

## Early Maastrichtian (Late Cretaceous) methane seeps and their associated fauna from southern Alaska

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We investigated early Maastrichtian (Late Cretaceous) methane seep deposits hosted by the upper Matanuska Formation in the Talkeetna Mountains, southern Alaska. During two field seasons (2006 and 2007), we encountered numerous carbonate concretions embedded in the mudstone-dominated formation that is exposed along the Alfred Creek. These carbonates can be classified into several morphotypes, i.e. rounded, irregularly rounded, doughnuts, pipe, and burrow types. At one outcrop, we studied the distribution pattern of the carbonate types. At outcrop, the pipe and doughnuts type carbonates were distributed in the lower horizon, whereas, the rounded type carbonates and the irregularly rounded and burrow type carbonates were found in the middle and upper horizons, respectively. Carbon and oxygen isotopic compositions of the carbonates range from -41.5 to -6.1 per mil (vs VPDB) and from -10.7 to -0.4 per mil (vs VPDB), respectively. Among the investigated carbonates, the burrow, irregularly rounded, and rounded carbonates have more negative values of carbon isotopes indicating that these carbonates were formed under the influence of anaerobic oxidation of methane. Dozens of crustaceans and solemyid bivalves, with lucinid bivalves as a minor component, were found from the upper part of the outcrop. The recent counterparts of solemyid and lucinid bivalves are known to host chemosymbiotic bacteria in their gills. The bivalves have been commonly found around ancient and Recent methane-seeps.

It is worth to note that this is the first record of the methane-seep dependent chemosynthesis-based ecosystems from the Maastrichtian worldwide. The fauna at the Early Maastrichtian seep of Alfred Creek is dominated by infaunal dwellers: crustaceans, solemyid and lucinid bivalves. This finding supports previously reported macroevolutionary pattern in chemosymbiotic bivalves, i.e. after the decline of modiomorphid Caspiconcha at the end of the Early Cretaceous and its last occurrence in the Campanian, the ecological niche of epifaunal to semi-infaunal seep endemic bivalves was largely vacant and not reoccupied until the Eocene with the appearance of the vesicomid and bathymodiolin bivalves.

Keywords: methane seep, Late Cretaceous, solemyid, lucinid, stable carbon isotope, authigenic carbonate

## Reconstruction of seepage site with *Calyptogena* colonies in the Upper Miocene Morai Formation, Hokkaido, Japan

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The Upper Miocene Morai Formation, Hokkaido, Japan, consists of alternating beds of semi-consolidated and hard mudstone, and intercalates calcareous concretions associated with abundant fossils of *Calyptogena pacifica*. This study reconstructs habitat conditions of *Calyptogena pacifica* on the basis of field observation of a 20-m-high sea-cliff outcrop.

The calcareous concretion blocks bearing *Calyptogena pacifica* fossils are restricted in several horizons in the upper part of the sea cliff. We surveyed the shape, size and distribution of the concretions and observed the carbonate lithology and modes of fossil occurrence, hanging from the cliff with a rope. The concretion blocks are lenticular in shape, attaining over 10 m in horizontal length and less than 2 m in thickness, and laterally changed into hard mudstone beds which also yield abundant *Calyptogena* shells. The concretion blocks have nearly flat roof and base, but its sides sometimes interfinger with neighboring mudstone.

The concretion blocks consist of monotonous muddy dolomicrite, associated with small calcitic nodules, several centimeters in diameter, just below the blocks. The dolomicritic concretions sometimes show auto-brecciation, which shows break of mudstone into angular pieces to form jigsaw-puzzle fabric. The space between breccia was filled with dolosparitic cement. It is noted that the brecciated fabric is restricted to interior of the concretion blocks and does not continue to the surrounding mudstones, which does not show any fracturing. These suggest that the auto-brecciation was not due to hydrofracturing by pore-fluid pressure, but was maybe caused by contraction during dolomitization.

*Calyptogena* fossils form shell concentrated beds associated with *Conchocele bisecta*, *Acharax johnsoni*, and *Lucinoma* sp. The shell beds continue laterally more than 20 m. The thickness attains more than 50 cm in the concretion blocks, but becomes thinner (ca. 15 cm) in the surroundings. It is noted that all the bivalve species show high ratio of conjoined valves (ca. 60 % in case of *Calyptogena pacifica*). In particular, almost all of *Conchocele bisecta* fossils preserved their life position. Most of *Calyptogena* conjoined valves show life position or reclining orientation with their commissural plane horizontal, but some show an inverted orientation from life position, maybe fossilized on the way to escape from rapid burial. These suggest that the *Calyptogena* shell concentrations were not due to transportation, but preserved the original colony condition due to rapid burial maybe by muddy turbidity current.

Localized distribution of carbonate concretions and *Calyptogena* shell beds indicate that the *Calyptogena* colony was not formed by reducing mud due to oxygen-depleted water-mass, but was supported by methane seepage. The outcrop observation suggests that the seepage was not channelized but diffusive. The seepage influence expanded the bottom surface area over 20m in diameter, but was repeatedly interrupted by rapid burial of muddy turbidite.

Keywords: chemosynthetic community, cold-seepage, Miocene, Morai Formation, Hokkaido, *Calyptogena*

## Predation marks on *Calyptogena* dead shells off Hatsushima Island, Sagami Bay, central Japan

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Predation pressure is one of the important factors controlling community structure and evolution, but only few attempts have so far been made to quantitatively estimate predation pressure in chemosynthetic communities.

We analyzed breaking pattern of *Calyptogena* dead shells in a living colony at a cold-seepage site off Hatsuhima Island, Sagami Bay, central Japan. The sampling site is located at 856 m in bathymetrical depth, on slope of western side of Sagami Bay. The dead shells were collected by submersible *KAIKO 7000II* (Dive #546, KR12-05 cruise) using a Kumade-sampler with 15 cm x 18 cm mouth, 13 cm depth, and 7.6 to 7.8 mm sieve openings.

The dead shell assemblage consists of a mixture of various conditions from complete conjoined valves to abraded shell fragments. Among them, we paid attention to open valves with umbo intact, some of which are not abraded, preserved sharp and fresh break edge, and show remarkable contrast in preservation between the right and left valve. Almost all part of one valve (right or left) is missing except for the strong hinge and dorsal margin connected with ligament, whereas the other valve is nearly complete with notch-like injury in ventral margin. This breaking pattern is difficult to be explained only by non-biological factors. We judge the shell breaking pattern to be a predation mark, and estimate the predator as decapod crab *Paralomis multispina*, which were frequently observed to gregariously habit around the sampling site. *P. multispina* was reported to catch and eat *Calyptogena* by Fujikura et al. (2008), who attached the photograph showing that *P. multispina* inserted its right crusher chelae into the ventral margin of a *Calyptogena* shell and tried wrenching open it.

In order to quantitatively estimate the contribution of predation to all death causes, we calculate the ratio of numbers of predation-mark specimens to all attached valves in each shell size class. We exclude detached or fragment specimens in the calculation because it is difficult to judge whether the specimens are results of predation crush or physical break.

Total 75 attached valves are examined. The predation marks are recognized from young to gerontic *Calyptogena* clams, shell length of which varies from about 2 cm to over 12 cm. The ratio of predation-mark specimens in each shell size class varies from 17 to 83%, and its average is 40%. This data shows no size-selective feeding behavior, and that the predators have crushing force enough to break the stout shells of gerontic *Calyptogena* clams. The predator, maybe *Paralomis multispina*, contributes 40% to all death cause of *Calyptogena* clams.

These suggest that *Calyptogena* colonies off Hatsuhima Island have been maybe under high predation pressure in spite of the deep-sea condition. Methane-seep sites are deep-sea oases not only for chemosynthetic animals but also for the predators.

Keywords: chemosynthetic community, cold-seepage, *Calyptogena*, predation pressure

## Biodiversity of deep-sea hydrothermal vent fauna and its relationships to environments in Okinawa Trough

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Flourish assemblages of deep-sea hydrothermal vent fauna associated with steep environmental gradient formed by high temperature venting fluid containing high concentration of metals and the other kinds of chemicals. The Okinawa Trough, is a backarc basin which has started rifting from the southern part 2My ago, and at least nine hydrothermal vent fields has been discovered along the NE-SW spreading axis. Multi-disciplinary investigation was carried out in five of nine vent fields to find out the relationships between biodiversity and environments in the Okinawa Trough.

In the five hydrothermal vent fields, quantitative faunal sampling and simultaneous environmental measurements using sensors including a newly developed D-Pote (a Deep-sea Potentiostat) were carried out at more than two points in each vent field, during the NT11-20 cruise of R/V Natsushima / ROV Hyper-Dolphin. Biodiversity was evaluated based on the collected faunal samples and the relationships between faunal composition and environmental factors such as temperature, depth, salinity, and concentrations of oxygen and sulphide compounds were examined statistically.

Biodiversity of the vent fauna was relatively low in the northern part of the Okinawa Trough, where vent fields are located in relatively shallow area (< 1000 m depth). On the other hand, in the southern, relatively deep (> 1000 m depth) and older part, vent communities of relatively high biodiversity corresponding with great variation of concentration of sulphide compounds were observed. The present results suggested that the correspondence of the biodiversity, environmental diversity, and geological history of hydrothermal vent fields in the Okinawa Trough.

Keywords: hydrothermal vent, biodiversity, similarity, chemosynthetic community

## Variation of lipid biomarker composition in *Bathymodiolus sp.* at hydrothermal vents in Okinawa Trough

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### <Introduction>

*Bathymodiolus sp.* host chemoautotrophic bacterial endosymbionts such as sulfur-oxidizing bacteria and/or methane-oxidizing bacteria in their gills. However, no studies have revealed how the mussels incorporate chemoautotrophic bacteria. Tyler (1999) reported that larval of *Bathymodiolus sp.* take planktons and dissolved organic matters in sea water. To investigate the relationship between deep-sea mussels and endosymbionts in Okinawa Trough as the mussels grow, lipid biomarkers and carbon, nitrogen and sulfur isotopic studies of *Bathymodiolus sp.* are analyzed in this study.

### <Samples & analytical methods>

Deep-sea mussels were collected at two different hydrothermal vents: Hatoma Knoll (HTM) during the NT09-11 cruise and Iheya North Knoll (INK) during the NT09-17 cruise. *Bathymodiolus sp.* were frozen (-20°C) immediately and sent to Kyushu University. After gill tissue segregation, each sample was lyophilized and extracted with organic solvents to analyze lipid biomarkers including hopanoid hydrocarbons and fatty acids by gas chromatography/mass spectrometry (GC/MS) and GC isotope-ratio/mass spectrometry (GC/IRMS).

### <Results & discussion>

Gill tissue of all bivalves contained diploptene, which is known as one of specific biomarkers of methanotrophs. The presence of diploptene indicates that all deep-sea mussels of this study host methanotrophic bacteria in the gills. Saturated and unsaturated fatty acids from C<sub>15</sub> to C<sub>22</sub> were identified. The most abundant fatty acids was C<sub>16:1</sub> except the HTM2 sample. However, relative compositions of fatty acids were different each other. The fatty acids from the INK mussels became depleted in 2.5 to 3.7 per mil (relative to PDB) as the mussels grow. These variations in abundance and isotopic compositions could reflect growth stages associated with the activity of bacterial endosymbiont. On the other hand, the carbon isotopic composition of fatty acids from the HTM mussels were very similar each other, probably because they might have already grown up enough.

Keywords: *Bathymodiolus sp.*, Lipid biomarkers, Methanotroph, Okinawa Trough, Fatty acid composition, Carbon isotopic composition

## Evolution of Chemosynthetic Community: From an Evo-Devo perspective

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Recent paleontological, phylogenetic and phylogeographic researches have revealed the evolutionary patterns of chemosynthetic ecosystems. However, the mechanisms of their evolution remain to be solved. What were the essential genetic mechanisms driving to their evolution. Whether ancestral organisms had the molecular mechanisms responsible for the adaptation to the environment, or not. If not, how did the mechanisms evolve? To understand the origin of organisms in the chemosynthetic ecosystem, we should elucidate both the patterns and processes of their evolution.

Evolutionary and developmental biology (Evo-Devo) is a field of biology that addresses the mechanism of the evolution through the development of characteristics. To understanding the mechanisms of evolution, we investigate how the characteristics, such as shape, behavior and physiology, arise and compare the results with that of related taxa. To carry out these researches, fundamental biological information, such as life cycle, behavior, about organisms of interest is necessary. However, because of the difficulty of collecting and rearing animals in chemosynthetic ecosystem, these kinds of knowledge are limited. To overcome the situation, we have established methods to culture animals in a laboratory condition. And also, we have succeeded in applying some modern techniques of developmental and cellular biology to them. In this presentation, we will introduce some results about the Deep-sea Evo-Devo in siboglinid polychaetes.

## How can environmental DNA help understanding chemosynthetic communities evolution and ecology?

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Chemosynthetic environments in the deep sea harbor highly specialised communities. Those communities are often dominated by a few characteristic species. While the dominant macrofaunal species received lots of attention from the scientific community, other groups such as the meiofauna remain largely unknown. Benthic meiofauna groups organisms between 500 (1000) and 44 (31)micrometers living on or in the sediments. Meiofauna has been reported to be rare or less diverse in chemosynthetic environments such as hydrothermal vents compared to bathyal or abyssal plains. This is likely explained by the little availability of sediments samples as well as environmental characteristics of hydrothermal vents ecosystem. In shallow environments, meiobenthic communities have been shown to be sensitive to a variety of environmental parameters. In response to the steep gradients in multiple environmental parameters encountered in hydrothermal vents fields, it is expected that the patterns of chemosynthetic communities will be complex and meiobenthos will be reflecting such environmental changes accurately. However, low abundance of organisms and difficulty to collect samples from these environments are a major issue towards the investigation of meiofaunal diversity and biogeographical patterns.

Metagenetics is the part of metagenomics consisting in sequencing one homologous marker from environmental DNA. This method offers new perspectives to investigate the taxonomic composition of the communities inhabiting ocean seafloor. In comparison to more traditional methods based on sorting of organisms, environmental DNA allows to detect rare species in an environment, even if only fragment of organisms or DNA are present in the sampled sediments. Moreover, metagenetics approach allows the estimation of taxonomic richness and distribution across multiple phyla in parallel.

Our study focused on environmental DNA extracted from sediments samples collected in the Iheya North hydrothermal vent field in the Okinawa Trough. In the research cruise NT12-27, eight sediment cores were obtained from 3 sites within the vent field. One is located near a site of active hydrothermal venting, while the other two sites are distant from the active vent. The core samples were sliced in five layers of 1cm thickness, and three replicates were sub-sampled from each layer. Environmental DNA was extracted independently from less than 1 g sediments from each replicate.

Large amounts of DNA were obtained from the core sample from the active area covered by white matter, while the samples collected in inactive zones yielded very low amounts of DNA. Here we will present the preliminary results obtained from the sequence data of this environmental DNA, and discuss on the usefulness of this metagenetic protocol to determine the diversity of meiobenthic community.

Keywords: Biodiversity, Environmental DNA, Meiobenthos, Iheya North

## CT scanned deep-sea creatures -using CT data of chemosynthetic animals for research and education-

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We took three dimensional images of deep sea creatures, including many chemosynthesis-based animals, using x-ray CT scan (ScanXmate-D150-S270) at Comscantecno Co.,Ltd. We have opened the CT data on the web site of Atmosphere and Ocean Research Institute, The University of Tokyo (<http://www.aori.u-tokyo.ac.jp/project/3D/index.html>). We will demonstrate how to use the CT data for research and education.

Keywords: X-ray, chemosynthetic animals, biomineralization, 3D



## Benthic foraminiferal assemblages associated with chemosynthetic bivalves from the Plio-Pleistocene in the Leyte Island

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We studied benthic foraminiferal assemblages that are associated with vesicomid communities from massive mudstones at the Cambuntug Point and lucinid community from massive sandy mudstones at the Antipolo Point, northwestern parts of Leyte Island, Philippines. The ages of both fossil horizons are 3.97-1.77 Ma based on nanofossils and planktic foraminifers.

At the Cambuntug Point, we examined 10 sediment samples: four samples from the matrix sediments around the vesicomid bivalve fossils (S551 and S556 from locality 1 of Majima et al., 2010; and B2-B3 and C2-C3 from locality 3 of Majima et al., 2010), and six samples from the locations where no vesicomid bivalve occurs (B1, C1, B4, C4, S605, and S606 from locality 3). At the Antipolo Point, we observed five sediment samples: three samples (S703, S704, and S707) from the matrix sediments around the lucinid bivalve fossils, and two samples (S701 and S702) from the locations where no lucinid occurs. From each sample, about 200-300 benthic foraminiferal fossils (larger than 125 micrometer), were picked up and analyzed.

The results of Q-mode cluster analysis indicate that the examined 15 samples are clearly divided into the two groups: one is composed of the samples from the Cambuntug Point (S551, S556, B1, C1, B2-B3, C2-C3, B4, C4, S605, and S606) and the other is composed of the samples from the Antipolo Point (S701, S702, S703, S704 and S707). This result suggests that the benthic foraminiferal assemblages were primarily controlled by the lithology of the examined horizons (mudstones and sandy mudstones), which would be related to the depositional environments. The samples from the Cambuntug Point are subdivided into two subgroups: (1) B4, C4, S605, and S606, and (2) S551, S556, B2-B3, C2-C3, B1, and C1. The samples of S551, S556, B2-B3 and C2-C3 were collected from matrix sediments around the vesicomid bivalve fossils, although the samples of B1 and C1 were not associated with the vesicomid fossils. The vesicomid occurrence gradually changes from the absence in B1 and C1 horizons into the abundance in B2-B3 and C2-C3 horizons. Therefore, we infer that the methane seepage had already begun at the B1 and C1 horizons. At the Antipolo Point, however, there is no difference of the benthic foraminiferal assemblages, irrespective of the occurrences of lucinid bivalves.

We consider that the benthic foraminiferal assemblages associated with vesicomid fossils in the Cambuntug Point had been influenced by hydrogen sulfide, diet of chemosynthetic bivalves, originated in the anaerobic oxidation of methane. However, in the Antipolo Point, where lucinid fossils occur, the benthic foraminiferal assemblages had not been influenced by hydrogen sulfide. Living Vesicomids are half buried in the sea bottom sediments with hydrogen sulfide, although living lucinids burrow deeply in the sediments, so that hydrogen sulfide are possibly limited in the deeper sediments. Therefore, the benthic foraminiferal assemblages of sediment surface were not possibly affected by the hydrogen sulfide.

Keywords: benthic foraminifera, methane seepage, chemosynthetic assemblages, Philippines, Pliocene, Pleistocene

## A fossil cold-seep assemblage from the Pleistocene Shioda Formation, Nakatsu Group, central Japan

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Lucinid and thyasirid bivalves-dominated fossil cold-seep assemblage occur in the Pleistocene Shioda Formation, Nakatsu Group, central Japan. In the studied outcrop (11.4 m in total thickness), sandy mudstones with intercalated muddy sandstones (tens of cm in thickness) and pumice-rich lapilli tuff beds (several cm to tens of cm in thickness) are dominated, and mudstones are exposed in the uppermost part (1.4 m in thickness). The fossils occur sporadically in the muddy sandstones, sandy mudstones and mudstones. They are composed of *Lucinoma* sp. and *Conchocele* sp. which are major component members of Neogene chemosynthetic fossil assemblages in Japan. Most of the large bivalves are articulated (85% articulation ratio) in the interval from the base of the outcrop to 2.86 m-upper horizon, which indicate that these bivalves had not been transported long distances from their living places.

Authigenic carbonate concretions are developed sporadically in the sandy mudstones, muddy sandstones and pumice-rich tuffs. Three carbonate concretions were sampled: A) a bedded concretion immediately above pumice-rich tuff, which locates at 3.7 m above from the base of the outcrop (ABO); B) a nodular concretion at 2.9 m ABO; C) a nodular concretion at 1.7 m ABO. In terms of carbonate mineral composition, A-1, A-2 (both from sample A) are composed of high-Mg calcite, B, high-Mg calcite (88.6 wt%) and dolomite (11.4 wt%) and C, dolomite. Stable isotope values ( $d^{13}C$  and  $d^{18}O$  per mill vs PDB) of the samples are following: A1, -31.2 and 1.3; A-2, -33.3 and 1.3; B, -30.6 and 1.2; C, -22.6 and 2.6, respectively. The  $d^{13}C$  values indicate that the high-Mg calcites had been precipitated under the influence of anaerobic methane oxidation and also indicate that the co-occurring fossil *Lucinoma* sp. and *Conchocele* sp. depended upon methane-containing cold seep.

Keywords: Nakatsu Group, fossil cold-seep assemblage, Pleistocene