

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