEAN REVEALED BY BROMODEOXYURIDINE LABELING AND 454 TAG SEQUENCING

Dr. Koji Hamasaki
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Tokyo, Japan

Dr. Koji Hamasaki is an associate professor working with marine microbes especially on their ecology. After he completed PhD in marine microbiology at the Ocean Research Institute, he had a faculty researcher position in biological oceanography at the Soka University in 1995 and then moved to the Hiroshima University in 1997. He also used to working at Azam's lab in the Scripps Institution of Oceanography from 2000 to 2002. He has been appointed the current position since 2005, mainly working on bacterial diversity and community structures in pelagic environments. Akito Taniguchi just completed PhD last year. Koji and Akito have been developing a set of culture-independent methodology to study bacterial growth response in seawater using bromodeoxyuridine incorporation.

Other authors: Akito Taniguchi

WE APPLIED the 454 tag sequencing technology to DNA samples retrieved from actively growing bacterial communities in order to assess contemporaneous growth of diverse bacterial taxa in "rare biosphere" and determine its variability along a north-south transect in the Pacific Ocean. Bromodeoxyuridine (BrdU), halogenated nucleoside that can serve as a thymidine analogue, was used to label bacteria and archea with detectable DNA *de novo* synthesis. The massive tag sequencing technology in combination with BrdU-labeling and immunocapture technique was expected to reveal the extent and variability of bacterial diversity in "active-but-rare" biosphere. Based on the difference in physical and chemical characteristics of seawater and PCR-DGGE banding patterns of bacterial communities, we selected a subset of 4 samples from the Southern Ocean to the equatorial Pacific. The number of reads per bacterial sample ranged from 12398 to 25094 sequences. Total bacterial communities showed c.a. 1400 to 4000 unique V6 tag sequences, whereas 'active' communities showed nearly 1000 to 2300 unique sequences. Chao1 estimation of richness at 3 % difference gave nearly 1000 to 3000 species in the total communities and nearly 630 to 1200 species in the 'active' communities. This study showed distinctive patterns of species diversity and also community structures in active parts of rare biosphere, which gave an insight into understanding how environments selected bacterial taxa. Also a large number of singletons in total communities represented higher abundances in 'active' communities, implying an importance of rare biosphere as either active gene pools or mediators of various elemental cycles.

ZOOPLANKTON METAGENOMICS AND ITS COMPARISON WITH CHAETOGNATHA BARCODE DATA

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Dr. Ryuji Machida is currently 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. Dr. Machida has pioneered applications of community metagenomic analysis for marine zooplankton.

Other authors: Hiroomi Miyamoto, 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 comparison 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.

POSTER SESSION

Population genetic structure and distributional pattern of the pelagic copepod family Scolecitrichidae

Mikiko Kuriyama[1], Ryuji J. Machida[2], Shuhei Nishida[2]. *[1]National Research Institute of Fisheries Sciences. [2]Ocean Research Institute, the University of Tokyo*

Recent genetic analyses are progressively revealing cryptic species within planktonic animals that had been referred to as cosmopolitans or known to have ocean-wide distributions. However, most studies have focused on epipelagic species, hence little is known of the gene flow of populations in the meso- and/or bathypelagic zooplankton. We examined genetic distances of populations of the pelagic-copepod family Scolecitrichidae, collected from the Pacific and Atlantic Oceans. Mitochondrial COI and 12S genes were used as genetic markers. The family Scolecitrichidae is among the most species-rich families in calanoid copepods. Many species of this family, which are widely distributed throughout the world oceans, exhibit specific patterns in their vertical distribution. The mitochondrial DNA analyses showed that the patterns of gene flow between the Oceans varied with species even within a genus. There was a tendency that more widespread species, not only vertically but also horizontally, shows larger genetic distance between populations, though there is ongoing or recent gene flow between the Oceans in most species. As meso- and bathypelagic species usually have wide distribution, it appears that the meso- and bathypelagic species shows larger genetic diversity than the epipelagic species. This result suggests that the distributional range of species is an important factor for the intraspecific genetic divergence.

Taxonomic reexamination of the pelagic chaetognath *Solidosagitta zetesios* (Fowler, 1905) using DNA barcodes and morphology

Hiroomi Miyamoto, Ryuji J. Machida, Shuhei Nishida. *Ocean Research Institute, the University of Tokyo*

Solidosagitta zetesios is widely distributed in the meso- and bathypelagic zones of the oceans. We reexamined the taxonomy of *S. zetesios* on the basis of mitochondrial COI gene (mtCOI) sequences and morphological features. Specimens were collected in the western North Pacific and Atlantic Oceans. Four distinct lineages with robust statistical support were indicated by phylogenetic analysis of the mtCOI sequences. Of the four lineages, the two lineages (A1 and A2) were composed of specimens from Atlantic Ocean, and the others (P1 and P2) were comprised of Pacific specimens, and each linage from the two oceans (P1+A1 and P2+A2) comprised monophyletic lineages. Morphologically the A1+P1 linage is distinguishable from the A2+P2 linage by the position of anterior fins. A1 specimens were further discriminated from those of P1 by color of grasping spines. However, we could not find morphological differences between A2 and P2 specimens. These observations suggest presence of several cryptic species within *S. zetesios* as currently defined.

The mitochondrial genome of the calanoid copepod, *Calanus sinicus* and its application as molecular markers

Chaolun Li. *Key Laboratory of Marine Ecology and Environmental Sciences. Institute of Oceanology, Chinese Academy of Sciences (IOCAS), China*

Sequences from mitochondrial genome are always used as molecular markers to study interspecific/intraspecific diversity and high-level phylogenetic position of the given species. In this study, we sequenced the nearly complete mitochondrial genome of *Calanus sinicus* (Copepoda: Calanoida: Calanidae) to select the suitable genes for molecular markers. Its size is relatively large (>20kb) for crustacean mtDNA due to the presence of many intergenic non-coding sequences (IGNs), 2 of which abutting on ATP6 coding region contained large repeat regions. Within the sequences obtained, 36 genes (13 protein-coding genes, 2 rRNAs and 21 tRNAs) were found. Compared to typical mitochondrial genomes of crustacean, gene order of *C. sinicus* mito-genome is highly rearranged with a novel gene structure. Relative to ITS region in nuclear genome, sequences in mito-genome are more variable. Non-coding region has maximal variation followed by ND4L region. Thereby, the above 2 regions are potential molecular markers for intraspecific genetic diversity research in *C. sinicus*.

Molecular identification and population genetics of a colonial ascidian (*Didemnum vexillum*) in New Zealand

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Determining whether a newly discovered species is non-native or a previously unreported indigenous species is sometimes problematic, particularly if taxonomic assignments are based on highly variable morphological characteristics. Correct identification is critical for a rapid response and prevention of spread if a new species is potentially invasive. In the marine environment new colonizers may go unnoticed particularly if it is morphologically indistinguishable from a native species or lacks obvious morphological characteristics enabling easy identification. Ascidians are common invasive species in marine environments around the world. The ascidian *Didemnum vexillum* was first identified in New Zealand. The species was identified, using morphological characteristics, as *Didemnum vexillum* and declared a native species. Identification of ascidians in the genus *Didemnum* is problematic due to the small sizes of zooids, larvae and spicules of most species, as well as poor preservation techniques and inadequate sampling. This work describes the use of the mitochondrial gene, cytochrome oxidase 1 (mtCO1), for the unambiguous identification of the *D. vexillum* in New Zealand. Molecular phylogenetic analyses placed populations of *D. vexillum* in New Zealand in the same clade as other populations around the world and confirmed that *D. vexillum* should be classified as a non-indigenous species in New Zealand. This interpretation receives support from the relatively low mtCO1 haplotype diversity amongst *D. vexillum* in New Zealand indicating a 'genetic bottleneck' possibly associated with recent colonization. These findings have significant implications for any efforts to mitigate negative affects that *D. vexillum* may have on the New Zealand aquaculture industry.