Chemostratigraphy of the *Calyptogena*-bearing, Plio-Pleistocene Miura and Kazusa Groups, central Japan

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We studied the stratigraphy and geologic ages of the *Calyptogena*-bearing, Plio-Pleistocene Miura and Kazusa Groups, the fore-arc basin-fills, based on calcareous nanno fossil biostratigraphy and magnetostratigraphy.

Samples examined were obtained from twelve horizons of the Ikego Formation (Pliocene) of the Miura Group, five of the Urago Formation (upper Pliocene to lower Pleistocene) and six of the Nojima Formation (lower Pleistocene) of the Kazusa Group exposed in the northern Miura Peninsula.

The following five nanno-fossil datums have been recognized in ascending order (ages from Raffi et al., 2006): FO (First Occurrence) of *Pseudoemiliania lacunosa* (applied as CN11a - 11b boundary: 4.13 to 4.12 Ma) and LO (Last Occurrence) of *Reticulofenestra pseudoumbilicus* (CN11b - CN12a boundary: 3.79 Ma) in the Ikego Formation; LO of *Discoaster tamalis* (CN12a - CN12b boundary: 2.87 Ma) and LO of *Discoaster surculus* (CN12b - CN12c boundary: 2.52 Ma) in the Urago Formation; LO of *Discoaster pentaradiatus* (CN12c - CN12d boundary: 2.39 Ma) in the lowermost Nojima Formations. Gauss - Matuyama boundary (2.58 Ma) has been recognized in about 30 to 60 m beneath LO of *Discoaster pentaradiatus*.

Based on the results above, the age of the *Calyptogena*-dominated assemblages from the Ikego Formation can be dated in 4.50 to 3.79 Ma, and those from the Urago Formation in 2.52 - 2.50 Ma.

Keywords: chemosynthetic fossil assemblage, Miura Peninsula, Miura Group, Kazusa Group, calcareous nanno fossil stratigraphy
Distributional gradient of sister species of vesicomyid bivalves in chemosynthetic fauna in Japan

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Vesicomyid bivalves is one of the important components of the deep-sea chemosynthetic biological communities due to their high biomass based on the chemosynthetic primary production of their symbionts. The restrictive distribution of vesicomyid clams by chemical gradient such as sulfide concentration in sediment has been discussed, but the relationships between general environmental properties such as temperature, salinity and oxygen concentration were unclear. With a development of a specific primer set for mPCR to identify concurrent vesicomyids, C. okutanii and C. soyoae, in chemosynthetic biological communities in Sagami Bay, distributional bias of two vesicomyids along the environmental factors was examined. Ratio of C. okutanii and C. soyoae in Calyptogena assemblages in Sagami Bay were correlated with depth, temperature and salinity but were not correlated with DO. However, the environmental information of the habitats of C. okutanii in Okinawa Trough hydrothermal vent fields revealed that the depth was not the factor to discriminate the habitat of the two Calyptogena clams. The present results showed that the distribution of the Calyptogena clams which highly depending on chemosynthetic symbionts was also affected by ordinal oceanographic environmental factors.

Keywords: vesicomyid, chemosynthetic fauna, Sagami Bay, Okinawa Trough, mPCR
Ethological observation in a Calyptogena colony off Hatsushima Island in Sagami Bay, central Japan

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Ethology of Calyptogena clams is analyzed on the basis of a serial video-capture images with three-minutes interval from 14th March to 19th June, 1988 on the Long-term Deep Sea Floor Observatory off Hatsushima Island in Sagami Bay. The video camera was fixed to observe the Calyptogena colony (yellow bacteria mat and its surroundings of Calyptogena gregarious zone).

During the video-capture working time, more than three mud-flows stroke the colony, but the thickness of mud-flow sediments were maybe not over 10 cm. The Calyptogena clams pushed up their shells and escaped from the burial by pushing their foot against underground firm bottom. After the mud-flow blanketed the colony, some Calyptogena clams randomly moved and disturbed the reducing bottom breaking the bacteria mat, but its significance is unknown.

It is noteworthy that this serial-images record a complete account of prey/predation processes on a Calyptogena clam from beginning to end. Calyptogena clams were attacked by large buccinid and small turrid gastropods, which wandered in and around the living Calyptogena colony and rode on some clams. Calyptogena clams escape or protect from their predation by the following three methods: 1) closing the valves; 2) burrowing into sulphide-rich bottom where is not preferable condition for aerobic animals; and 3) hiding behind large dead shells.

Small gastropod-attacks did not cause dynamic escape activity of Calyptogena bivalves. Gregarious small gastropods had swarmed on some living Calyptogena clams during over 20 days, but the Calyptogena clam only once crawled into the reducing bottom and mainly resists the small-gastropod attacks by closing their valves.

On the other hand, attacks by large buccinid gastropods promptly cause escape actions of Calyptogena bivalves. We confirm that a Calyptogena clam instantly burrowed into the bottom just after large buccinid-attacks. The Calyptogena clam stayed in the black reducing sediments over nine hours. During the burrowing time, buccinid gastropods also burrowed into the sediments with their long siphon above the sediment surface, and struggled and tried to prey the clam. After all some buccinid gastropods gave up and lefted the clam burrowing point although others retained around the point. The Calyptogena clam once succeeded in escaping from the predation, but unfortunately the clam received next attack by another buccinid gastropod after the clam rose to the surface. The clam maybe had little reserve of energy to burrow again, and alternatively hided behind a large dead Calyptogena shell. This way was not efficient for the protect from their predation. After the hiding, the living Calyptogena received intensive attacks by many large and small gastropods. It took about 4 days and a half from the start of intensive attacks to completion of the predation. This predation time suggests that old-aged Calyptogena colony has enough potential to attract the high-density gastropod population.

Keywords: Calyptogena, methane seepage, ethology, Sagami Bay
**Nucinella** found in a chemosynthetic community off the Sanriku coast, northeastern Japan at 123 m depth

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Nucinellids are very small bivalves, generally less than 5 mm in length, related to the Solemyidae, which are typical chemosymbiotic bivalves. Reid (1990) and Amano et al. (2007) hypothesized, on the basis of the Nucinellidae’s gutless state and their occurrence in Cretaceous cold-seep deposits, that they have chemosynthetic bacteria in their body. This hypothesis has been partially confirmed by Oliver and Taylor (2012). They found bacteria-like microstructures in their gills. But it has still not yet been fully confirmed that the Nucinellidae have chemosynthetic bacteria or not.

We recovered many dead shells of *Nucinella* sp. with living chemosynthetic lucinid and thyasirid bivalves from the sea off the Sanriku coast at a depth of 123 m during the Tansei-maru (JAMSTEC) cruise KT-11-17 in the summer of 2011. The finding indicates that the Nucinellidae might be a member of chemosynthetic communities even in the Recent, not only in the Cretaceous. Our finding supports the hypothesis that the Nucinellidae have chemosynbiotic bacteria.

Keywords: Nucinellidae, methane seep, cold seep, symbiosis
Evaluation of nutrient sources for the sponges inhabited around seafloor hydrothermal areas in the Okinawa Trough

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Since discovery of seafloor hydrothermal vents the dense and endemic animal communities inhabited around the hot vents have been the most impressive feature for many scientists. Such animals have been known as chemosynthesis-based species and studied many investigators. On the other hand, some benthic animals foundon abyssal plain have been observed slightly high density at the adjacent area to active vent sites. It implies that those opportunistic benthos may also rely on the chemosynthetic primary production and the hydrothermal chemosynthetic ecosystem may extend widely rather than previous expectation.

In that case, it is an interesting issue how the dense sponge community is sustained around the hydrothermal fields. For clarifying the issue isotope geochemical study has been performed to evaluate food ecology of the sponges and some other animals obtained from the deep seafloor in the Okinawa Trough.

Stable isotope analysis of carbon, nitrogen, and sulfur of the sample organisms obtained from the Tarama knoll show significant low delta^{13}C and delta^{34}S values for the sponge samples. Those results suggest plausible contribution of sulfur oxidizing bacteria as food source for the sponges because such low delta^{13}C and delta^{34}S values are often observed for thioautotrophic chemosynthesis-based animals. The sulfur isotope ratios of the sponges also imply that the source of sulfur for sulfur oxidizing bacteria is possibly magmatism in origin. It also implies that the observed turbid water at the Tarama knoll is hydrothermal plume. Therefore, active high-temperature hydrothermal emission supplying hydrogen sulfide is expected at the Tarama knoll.

On the other hand, the sponge sample obtained from the Daiichi Kohama knoll shows similar isotopic characteristics observed for the sponges from the Tarama knoll. It may also imply the importance of sulfur oxidizing bacteria as food source for the sponge at the Daiichi Kohama knoll, therefore, hidden hydrothermal emission may be expected at the knoll.

Keywords: stable isotope, sponge, deep sea, seafloor hydrothermal system, Okinawa Trough
Estimation of trophic position in marine ecosystems based on nitrogen isotope of amino acids: prey-predator interaction

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Estimate of the accurate trophic position of organisms in food webs allows better understanding of not only biomass flow and trophic linkages in complex networks of natural ecosystems but also function and niche of individual organisms, species, and communities in the ecosystems. Combination of ultra-sensitive carbon and nitrogen isotope analyses of bulk whole samples (or tissues) and nitrogen isotope analysis of amino acids of the samples is a potential powerful tool to access the above subjects.

In this presentation, we will show a comprehensive data set on the isotope signatures for various organisms collected from marine surface photosynthetic and deep-see chemosynthetic communities in Sagami bay, and will discuss 1) applicability of this method to chemosynthetic communities, 2) nitrogen flow into the deep-see food web from photosynthetic (i.e., sinking particle) and chemosynthetic (i.e., seep water) communities, and 3) characteristics of specific nitrogen (or amino acids) transfer system during symbiosis between chemosynthetic bacteria and host organisms.
Estimation of trophic position in marine ecosystems based on nitrogen isotope of amino acids: host-symbiont relationship

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This study aims to clarify the trophic position (TP) of marine organisms to understand food web structures in marine ecosystems based on the stable isotope analysis of amino acid that we mainly focused on 1) the food web structures of marine ecosystems, and 2) the host-symbiont interactions. Combined analyses using stable isotopes along with ecologic, biochemical, and genetic analyses will clarify the relationships among prey-predator interactions in marine organisms and illustrate the dynamics and evolution of marine ecosystems.

We clarified the host-symbiont interactions between Calyptogena clam, and endosymbiotic bacteria. TP of Calyptogena muscle and symbiotic bacteria shows 2.0 and 3.1, respectively. TP increases 1.1 from symbiotic bacteria to muscle. We assumed that host Calyptogena muscle obtained almost 100% of nutritional source from symbiotic bacteria as amino acid. On the other hand, Bathymodiolus species have same TP value (=1.2) among the host Bathymodiolus (muscle, mantle and foot), symbiont-bearing gill, and symbiotic bacteria. It means that Bathymodiolus may have different mechanisms of nutrient requirement than Calyptogena.

Keywords: nitrogen isotope of amino acids, trophic position, host-symbiont relationships, chemosynthetic ecosystem, Calyptogena species, Bathymodiolus species