

Geology and biology of the Shinkai Seep Field in the Southern Mariana Forearc

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The Shinkai Seep Field (SSF), located in the inner trench slope of the southern Mariana Trench, ~80 km northeast of the Challenger Deep, is a serpentinite-hosted ecosystem mainly consisted of vesicomid clams. Although vesicomid clams are among the dominant invertebrates of chemosynthesis-based communities found principally at methane cold seeps derived from sediment diagenesis (such as at the Japan Trench, Nakai Trough, and Sagami Trough) and high-temperature hydrothermal vents (such as at the Galapagos Rift and Okinawa Trough), there have been no live examples from a serpentinite-hosted hydrothermal system including serpentinite mud volcanoes.

The SSF was serendipitously discovered by a Shinkai 6500 dive to map the mantle peridotite in the southern Mariana forearc, during YK10-12 cruise of R/V Yokosuka in September 2010. Although the dive was successful in collecting mantle peridotites and vesicomid clams, no water and sediments were collected. TN273 cruise of R/V Thomas G. Thompson in January 2012 performed Deep-towed IMI-30 sonar backscatter imaging. The result indicates that the SSF is associated with a small, low backscatter feature that may be a small mound. Such low backscatter features can be widespread in the mapped area.

In order to understand the SSF, YK13-08 cruise had the following objectives:

- (1) Finding and locating active fluid venting in the SSF. If successful, sampling the vent fluid and associated sediment for chemical and microbiological study.
- (2) Finding seep fields other than the SSF in the southern Mariana forearc, using the low backscatter feature on IMI-30 image as a guide.
- (3) Comprehensive understanding of the geology of the SSF. It is important to understand the geological background of the SSF including tectonic development of the southern Mariana forearc.

During YK13-08 cruise, Shinkai dives 1362, 1365 and 1366 successfully revisited the SSF, obtaining core samples for investigation of faunal composition, microbial and geochemical analyses in sediments, Niskin and pressure-tight water samples for geochemical analyses, and discovering chimneys. Shinkai dives 1363 and 1364 investigated the landward slope of the southern Mariana Trench ~7 km west of the SSF, revealing that the mapped slope is entirely consisted of serpentinitized harzburgites. New seep fields were not discovered during the cruise, indicating that not all low backscatter features on IMI-30 image correspond to seep fields. In this talk, we will show the preliminary results of YK13-08 cruise and discuss the geology and biology of the SSF.

Keywords: chemosynthetic community, serpentinite, Shinkai Seep Field

Deep-sea hydrothermal vent fauna on the Central Indian Ridge

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In deep-sea hydrothermal vent fields, faunal distribution is associated with the geochemical environments generated by hydrothermal vent activity. Hydrothermal vent fields on the Central Indian Ridge (CIR) are associated with vent fauna which is a mixture of Atlantic and Pacific and are discretely distributed along the ridge axis of more than 1000 km apart. In this presentation, faunal distribution in hydrothermal vent fields on the CIR is summarized at the intra- and inter-field levels. The species composition of the vent fauna in the four vent fields hitherto known is reviewed and updated, and faunal resemblance among the four vent fields of the CIR appears to reflect the number of species recorded, indicating that faunal surveys are not sufficient in describing the total vent fauna on the CIR. All the genetic studies of the CIR vent fauna have indicated a high genetic connectivity among the local populations, despite the many potential dispersal barriers existing between the vent fields. On the basis of the spatial distribution of vent species in a vent field, typical vent fields on the CIR were classified into six zones, of which the central two zones are often covered by *Rimicaris* swarms in the Kairei and Edmond fields. The close relationship between vent fauna from the CIR and the western Pacific, compared to those from other regions, is highlighted. Knowledge of the Indian Ocean vent fauna is limited, and further quantitative information on the biodiversity of vent fauna will provide clues to the formation of biogeographical regions and the dispersal of vent fauna among deep-sea hydrothermal vent fields.

Keywords: chemosynthetic biological community, biogeography, faunal similarity

A trial on evaluating hydrothermal system evolution using geochronological dating and biological diversity analyses

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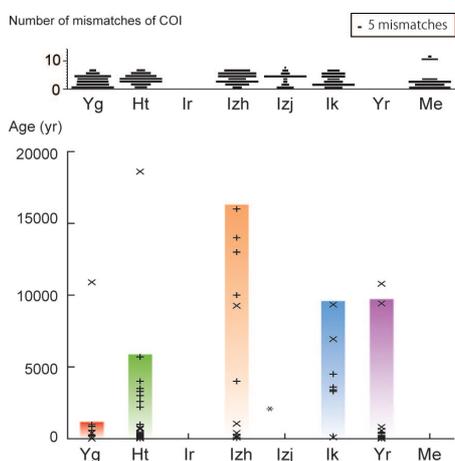
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To elucidate the evolution of hydrothermal activities, we conducted an interdisciplinary study including geochemistry and biology to develop a method of obtaining reliable age information. Because there was a small amount of constraint on the lifetime of activity at hydrothermal sites, this study is one of the principal goals of the TAIGA-project, "Trans-crustal Advection and In-situ biogeochemical processes of Global sub-seafloor Aquifer" funded by a Grant-in-Aid for Scientific Research on Innovative Areas. As geochemical dating techniques, two methods applicable for hydrothermal ore minerals were developed and improved to fill the gap of the time-ranges in the conventional dating methods: electron spin resonance method and uranium-thorium disequilibrium method. Cross checks between the two methods generally showed good agreement for the range of hundreds to thousands of years. Except for the extreme values for each hydrothermal site, geochemical ages exceed 9ka for the southern Mariana Trough and for 16ka for the Okinawa Trough, respectively. As biological analysis, the biodiversity among faunal communities in the targeted areas was analyzed at the species and DNA levels. In the southern Mariana Trough, *Alviniconcha* gastropods and *Neoverruca* barnacles clearly show the greater genetic diversity with greater distances from the ridge axis, which fairly corresponds to the geochemical ages for ore minerals. In the Okinawa Trough, *Bathymacrea* limpet showed greater genetic diversity at the Hakurei site in the Izena Hole where the ore minerals show oldest ages among the studied sites (Fig.).

Species and genetic diversity of the local fauna were not always correlated to geochemical dating, either in the southern Mariana Trough region or in the Okinawa Trough region. Although the results are not simple, comparison of age information obtained from analyses of these two disciplines potentially provides important constraints for discussion of the history and evolution of hydrothermal activities.

Figure caption (upper): Genetic divergence of COI gene indicated as mismatches in base sequences of *Bathymacrea secunda* limpet of the Okinawa Trough. Scale bars are shown as five mismatches of partial COI sequences. (lower) Geochemical age range determined from the sulfide and sulfate deposits in Okinawa Trough. Active sites are shown from approximately SW to NE. The left-hand side is the southwestern end. Colored bars represent reliable age ranges for respective sites. The localities are denoted as follows: Yg, Daiyon-Yonaguni Knoll; Ht, Hatoma Knoll; Ir, Irabu Knoll; Izh, Hakurei-site in Izena Hole; Izj, JADE-site in Izena Hole; Ik, Iheya North Knoll; Yr, Yoron Hole; Me, Minami-Ensei Knoll.

Keywords: geochronology, biodiversity, TAIGA-project, ESR, U-Th disequilibrium, mitochondrial mismatch analysis



Chemosynthesis-based ecosystem discovered on a Cretaceous sea turtles from Japan

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One of the basic types of chemosynthetic ecosystems is known to develop on vertebrate carcasses. Within the framework of efforts to trace the evolution of chemosynthetic animals thriving in the modern vents and seeps, it has been hypothesized that these chemosynthetic animals adapted to the vent and seep environments via the transient environment formed by the decomposition of bones of vertebrate animals (e.g. Distel et al., 2000). Thus a study of the geological record of chemosynthetic ecosystems on vertebrate carcasses became of increasing importance in understanding the evolution of chemosynthetic animals. However, such studies were not fully assessed so far. Kaim et al. (2008) reported the existence of chemosynthetic ecosystems on plesiosaurid carcasses, marine reptiles which flourished in the Cretaceous oceans. However, we still were uncertain whether any other marine reptile carcasses could support chemosynthetic ecosystems. Here we document the first chemosynthetic community found on carcasses of the Cretaceous sea turtles.

The fossil sea turtle (*Mesodermochelys* sp.) has been collected from the Upper Cretaceous Campanian deposits cropping out along the Nio River, Nakagawa Town, Hokkaido. Sediments surrounding the turtle yielded provannid gastropods and thyasirid bivalves, both known to be members of chemosynthetic communities. Those chemosynthetic molluscan fossils have also been found in Cretaceous hydrocarbon seeps and on plesiosaurid carcasses (Kaim et al., 2008; 2009; Kiel et al., 2008).

This finding indicates that the chemosynthetic communities were supported not only by plesiosaurid carcasses but also by decomposing sea turtles. The sea turtles are a rare example of Cretaceous marine reptiles surviving the Cretaceous/Paleocene extinction event. Thus, it is reasonable to assume that sea turtle carcasses could continuously support chemosynthetic ecosystems linking the Mesozoic reptile fall communities with Cenozoic and modern whale fall communities, the latter occurring in the fossil record not earlier than Eocene.

A chemosynthetic community on plesiosaurid carcass: with focus on distributions of microbes and invertebrate fossils

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Chemosynthesis - based communities are known to have been established not only in hydrocarbon seeps and/or hydrothermal vents but also on Cretaceous plesiosaurid carcasses (Kaim et al., 2008a). However, no detailed reconstruction of chemosynthetic ecosystems on plesiosaurid carcasses has yet been undertaken. To reconstruct the detailed development of ecosystems, we examined distribution patterns of chemosynthetic molluscs and micro- and macroborings around/on a plesiosaurid carcass. The examined carcass derived from a Cretaceous marine deposit distributed in Haboro Town, Hokkaido, and thought to have perhaps supported chemosynthetic ecosystems (Kaim et al. 2008a).

We observed the surface and a cross section of the plesiosaurid specimen. Chemosynthetic gastropods (Abyssochrysoidea) were densely distributed around the plesiosaurid bones (especially on the upper side). Several types of borings (e.g. micron-sized filamentous microborings and rounded boring holes with apertures) could be found on the plesiosaurid bones. On the basis of their genera shapes and juxtaposition to pyrites, we hypothesize that the filamentous borings might have been formed by sulfur-oxidizing bacteria. The rounded boring holes with apertures within the bones are similar to modern borings made by *Osedax*.

The borings were distributed on the upper side of the bones relative to the lower side, resembling the distribution pattern of chemosynthetic gastropods. Most Recent abyssochrysoid gastropods are known to graze bacterial mats. The coherent distribution patterns of abyssochrysoid gastropods and microborings on the plesiosaurid bones indicate that the gastropods grazed bacterial mats even in the Cretaceous age. In addition, bone-eating animals also accumulated on the upper side of the bones. These distribution patterns might be influenced by the difference in exposure duration times of the upper and lower bone surfaces (upper side exposed on sea floor for a longer time than the lower side due to continuous sedimentation).

Keywords: Reptile fall, Plesiosauridae, distribution patterns, borings, chemosynthetic molluscs

Paleoecology of the Upper Cretaceous echinoderms from cold seep carbonates in South Dakota, USA

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Echinoderms were thought to be rare in a cold seep environment and had not been considered as a member of the chemosynthetic community until recent years, whereas the chemosynthesis community consists of a variety of other taxa. In the last 10 years, some species of echinoderms have been reported as a member of the modern chemosynthetic community, and some fossil echinoderms have also been found from or near carbonate mounds associated with cold seep. However the taxonomic and paleoecologic studies about these echinoderms have not been sufficiently done, and the ecologic relationship between these echinoderms and cold seeps has been also unsolved. The purposes of this study is to discuss paleoecology and process of adaptive evolution of echinoderms associated with a cold seep environment found from the Upper Campanian Pierre Shale in South Dakota, by field surveys, taxonomic of and morphological observation of fossil echinoderms. Chemical analyses of fossil echinoderm skeletons were also conducted, including element analysis for estimating the degree of diagenesis, and stable carbon isotopes analysis for clarifying the degree of relation between the echinoderms and the seep hydrocarbon.

As a result of field surveys, it is proved that the diversity of fossil species from carbonate mounds associated with cold seeps is different among mounds, even between adjacent mounds. Such a difference of species diversity is considered to reflect the difference of environments during the time when the carbonates were formed. It is presumed that the carbonate mounds with high diversity were exposed on the sea floor for a long time and provided a suitable environment for epifauna such as many echinoderms. Fossil crinoid from seep carbonates has low values of $\delta^{13}\text{C}$ (-20 ‰ or less). These values are considerably lower than modern crinoids which inhabit non-seep environments, and are also lower than the values of other fossil echinoderms from seep carbonates of the Pierre Shale. The crinoid from seep carbonates also has very strange, characteristic morphology, not seen in other stalked crinoids. Considering these chemical and morphological data, the crinoid from seep carbonates had probably adapted to the environments of cold seeps. On the other hand, echinoids from cold seeps do not have low values of $\delta^{13}\text{C}$, and morphologically they are not significantly different from those found from non-seep environments. Therefore, it is considered that the echinoids from seep carbonates are not regarded as a true member of chemosynthetic community, but they came into cold seeps to benefit irregular, hard substrate to live on, or to obtain ample food sources from this cold seep environment.

The degrees of adaptation to cold seeps are therefore different among echinoderm species.

Keywords: cold seep, echinoderms, paleoecology, chemosynthetic community

Molecular phylogenetic evidence for host switching in chemoautotrophic symbionts of deep-sea *Calyptogena* clams

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Calyptogena clams are living in deep-sea chemosynthetic habitats and globally distributed in seeps and hydrothermal vents. They are nutritionally dependent on chemoautotrophic sulfur oxidizing bacteria, which are harbored within their gill epithelial cells. The *Calyptogena* symbionts are thought to be vertically transmitted via clam's egg to the next generation. Both host and symbiont are thought to coevolve, because topologies of the phylogenetic trees of them form a mirror image. However, their phylogenetic trees have not been robust enough for analyzing their coevolutional relationship, because of using partial gene sequences of host (mitochondrial *cox1* and *rrnL* genes) and symbiont (16S rRNA gene). The possibility of lateral acquisition of the symbiont has been reported in some *Calyptogena* lineages. To improve the phylogenetic trees of *Calyptogena* clams and of symbiont, we sequenced the mitochondrial genomes of *Calyptogena* clams, and several their symbiont genes, and analyzed the phylogenetic trees by using the concatenated sequences.

Mitochondrial genomes of *C. phaseoliformis*, *C. okutanii* and *C. fossajaponica* were sequenced. Based on these mitochondrial genome sequences, primer sets for PCR of mitochondrial genes of other *Calyptogena* clams were designed. Using them, 11 mitochondrial genes (*cox1*, *cox2*, *cox3*, *nad1*, *nad3*, *nad4*, *nad5*, *cytb*, *atp6*, *atp8* and *rrnL*) of other 8 *Calyptogena* species (*C. fausta*, *C. kawamurai*, *C. kilmeri*, *C. laubieri*, *C. nautilei*, *C. pacifica*, *C. soyoae*, *C. stearnsii*) were amplified by PCR and sequenced. Eight genes (16S rRNA, 23S rRNA, *uvrA*, *uvrD*, *mfD*, *groEL*, *groES* and *gyrB*) of symbionts of these *Calyptogena* clams were also sequenced. Phylogenetic trees of clams and symbionts were constructed by maximum likelihood and bayesian analysis based on concatenated 11 mitochondrial and 8 symbionts genes, respectively.

The reliabilities of phylogenetic trees of the hosts and their symbionts were significantly improved by using the concatenated genes sequences (Fig.1). Bootstrap values and posterior probabilities of internal nodes were better supported than those of the previous phylogenetic trees using partial gene sequences. Topological congruence of host and symbiont that was supported by bootstrap value (100%) and posterior probabilities (1.0), was shown in *C. okutanii*, *C. soyoae*, *C. kilmeri*, *C. pacifica* and *C. fausta*. These results suggested that these symbionts were cospeciated with their host clams (green boxes in Fig.1). Although the topologies of host and symbiont were congruent with *C. fossajaponica* and *C. phaseoliformis*, there were the low bootstrap values and low posterior probabilities in the host clade.

Topological incongruence between host and symbiont trees was shown in *C. kawamurai* - *C. laubieri* clade and *C. nautilei* - *C. stearnsii* clades (Fig.1) Congruence of topologies was rejected by approximately unbiased test using sitewise log-likelihoods (red branches in Fig.1). This result suggested that these symbionts have not cospeciated with their host clams. Host switching of the symbionts in the clades of *C. kawamurai* - *C. laubieri* and *C. nautilei* - *C. stearnsii* were examined by coevolution software, which compared the topologies of host and symbiont. Host switching is the event that symbiont is transferred from a host to a new host in a different lineage during speciation. The host switching of symbiont between *C. kawamurai* and *C. laubieri* was suggested by this software. Moreover, both clams are living in different depths of the same area (blue box on Fig.1). However, this software did not suggest the host switching of symbionts between *C. nautilei* and *C. stearnsii*. They are living in different areas. In this study, we show the phylogenetic relationships of cospeciation and non-cospeciation species with the symbionts among examined 11 *Calyptogena* species. It was suggested that topological incongruence of host and symbiont trees in clade of *C. kawamurai* - *C. laubieri* may be due to the host switching

Keywords: symbiosis, deep-sea *Calyptogena* clams, coevolution, host switching

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Time:April 29 11:00-11:15

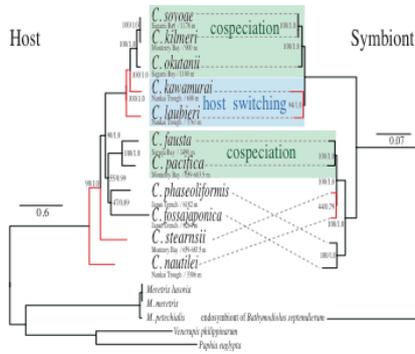


Fig1. cospeciation and host swithing on *Calyptogenia* clam and symbiont trees.
 Numbers in the nodes correspond to maximum likelihood bootstrap values and posterior probabilities.
 Reg branches correspond to topological congruence rejected by approximately unbiased test using
 sitewise log-likelihoods.

Two forms of *Calyptogena (Ectenagena) nautilei* recognized in shell morphologies

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Calyptogena (Ectenagena) nautilei was originally described by Okutani and Métivier (1986) from the cold seep sites in the Tenryu Canyon at the Nankai Trough based on six living specimens. After that, this species has been reported from the continental slope off Kumano, the Daiichi-Minami-Muroto Knoll, Zenisu Ridge, and Shionomisaki Canyon in the Nankai Trough (Fujikura et al., 2000; Okutani et al., 2002; Kojima et al., 2004; Anma et al., 2010). Okutani et al. (2002) examined the species from the Tenryu Canyon, the continental slope off Kumano, and the Daiichi-Minami-Muroto Knoll, and described that the species had a great variety in the shell outline. We observed the shell morphologies and structures of *C. (E.) nautilei* from some localities above including the type materials, and concluded that this species can be divided into two forms (form 1 and 2) by the shell morphologies and the shell structure.

We examined three type specimens from the Tenryu Canyon (Nautile Dive KD-3 and KD-5: Holotype, MNHN 26983, Paratype, MNHN 26984, Paratype, MNHN 26985), four specimens from the continental slope off Kumano (Shinkai 6500 Dive 615), five specimens from the Shionomisaki Canyon (Shinkai 6500 Dive 889, 890, and 891), and eight specimens from the Daiichi-Minami-Muroto Knoll (KAIKO Dive 189, 192, and 193). All specimens were observed with an optical microscope and bare eyes, and two specimens from the Shionomisaki Canyon were observed with a scanning electron microscope in the shell surfaces and cross sections.

The specimens from the Tenryu Canyon are assigned to form 1, and the other specimens are assigned to form 2. Two forms are most easily distinguished in the shell inner surface characters. That is, form 1 has smooth inner surface whereas form 2 is ornamented nearly overall the surface in hole-like structures that consist of about 61-548 μm in diameter. In addition to the inner surface ornamentation, form 1 is distinguished from the form 2 in having a subumbonal pit in the hinge plate of both valves, anterior ramus of right subumbonal cardinal tooth, and pallial sinus.

Keywords: Vesicomidae, *Calyptogena (Ectenagena) nautilei*, Shell morphology, Hole-like structure

Archives of long-term deep seafloor videos at chemo-synthetic biological community off Hatsushima Island in Sagami Bay

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More than 20 years of multidisciplinary long-term observation, including visual observation, has been carried out with a cabled observatory on deep seafloor at a depth of 1175 m off Hatsushima Island in Sagami Bay since the first deployment in 1993, experiencing entire replacement for upgrade in 2000. The observatory was installed at a cold seepage site where large chemo-synthetic biological communities mainly consisted of vesicomyid clams exist. The observatory is composed of several kinds of sensors, including video cameras, a hydrophone, CTD sensor and seismometer in order to observe biological phenomena visually and also to investigate environmental fluctuation on deep seafloor.

All those data obtained with the underwater unit are transmitted through a submarine cable to the shore station in Hatsushima Island. The video signal was recorded on S-VHS videotape before the replacement of the observatory in 2000 and mainly on DVCAM videotape after the replacement, both with acoustic signal obtained with a hydrophone on soundtrack as audible sound. The shore station is usually uninhabited, and daily visual monitoring of seafloor, 30 minutes a day before the replacement and 26 minutes a day after the replacement, has been performed automatically. The videotape has been replaced once a week on the day when manual observation is performed usually for 6 hours. As for lighting, six halogen lights were attached at first and two of them were turned on simultaneously by turn for usual observation considering lifetimes. However, most of those lights were broken by 2008 and since then an LED light is used which is darker but has longer lifetime than the halogen lights, resulting narrow view.

Although visual observation has been performed about ten hours a week, more than 20 year observation produced thousands of videotapes. Archiving those videotapes becomes important because they degrade over time and the devices to replay them are going out of production and the opportunities to utilize them are being lost.

Meanwhile, vocalizations of sperm whales were found in the acoustic signal recorded on the soundtrack of the videotapes and, in order to utilize them as one of the *in situ* data for the remote species identification, archiving the videotapes started under one of the research project in Core Researches for Evolutional Science and Technology (CREST) founded by Japan Science and Technology Agency (JST) since December 2011. At the end of the fiscal year 2013, more than half of those videotapes will be archived. Although the main target of the CREST project is acoustic data, video signals on the videotapes are converted to MPEG-2 files for S-VHS tapes and both AVI and MPEG-2 files for DVCAM tapes before extracting acoustic data.

In those video images, not only the long term change of the clam colony but also some episodic events, such as spawning of the clams, sudden increase of snails and other unidentified events have been recognized, which would be invaluable data for the investigation of chemo-synthetic ecosystems. Those archived video images will be able to supply researchers outside the project in near future. However, there still exists a problem that the number of hard disks in which the video images are stored is very large even though it is less than a thousand.

Keywords: off Hatsushima Island in Sagami Bay, long-term visual observation, archives of videos

Paleoecology of Neogene vesicomyids from Niigata, Japan and their adaptations to geochemical environments of cold seeps

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Living vesicomyid bivalves are known to adapt to different hydrogen sulfide concentration and various habitats such as methane seeps, hydrothermal vents, whale falls, and petroleum seeps depending on species. Fossil vesicomyids are reported especially from Neogene seeps worldwide, but their adaptations to the geochemical environments of ancient seeps, which can help to understand the diversity and evolution of them, remain unrevealed. This study examined the paleoecology of fossil vesicomyids and geochemical environments of seeps to which they adapted by investigating their modes of fossil occurrence and geochemistry and petrography of seep carbonates from the two Neogene seep localities in Niigata Prefecture.

The lower Pliocene part of the Kurokura Formation mainly consists of gray to dark gray siltstones which deposited in upper bathyal depth. At the riverside cliff of Echido River at Matsunoyama-Matsuguchi, Tokamachi City, pebble-sized carbonate concretions are contained in 60 cm-thick massive gray siltstone. Fossil vesicomyids, *Archivesica kannoi*, are contained in the concretions some of which are gradually bounded by surrounding siltstone. A large individual (ca. 90 mm length) and surrounding small individuals (ca. 20 mm in the mean length) of *A. kannoi* are contained in the same concretions with various other bivalves, gastropods, and scaphopods which are not unusual to the modern cold-seep communities. Lucinid bivalves are contained in surrounding siltstone and burrows filled with carbonates are also observed in the siltstone. Concretions are mainly composed of micritic Mg-calcite, containing abundant pyrite crystals, and stable carbon isotopic compositions of them are very low values (-43.3 to -27.1 ‰ vs. PDB), showing their derivation from methane, whether they contain fossils or not. Only fossil-bearing concretions contain clast-like carbonates (ca. 5 mm in diameter) which are triangular or oval-shaped in cross section and composed of many fine dolomite crystals surrounded by Mg-calcite matrices in thin section. Dolomite formation is related to the removal of dissolved sulfate by sulfate reduction, thus it may suggest active produce of hydrogen sulfide. It can be concluded that *A. kannoi* was adapted to the habitat where hydrogen sulfide concentration was relatively higher due to more active sulfate reduction than surroundings, or pumping activity of *A. kannoi* supplying sulfate was active enough to promote active sulfate reduction.

The upper Miocene Nodani Formation consists of alternation of gray sandstone and dark gray siltstone which deposited in upper bathyal depth as submarine fan turbidites. At the river cliff of Nakanomata River at Nakanomata, Joetsu City, pebble-sized carbonate concretions are contained in dark gray siltstone just below gray, oily sandstone. Fossil vesicomyids, *Calyptogena pacifica*, are contained in these concretions or surrounding siltstone. Some of them are preserved in life position. Fossils and concretions are contained in a narrow range of 30 cm wide and 5 cm thick, and pipe-shaped carbonate concretions are contained parallel to bedding in siltstone 50 cm below. These concretions are mainly composed of micritic calcite and carbon isotopic values of them are moderately low (-21.7 to -13.2 ‰), suggesting their derivation from crude oil. In thin section, the fossil-bearing concretion contains many micritic peloids. Central void space of pipe-shaped concretion is fringed with bladed calcite which also shows low carbon isotopic value (-22.6 ‰), suggesting that these pipes acted as conduits of seepage. *C. pacifica* lives in the Recent methane seeps, but it is suggested that this species was also adapted to narrow-ranged, local petroleum seep in the Miocene.

Keywords: vesicomyids, Neogene, sulfide concentration, petroleum seep

Recent trials of laboratory culture with chemosynthetic organisms

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Chemosynthetic ecosystem is dominated by the organisms what has symbiotic bacteria in their body. Deep-sea bivalve *Bathymodiolus septemdirum* have been hosting some sulfur oxidizing bacteria in their gills. The bacteria have ability to synthesize such organic compounds as sugars from inorganic carbon source with hydrogen sulfide (H_2S). Many questions have been still remaining about the emergence and maintenance mechanisms of such symbiotic relationship between host animal and bacteria. Even though the development of laboratory culture techniques of such chemosynthetic bivalves are very useful approach to understand the detailed ecology and for further experiments, the technique is not developed very well. Our research group try to set chemostat water bath up with hydrogen sulfide to keep *B. septemdirum* as healthy condition. We try to use the culture system to evaluate the bivalves can keep their symbiotic bacteria to make much longer life time in laboratory. The activity of symbiotic bacteria has been tested by the uptake ability of ^{13}C labeled inorganic carbon into their body.

Individuals of *B. septemdirum* are captured during dive series of ROV Hyper-dolphin system of two cruises of R/V Natsushima operated by Japan Agency for Marine-Earth Science and Technology (JAMSTEC) in April of 2012 and in March of 2013. The samples are collected around Myojin-Sho submarine volcano on the Izu-Ogasawara Ridge. Collected samples were kept under 4 °C water tank in an on-board low-temperature room till the end of cruise. Then, the individuals are immediately transfer to on-land laboratory water tank after cruise to avoid the unfavorable environment. The water tank has been designed as chemostat system with H_2S supply to maintain symbiotic bacteria of deep-sea chemosynthetic animals. The individuals are cultured in this system for three months and fourteen months respectively. Here, previous study shows the symbiotic bacteria disappeared within three months without H_2S source. Therefore, we prefer to confirm the bacteria have been hopefully maintained more than three months in our chemostat system or not. For this purpose, carbon isotope labeling experiments were carried out to clarify the existence of symbiotic bacterial activity. The carbon isotope will be taken into organic matter of *B. septemdirum* if the symbionts are active after laboratory culture. We have compared the carbon isotopic uptake between under H_2S positive and under H_2S negative (control) conditions, respectively. Meantime, dissolved oxygen (DO) of each cultivation was monitored to check health and activity of individual bivalves. The results show the labeled ^{13}C signals were detected on the organic matters of both gills and foot especially under H_2S positive condition. Surprisingly, the activity was much positive even the individuals were kept for fourteen months in the chemostat system.

Keywords: laboratory culture, chemosynthetic organisms, *Bathymodiolus septemdirum*