Archives of long-term deep seafloor video image offshore of Cape Muroto in Nankai Trough

*Ryoichi Iwase[1]

1. National Research and Development Agency, Japan Agency for Marine-Earth Science and Technology

Offshore of Cape Muroto in Nankai Trough at a depth of 3572 m the multidisciplinary cable-end station of the cabled observatory was deployed in 1997. The cable-end station was located at the cold seepage site where several vesicomyid clams existed. It was composed of a video camera, a CTD (Conductivity, Temperature and Depth of sea water) sensor, sub-bottom thermometer, an electro-magnetic current meter and an ADCP (Acoustic Doppler Current Profiler). Visual observation of seafloor with the video camera was carried out from April 1997 to January 2003 before the breakdown of all of the lights and the video camera. The outline of the visual observation was already introduced in the video "LIFE IN THE ABYSS - 4 Year Observations in Nankai Trough, Japan" (Iwasaki and Iwai, Tokyo Cinema, inc., 2002). The observed video images were recorded on 800 S-VHS videotapes. This time, all of the videotapes were digitized and were converted to MPEG-2 format files.

The video camera had pan and tilt control. All through the observation period, a specific place, where two vesicomyid calms existed in most of the period, has been focused as a home position. Although the condition of the video image was not same because of the successive fault of the lights, the long-term change of two clams has been viewed. One of the clams died in May 2001, and the other clam moved out of the sight in August 2001. Periodic appearance and disappearance of bacteria mat beside one of the clams could be seen in the early stage of the observation period.

Although online providing of those MPEG-2 video image files is not planned at present, offline providing could be possible in near future. Contribution to the research on chemo-synthetic ecosystem is expected.

Keywords: offshore of Cape Muroto in Nankai Trough, long-term visual observation, archives of videos

Revision of taxonomy of barnacles in deep-sea chemosynthetic environments

*Hiromi Kayama WATANABE[1], Benny K. K. Chan[2]

1. Japan Agency for Marine-Earth Science and Technology, 2. Academia Sinica, Taiwan

Barnacle is one of the abundant animals associated with deep-sea hydrothermal vent in the northwestern Pacific, Indian and Southern Oceans. Currently each of four suborders of thoracican barnacles is reported from deep-sea hydrothermal vent fields, although recent molecular phylogenetic analyses on the barnacles in deep-sea chemosynthetic environments provided new insights into barnacle phylogeny. Molecular phylogenetic analyses showed; 1) molecular phylogeny of the stalked eolepadid barnacles was not correlated to genus-level taxonomy, 2) hydrothermal vent barnacles of genus Neoverruca is not belong to the lineage of suborder Verrucomorpha, and 3) the solitary brachylepadomorph, *Neobrachylepas relica* is not consist a monophyletic clade, but consist a part of scalpellomorph, as same as the genus *Neoverruca*. Shell morphology, that is important morphological characters of barnacles, is not always appropriate characters for taxanomy, because it is variable according to density of the population.

Keywords: Biodiversity, morphology, molecular phylogeny

Meta-transcriptomic analysis unveils the symbiotic relationship between methane seep tubeworm and its symbiont

*Yi YANG[1], Jin Sun[1], Ting Xu[2], Jian-Wen Qiu[2], Pei-Yuan Qian[1]

1. Hong Kong University of Science and Technology , 2. Hong Kong Baptist University

Deep-sea chemosynthetic ecosystems are known for abundant reduced chemicals and thus support extensive megafauna including tubeworms (Annelida, Siboglinidae) which are mouthless and gutless and reliant on their symbiotic sulphide-oxidizing bacteria (SOB) as an energy and nutrient source. Most studies of chemosynthetic tubeworms have focused on *Riftia pachyptila* and *Lamellibrachia* spp., while other tubeworms have received little attention. We analyzed the meta-transcriptome of *Paraescarpia echinospica* collected from a newly discovered methane seep in the South China Sea to understand the molecular mechanisms of symbiosis between the tubeworm and its symbionts by sequencing three tissues, i.e. plume (a gill-like organ), vestimentum and trophosome (the organ that harbors the symbionts). The transcriptome sequences were assembled, annotated, and species sorted. The bacterial transcripts were involved in chemoautotrophy activities which were characteristics of SOB, such as sulphide oxidation, ion-regulation, and sulfurtransferase activity, etc. Host genes that were highly expressed in trophosome were represented by chitin metabolism, oxygen transport, hemoglobin complex and cell adhesion. These results suggest mutual benefits between *P. echinospica* and its symbionts. Our study has revealed the importance of thioautotrophy regulatory pathways in the symbionts, the dominant regulatory pathways in the host, and demonstrated the utility of meta-transcriptome sequencing in unveiling the relationship between deep-sea invertebrate hosts and their uncultured symbionts.

Keywords: Deep-sea, Chemosynthetic, Tubeworm, Symbionts

Paleoecology of serpulid worm tubes in the Late Cretaceous Sada Limestone seep deposits in southwestern Shikoku, Japan

*Takami Nobuhara[1], Kayoko Matsumura[1], Yasuo Kondo[2], Toyoho Ishimura[3], Akira Ijiri[4], Urumu Tsunogai[5], Olev Vinn[6], Steffen Kiel[7], Crispin Little[8]

1. Science Education (Geology), Faculty of Education, Shizuoka University, 2. Science Unit, Natural Sciences Cluster, Kochi University, 3. Department of Chemistry and Material Engineering, National Institute of Technology, Ibaraki College, 4. Japan Agency for Marine-Earth Science and Technology, 5. Graduate School of Environmental Studies, Nagoya University, 6. Department of Geology, University of Tartu, Estonia, 7. Department of Palaeobiology, Swedish Museum of Natural History, 8. School of Earth and Environment, University of Leeds

The Sada Limestone consists of a group of seep carbonate deposits enclosed in the Late Cretaceous slope mudstone facies of the Northern Shimanto Belt, Shikoku, SW Japan. The seep deposits are characterized by mass occurrence of large-sized chemosynthetic thyasirid bivalves and serpulid worm tubes, but the taxonomy and paleoecology of the worm tubes has not yet been fully examined. We report tube shell characters, lithology of the surrounding sediments, and the detail mode of fossil mass occurrences.

The tube fossils are slightly curved with circular cross section, 2.11 to 8.00 mm in diameter, over 10 cm in length, often attached to each other. The outer surface is ornamented with straight or corrugated growth lines and perpendicular ridges. The attached part forms a widened base with single thick keel or three keels. The tubes lumen contains few to numerous irregularly spaced septae, concave in cross section. The tube wall is moderately thick, and consists of a thin inner layer and a thick outer layer with chevron growth pattern, which suggests the worm tube to be assigned to serpulids. The inner layers are composed of two organic(?) sheets binding carbonaceous fill. The septae also have similar structure to inner layers, but sometimes form multiple stacking, between which elliptical pellets were often sealed. The inner layers and septae were under flexible deformation and delamination during calcification in early diagenesis. Such delamination has been often confirmed in other tubes having organic walls in several seep deposits.

The tubes occur in muddy micrite, sometimes mixed with thyasirid fossils, but the exclusive mass occurrence of tubes is lithorogically characterized with rich sparitic fabrics enclosing abundant undeformed pelloids. The δ^{13}C values of the micritic matrices are not so depleted (-10 to -15 ‰). The mass of tubes forms beds and mounds, over 1 m thick, composed of well-preserved tubes standing vertically to beddings in matrix-supported condition. Such autochthonous tube concentration directly covers turbiditic sandy layers rich in shell fragments, indicating that abundant serpulids simultaneously settled by attaching to shell fragments or coarse clastics just after the physical disturbance. Top of the mass occurrences is often truncated by sandy flow deposits, which broke and pushed down upper parts of the tube individuals. Thin sandy layers also sometimes abut tube-cluster mounds, which maybe formed slight topographic rises.

On the basis of the mode of fossil occurrence noted above, the Sada serpulids were opportunistic and semi-infaunal sessiles, forming colonial mounds in seep site. Abundant pellets sealing between the septae suggest the possession of digestive system, and suspension feeding maybe on high production of chemosynthetic seep ecosystem.

Keywords: chemosynthesis, cold seep, worm tube, Serpulidae, Shimanto Belt

Population genetic connectivity and natural selection in deep-sea mussels: insights from mitochondrial genes and genome-wide SNP markers

*Ting Xu[1], Jin Sun[2], Hiromi Kayama WATANABE[3], Chong Chen[3], Masako Nakamura[4], Pei-Yuan Qian[2], Jian-Wen Qiu[1]

1. Department of Biology, Hong Kong Baptist University, 2. Division of Life Science, The Hong Kong University of Science and Technology, 3. Japan Agency for Marine-Earth Science and Technology, 4. School of Marine Science and Technology, Tokai University

Understanding the genetic connectivity among hydrothermal vent and methane seep ecosystems can help to develop informed-management for these deep-sea resources. Nevertheless, little is known about the genetic connectivity of these special chemosynthesis-based ecosystems in the Western Pacific Province. Here, we applied a combination of three mitochondrial genes (i.e., *cox1*, *nad4* and *atp6*) and 7227 genome-wide SNPs identified based on restriction site-associated DNA genotyping-by-sequencing (RAD-seq) approach, to study the genetic connectivity of seven *Bathymodiolus platifrons* populations totaling 130 individuals in the Northwestern Pacific. These populations are distributed over a distance of 3486 km, and are between 858 m and 1482 m in water depth. Among them, two populations in the South China Sea (SCS) and one in Sagami Bay inhabit methane seeps, and four populations in Okinawa Trough inhabit vent fields. The mitochondrial markers showed that these populations were clustered into two genetic groups with one represented by the South Hainan (SH) population and another represented by the rest. In contrast, the genome-wide SNP markers showed that these populations were clustered into three distinct groups represented by the SH, the Jiaolong Ridge (JR) and the Okinawa Trough-Sagami Bay (OT-SB) populations. This pattern of genetic divergence revealed the importance of the Kuroshio Current and its intrusion into the SCS from the Luzon strait, rather than the water depth or types of habitat (vents vs. seeps), in shaping the genetic structure of this deep-sea mussel with a long pelagic larval period. Furthermore, an analysis of the SNP outliers detected 162 genes under natural selection, including genes with detoxification, endocytosis and apoptosis functions. Overall, our study has revealed the key role of the Kuroshio Current in maintaining the high genetic connectivity of the deep-sea mussel populations along its main course, the importance of the SCS as a marginal sea in promoting genetic divergence of deep-sea mussels, and highlighted the potential of high-throughput SNP discovery in resolving the fine genetic structure of deep-sea invertebrates with long-distance larval dispersal capabilities.

Keywords: Genetic connectivity, natural selection, hydrothermal vent, methane seep, Bathymodiolus, RAD-seq

Distribution pattern of minerals and iron-oxides in hydrothermal vent shrimp *Rimicaris* with special focus on its gut contents

*Kenta Kimura[2], Robert Jenkins[1], Hiromi Kayama WATANABE[3]

1. School of Natural System, College of Science and Engineering, Kanazawa University, 2. Graduate School of Natural Science and Technology, Kanazawa University, 3. JAMSTEC

The vent shrimp *Rimicaris*, dominated at many vent sites along the Mid-Atlantic Ridge and the Central Indian Ridge, harbors episymbiotic bacteria in their gill chamber. Because of those microbes, surface of the *Rimicaris* in gill chamber is fully covered by iron-oxides, and thus the *Rimicaris* has been considered one of the animals which do bioaccumulation of metals. In contrast to their gill chamber, however, gut contents haven’ t been investigated well. Here, we examined distributions of iron-oxides and minerals in digestive tract and in gill chamber of the *Rimicaris kairei* from the Edmond hydrothermal vent field on Central Indian Ridge.
We found Barite ($BaSO_4$), Sphalerite (ZnS), Chalcopyrite ($CuFeS_2$), Pyrite (FeS_2), Marcasite (FeS_2), Argentite (Ag_2S) and Hematite (Fe_2O_3) on the surface and in the gut of the *Rimicaris.* Nano- to micron sized iron-oxides were dominated in everywhere we examined, i.e. the surface, mouth part, stomach and gut. Relative amount of the iron-oxides largely increased between mouth and gut. In contrast, sulfide minerals decreased between them. Maximum diameters of those mineral particles drastically decreased through the digestive tract, especially at the mouthpart and the stomach. Those features suggest that the *Rimicaris* crushes the mineral particles at the mouthpart and within the stomach, and the sulfide minerals are consumed and/or deserted at the stomach.

Keywords: Hydrothermal vent, bioaccumulation, ore deposit, macrofauna

Biomineralization Toolkit in Deep-Sea Mussels: Insights from the Mantle Transcriptome and Shell Matrix Proteome of *Bathymodiolus platifrons*

*Jin Sun[1], Ting Xu[2], Pei-Yuan Qian[1], Jian-Wen Qiu[2]

1. Hong Kong University of Science and Technology, 2. Hong Kong Baptist University

As calcium carbonate is more soluble at lower temperature and higher pressure, understanding how deep-sea mollusks form their shells through biomineralization can reveal their adaptation to the deep-sea environment. Shell matrix proteins (SMPs) are known to play a key role in the shell formation of shallow water mollusks but so far there is no report of the composition and roles of SMPs in shell formation of deep-sea mollusks. In the present study, we analyzed the mantle transcriptome and shell matrix proteome of the deep-sea mussel *Bathymodiolus platifrons*. GO enrichment analysis of the highly expressed genes of the mantle revealed the enrichment of genes that are related to ion transportation and extracellular regions, indicating that the mantle was active in biomineralization. Nineteen SMPs were identified and their methiothine residues were exclusively oxidized. Among these identified SMPs, homologs of the 2 Chitin-bd-like proteins, 1 Perlwapin-like and 2 MSI60-like proteins have been reported in the shallow-water mussels *Mytilus* spp.. The 19 SMPs also included 3 with homology to proteins from non-mytilid bivalves, 1 from a gastropod, 1 from a nematode, and 9 novel proteins. Protein functional domain analysis showed that domain shuffling and *de novo* generation could be the major mechanisms for the evolution of SMPs in deep-sea mussels.

Keywords: deep-sea, bivalves, shell matrix protein, evolution, proteomics

Relationship between various elements in shells and crystal form of $CaCO_3$ constituting inside seashell All over Japan

*Chihiro Takayama[1], Mana Yasui[2], Hiroshi Hagiya[1]

1. Tokyo City University, 2. Waseda University

Seashell and coral contains mainly inorganic substances are consisted by calcium carbonate and partially organic substances.The seashell consists various combination of Calcite and Aragonite.
In this study, seashells collected on the coast of 10 areas in Japan have analyzed the crystal structure and the elements concentration.
And inorganic elements [Ca,Na,P,Si,Sr,Zr,Y,Rb,S] concentration of seashells are considered from relationship between the crystal structure and each element concentration.
Calcites contain more P and S than Aragonites, Aragonites contain more Sr and Zr than Calcites
There is a strong correlation between Sr and Zr concentration in the seashell.
Also Corals and Barnacles, contain more Sr and Zr than seashells sample.
Zr element is concentrated to 10^6 times the concentration of seawater.
Y element is also concentrated to 10^5 times the concentration of seawater. But Y element isn’t correlation the other elements.
It is considered that there are some unknown reason in biomineraligation of seashell.

Keywords: biomineral, calcite, aragonite, element

Boundary of Sakamotozawa formation, Nagaiwa formation and Kanokura formation in a part of Hikoroiti district

*Karin Taira[1], Mana Yasui[2], Hiroshi Hagiya[1]

1. Tokyo City University, 2. Waseda University

Sakamotozawa formation in a part of Hikoroiti district in Ofunato city, Iwate prefecture consists of basal conglomerate, slate rock, limestone and sandstone in the Permian early period.
Sakamotozawa formation is in contact with clino-unconformity of Nagaiwa formation(Mid Carboniferous) and conformity of Kanokura formation(Middle Permian).
In this research, to add a new interpretation to the previous research, we decided more accurate boundary of Nagaiwa, Sakamotozawa and Kanokura formation, and conducted a survey.
As a result, the sandstones in some areas considered to be Nagaiwa and Kanokura formation showed the very similar Ti-Nb ratio to sandstones of Sakamotozawa formation.
In addition, we measured undissolved residue of the muddy limestone from Sakamotozawa formation then it was identified that the amount of Cr·Ni contained in samples are particularly rich rather than the general sandstones of Sakamotozawa formation.
This suggests that there was a provenance which abundant Cr-Ni existed like serpentintite during sedimentation of limestone.

Keywords: limestone, Sakamotozawa formation, Nagaiwa formation, Kanokura formation, Ofunato