様　式　Ｃ－１９、Ｆ－１９、Ｚ－１９　（共通）

科学研究費助成事業　　研究成果報告書

平成　２８　年　　６　月　　６　日現在

機関番号：　３８００５
研究種目：　若手研究(B)
研究期間：　2013～2015
課題番号：　２５８７１０１５

研究課題名（和文）Xenophyophores as bioindicators for polluant concentrations and species diversity as sessment

研究課題名（英文）Xenophyophores as bioindicators for polluant concentrations and species diversity assessment

研究代表者

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交付決定額（研究期間全体）：（直接経費）　　1,600,000円

研究成果の概要（和文）：クセノフィオフォラは世界中の海洋の深海底に多く見られる巨大な単細胞の原生動物である。その大きく複雑な殻は、水中から有害金属のみならず、等脚類、多毛環虫、軟体動物、棘皮動物など、多種多様な生物を引き寄せる。本研究の目的は、深海生態系の健全性の生物指標としてクセノフィオフォラの妥当性を確立するとともに、環境汚染濃度及び在来種／地域種の多様性評価に利用できるかを見極めることである。当プロジェクトには、解析のためのサンプルが不可欠であり、サンプル採取のための航海に出たものの、必要なサンプルが得られなかったため結果として予定していた計画遂行がかなわなかった。

研究成果の概要（英文）：Xenophyphores are giant, unicellular protists that flourish on the deep-sea floor in all the word’s oceans. Their large and complex tests accumulate toxic metals from the water column, but also attract a wide variety of organisms, including isopods, polychaetes, mollusks, and echinoderms. This research was aimed to establish the relevance of xenophyphores as bioindicators of deep-sea ecosystem health and whether they can be used to assess environmental pollutant concentrations and local/regional species diversity. Unfortunately the project has been cancelled due to a lack of fresh specimen samples.

研究分野：　環境学

キーワード：　Xenophyphores　Molecular phylogeny　Microscopy

１．研究開始当初の背景

Xenophyphores are giant, unicellular protists that flourish on the deep-sea floor in all the word ' s oceans. Their large and complex tests accumulate toxic metals from the water column, but also attract a wide variety of organisms, including isopods, polychaetes, mollusks, and echinoderms.

The Deep Sea, which comprises the largest habitat on Earth, has a crucial position within the global ocean ecosystem, contributing significantly to its diversity and its overall productivity. Benthic foraminifera are core components of this system, and constitute one of the most abundant and diverse groups of deep-sea meiofauna. Calcareous, multi-chambered species have been extensively studied because their durable tests have left an excellent fossil record, and because of their usefulness as proxies for paleoclimatic reconstruction and for oil prospection. In contrast, recent molecular studies have revealed that the majority of deep-sea foraminifera are species with single-chambered (monothalamous) organic-walled or agglutinated tests (1). These are poorly preserved in the fossil record and thus have been studied relatively little. There is an urgent need to increase our general knowledge of these organisms, which play such a significant role in deep-sea ecosystems and in global geochemical cycles.

Xenophyophores are one of the monothalamous lineages that have been overlooked because of their lack of distinctive morphological characters and very brittle tests. Yet, they can be found throughout the world ' s oceans and even in the deepest trenches. Although they are some of the largest single-celled organisms (reaching up to 20 cm diameter) (2), and can occur in extremely high densities (up to 20 specimens per square meter) (3), information about this group, its diversity, biology, and ecology, remains scarce.
While they are growing, these giant foraminifera are known to concentrate high levels of heavy metals and radioactive isotopes from the environment by trapping particles and accumulating them in their tests within a network of fecal strings called stercomare (Fig. 1). For instance, Shinkaiya lindsayi can accumulate lead and uranium at concentrations twice and six times greater than those in the sediment, respectively, (4). In Occultammina profunda, 210Pb emissions from bioaccumulation are estimated to be among the highest of any living organism (several Sv.yr−1) (5). By correlating concentrations of toxic chemicals inside xenophyophores with those of the water column, such organisms can potentially be used as time integrative indicators for fluctuating pollutant concentrations.

Finally, xenophyophores are often described as " hot spots " of deep-sea meiofaunal and macrofaunal diversity, because they shelter a wide range of taxa (3, 6). Areas dominated by xenophyophores display 3-4 times the number of benthic crustaceans, echinoderms and mollusks seen in comparable areas that lack xenophyophores (7). It is unclear whether their complex test architecture is the only reason for their attractiveness as microhabitats, or whether they actually offer metabolic advantages to their tenants. Nevertheless, the density of xenophyophores and the diversity of associated species in a given area could offer a straightforward estimation of local and regional biodiversity, avoiding more time-consuming faunal studies. Therefore the aims of this study are to determine the bioconcentration factors of major pollutants from the water column, and to understand how well the local/regional faunal diversity is predicted by xenophyophore population densities.

２．研究の目的

This research was aimed to establish the relevance of xenophyphores as bioindicators of deep-sea ecosystem health and whether they can be used to assess environmental pollutant concentrations and local/regional

species diversity.
Very little is known about the mechanism of pollutant bioaccumulation into xenophyophores tests. This study was planning to quantify some of the connections between these giant protists and their environment. We were going to focus on 5 metallic pollutants: lead, mercury, barium, uranium and cesium. By measuring their respective concentrations in the water column, in the sediment, and inside the xenophyophore tests, we were going to establish Bioconcentration Factors for each. Rates and mechanisms of the bioaccumulation process itself would have been investigated by in situ incubation experiments and proteomics analyses. We were going to be looking especially for chelating proteins and ion transporter proteins involved in metal reduction and compartmentalization (exclusion of metal from the bulk of the cytoplasm to the stercomare). Another objective of the project was to determine to what extent the species diversity associated with xenophyophores reflects the regional diversity. Using molecular tools and environmental DNA from the sediment, we were going to address the following questions: Which taxa display a correlation between their regional population densities and their densities on the tests of or in the vicinity of xenophyophores? How does the strength of the correlation vary for different xenophyophores and different associated taxa? What are the environmental factors that should be considered when xenophyophores are used to monitor local/regional diversity?

3．研究の方法

This project was aimed to create a pipeline method to assess environmental conditions in a deep-sea ecosystem by sampling and analyzing xenophyophore specimens from that ecosystem. The research was initially planning to start by reviewing pollutant concentrations and γ-/β-diversity in areas where xenophyophores had been previously sampled. We would then perform new chemical analyses (for metal pollutants and radionuclides) and molecular experiments (for proteomics and environmental DNA/RNA) on xenophyophores, sediments, and water samples that have been collected during earlier studies. The last part of this project was going to be dedicated to compiling the results and to establishing a protocol using xenophyophores as bioindicators.

4．研究成果

In FY2013 - 2014, The project was focusing on

(1) Partial SSU rDNA from 3 specimens of xenophyophores from Ogasawara Trench has been amplified by PCR and sequenced. This is the first time that any sequence from stannomid xenophyphores is obtained. Preliminary phylogenic analysis suggests that xenophyphores are truly a monophyletic group.

(2) High level of Radon 222 and Lead 110 have been measured in the 3 specimens of xenophyophores, while Cesium 137 was under detection level except for 1 specimen.

(3) SEM microscopy of the 3 specimens of xenophyphores from Ogasawara Trench was performed

(4) Samples were collected during RV T.G. Thompson TN309 (April 10 to May 20, 2014) to the Kermedec Trench, Pacific Ocean. Surface undisturbed sediments were sampled using HROV Nereus manipulating push cores, and coupled with a 4K live camera system. After deployment and recovering on deck, cores were immediately place in a cold (4°C) environment to prevent deterioration of DNA and RNA material. Xenophyphore specimens were collected with fine tools. Two specimens have been collected from the Nereus dive #73 at 7137 m depth. Distal fragments of each specimen were collected for DNA analysis and some were frozen for eventual further analysis. The rest of each specimen was incubated in a solution of CelltrackerGreen (LifeTechnologies) in order to later illuminate any living associated

organisms. After incubation, specimens were fixed into fluteraldehyde solution to preserve its morphology and later perform microscopic analyses.

The plan was a first time changed according to opportunity of sampling (HADESK cruise TN309 to Kermadec Trench) occurring ealier than expected. We did not perform the deep-sea exploration movie review.

Unfortunately the project on xenophyphores had to change direction again and finally got aborted mainly for the two following reasons:

1) HADESK cruise TN309 to Kermadec Trench has faced many logistic problems impairing sampling effort. The submersible used during this cruise was destroyed in an explosion, which has reduced the number of specimen available for our investigation to only 2.

2) Despite my effort, I could not get any other xenophophore samples to work with nor any other sampling cruise to take part to. The fragments of xenophyophore specimen I had access to until now are too few to conduct the experiments I was planning to do. I have been trying to modify my research strategy for this project. My idea was to get the full genome of the specimen I already had by massively sequence its cytoplasmic DNA. This would have consisted in the first xenophyophore's genome ever retrieved. Xenophyophore specimen are in general good candidate for full genome sequencing, since they possess a large cytoplasm and many nuclei. Thus, I theoretically had enough material for this experiment, even without new specimen. Unfortunately, the morphology of my sample was somehow unusual and inappropriate for cytoplasmic DNA extraction.)

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計０件）

〔学会発表〕（計０件）

〔図書〕（計０件）

〔産業財産権〕
○出願状況（計０件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計０件）

名称：
発明者：
権利者：
種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等

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