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　２４　日現在

機関番号：　１８００１
研究種目：　若手研究(B)
研究期間：　2014～2015
課題番号：　２６８７０９１７

研究課題名（和文）Travelling DNA: a study in DNA dispersal and connectivity between marine ecosystem

研究課題名（英文）Travelling DNA: a study in DNA dispersal and connectivity between marine ecosystem

研究代表者

フレデリック　シニゲル（Frederic, Sinniger）

琉球大学・熱帯生物圏研究センター・研究員

研究者番号：１０６２５９４０

交付決定額（研究期間全体）：（直接経費）　　2,900,000円

研究成果の概要（和文）：異なる環境中の環境DNA（eDNA）を比較することで、環境中の遊離DNA断片がどのように海中を移動するのかを推定することができる。そこで、深海から浅海にわたる幅広い水環境より得た堆積物中のeDNAを、メタバーコーディング手法により解析し、堆積物中の生物多様性を推定した。異なる環境間での遊離DNA断片の流動を示唆する結果は現時点では得られていないが、未知の生物のシーケンスが深海の堆積物中より大量に得られ、未知の深海生態系の存在が示唆された。

研究成果の概要（英文）：I analysed environmental DNA (eDNA) from sediments from deep-sea and shallow water environments. I used a metabarcoding approach to sequence eDNA and estimate the biodiversity in sediments. By comparing different regions and environments, I could estimate how DNA can travel in the oceans.
My results first revealed a huge unknown biodiversity in marine sediments and largely contributed to evaluate the gap of knowledge in worldwide deep-sea biodiversity (Sinniger et al. 2016).
In addition, the results from Okinawa showed that my approach is able to detect different environments even if they are only few hundred meters apart. The results also showed that sediments from the same type of environment but from distant locations are distinct, but still group near each other (Sinniger et al. in prep). Overall my results indicate that while a transfer of DNA between environments certainly exists, it is not a major parameter affecting the estimation of genetic diversity in the sediments.

研究分野：　Biodiversity

キーワード：　Metabarcoding　Marine Biodiversity　Environmental DNA

１．研究開始当初の背景

Biodiversity is closely related to ecosystem functioning and is a key factor in ecosystem resilience to environmental change[1]. In a period of global change, it is essential to understand better the complexity and spatial organization of marine biodiversity. The deep sea covers over 65% of our planet, however we ignore most of the level and organization of eukaryotic diversity in the deep sea.

The deep sea acts as a sink; large part of the surface productivity (and pollution) is transferred from shallow ecosystems to the deep sea where it can be stored for geological times in the sediments. Metazoan communities living in the sediments play an active role in dispersion and re-mobilisation of this material. However, the diversity of many taxa of such communities is still poorly known due to the difficulty of taxonomic identification. Such difficulties come from the small size of organisms, morphological conservatism and the lack of taxonomists able to identify the high diversity of taxa living in sediments. In addition to these issues, the remote nature of the deep sea is a considerable limitation to extensive sediment sampling and often only very limited amounts of sediments are available for biodiversity studies.

In this context, the use of **environmental DNA (eDNA)** is a promising development towards understanding marine biodiversity, especially in the deep sea. eDNA comprises all the DNA present in an environmental sample. It includes DNA from living organisms, from dead organisms or fragments of organisms, from gut contents as well as extracellular DNA directly released in the environment after cellular degradation. It is assumed that **if an organism is present in an ecosystem, its DNA can be found in a sediment sample even if the organism itself is not collected**. In order to obtain good representation of the DNA diversity present in a sample, a large amount of DNA sequences must be obtained. High throughput DNA sequencing provides revolutionary options for investigating biodiversity using eDNA[2, 3, 4]. Such massive molecular identification of metazoan communities will significantly advance knowledge in benthic research[5, 6]. In comparison to classical ecological studies based on a few selected taxa, high throughput sequencing of eDNA allows the comparison of thousands of taxa in parallel. The eDNA approach potentially allows standardisation in sampling, data acquisition and analyses. Therefore it is ideally suited to develop monitoring methods for environmental impact assessments.

References

1. Dannovaro et al. (2008) Cur. Biol. 18: 1-8.; 2. Foncesca et al. (2010) Nat. Coms. 1:98; 3.Pawlowski et al. (2011) PlosOne 6 (4): e18169; 4. Bik et al. (2011) Mol. Ecol.; 5. Creer et al. (2010) Mol. Ecol. 19:4-20; 6. Creer & Sinniger (2011) Mol. Ecol. 21:1033-1035.

２．研究の目的 aims

The main objectives of the project were to apply new molecular tools to:

1. Estimate vertical transfer of organisms and detritus from shallow to deep-sea ecosystems.

2. Determine the connectivity between deep-sea ecosystems.

These objectives were realised through the testing of two main hypotheses (Figure 1):

Hypothesis 1 (H1): Benthic DNA from shallow ecosystems is not transferred to deep-sea ecosystems.

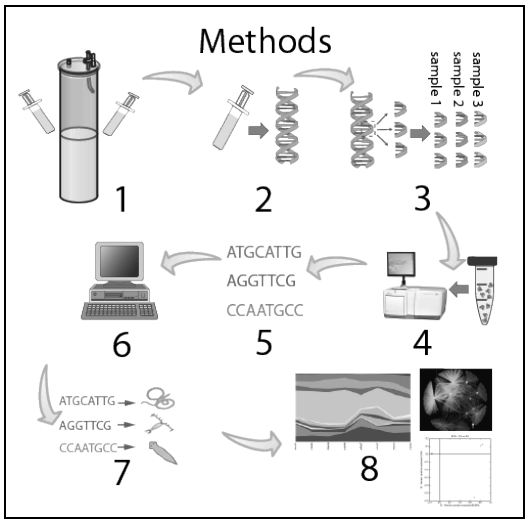
Hypothesis 2 (H2): Metazoan benthic communities living in sediments are not uniformly distributed among different deep-sea ecosystems.

３．研究の方法 methods

In this project I analysed eDNA extracted from sediments collected using cores at depths ranging from 3 to 11000 m. A metabarcoding approach was conducted using 18S rDNA and additional markers were used to sequence individual organisms in order to obtain baseline data on the biodiversity of the environments investigated at various levels of taxonomic resolution. For metabarcoding, sequences obtained using Titanium Roche 454 technology were used to characterize the taxonomic richness present in each sample. DNA from specific organisms (hydrothermal vents endemics or shallow corals) were tracked in the various samples to estimate the levels of eDNA dispersion. Measures of α- and β-diversity were used to compare similarities between the different samples and understand relationships between the different habitats. In addition, based on the results obtained in the first part of this project, the second part of the project focused to adapt this approach to Illumina sequencing and design new fusion primers to amplify targeted photosynthetic algae associated to shallow corals in the sediments to be used as a marker of dispersal of shallow water material to the deep-sea.

An overview of the metabarcoding approach is

illustrated below :

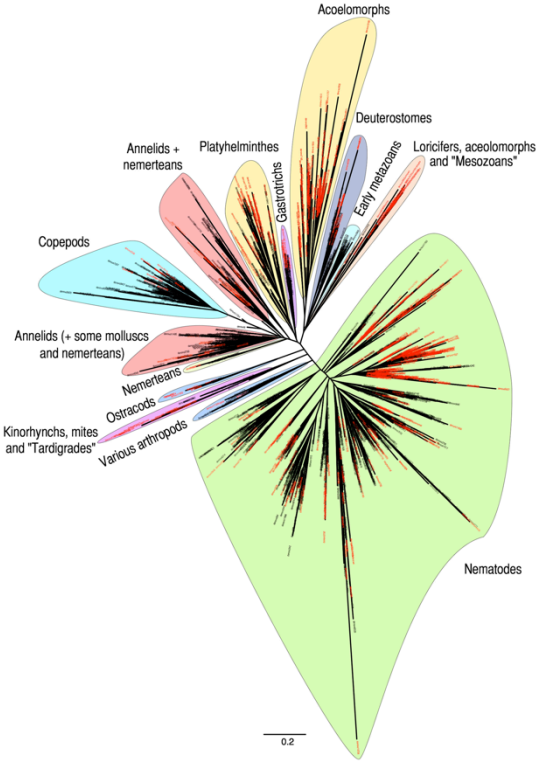


(1) Replicates of sediments are subsampled from cores and immediately frozen.
(2) Environmental DNA is extracted from the sediments using commercial MOBIO kits.
(3) Targeted gene is amplified from eDNA, including unique Molecular Identifier (MID) tag for each sample.
(4) All amplicons are pooled and sequenced using high throughput sequencing technique.
(5) MID tags are used to sort the sequences according to the original samples.
(6) Sequences are clustered into Operational Taxonomic Units (OTUs).
(7) Taxonomic lineages are assigned to the OTUs.
(8) Results are used for community analyses or to track specific taxa.

４．研究成果 results

The data obtained during this project were completed with data previously obtained from different locations. During this project, new eDNA data were obtained from the Japan Trench, the Okinawa Through as well as shallow coral reefs in Okinawa and deep-sea sediments from the South Pacific. The most important results came from massive sequencing of the partial nuclear small ribosomal subunit (18S) using next generation sequencing.

These results revealed a huge unknown genetic biodiversity in the deep-sea as well as in coral reef sediments and largely contributed to evaluate the gap of knowledge in worldwide deep-sea biodiversity (Sinniger et al. 2016). When compared with a reference database of sequences available on GenBank and phylogentically identified, the metabarcoding data obtained here clearly show a wide distribution of unknown taxa. The figure below (from Sinniger et al. 2016) illustrates the distribution of unknown taxa among metazoan, with unknown diversity indicated in red on the tree.



At a finer geographic scale, the results I obtained during this project based on sediments from the Okinawan region, showed that the approach I developed is able to distinguish different environments even if these environments are distant from less than a few hundred meters. The results also showed that sediments from the same type of environment (e.g. coral reefs) but from distant locations will be distinct, but still group near each other (Sinniger et al. in prep). The results from coral reefs at different depths showed a clear separation according to the depth with all the replicates samples from the same depth clustering together.

Overall these results indicate that while a transfer of DNA between environments certainly exists, it is not a major parameter affecting the estimation of genetic diversity in the sediments. This may be due to the large size of DNA fragments amplified, targeting in preference fresh DNA compared to the smaller fragments of DNA that would travel more easily.

In the second year of the project, I focused essentially to adapt the methods to the Illumina sequencing platform that allows a larger sequence output for a cheaper price. However, this technology also has its specific issues, especially regarding the formation of chimeras during the PCRs. To limit a maximum this issue, I designed fusion primers allowing library preparation in a single PCR (compared to 2 or 3 PCRs with the standard methods). The shorter DNA fragments targeted and the development of

specific primers for dinoflagellates algae allow to better trace heavily degraded extracellular DNA as well as photosynthetic organisms being transferred to the deep-sea.

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　1　件）papers

① Sinniger F, Pawlowski J, Harii S, Gooday AJ, Yamamoto H, Chevaldonné P, Cedhagen T, Carvalho G, Creer S. Worldwide analysis of sedimentary DNA reveals major gaps in taxonomic knowledge of deep-sea benthos. Frontiers in Marine Science, peer reviewed, 2016 vol. 3 article 92.
DOI : 10.3389/fmars.2016.00092

〔学会発表〕（計　6　件）presentation

① Sinniger F, From specimens to environmental DNA, perspectives and challenges in biodiversity studies. 5th JAMBIO Forum. 10th Feb. 2016 Tsukuba University, Tokyo Campus (Oral)

② Sinniger F, Mesophotic corals in Okinawa : a case of deep reef refugia ? Joint meeting of the Japanese Association of Benthology and the Plankton Society of Japan in 2015. 3rd Sep. 2015 Hokkaido University, Sapporo (Oral)

③ Sinniger F, From coral samples to eDNA, perspectives and challenges in mesophotic biodiversity. 2nd International Workshop on Mesophotic Coral Reef Ecosystems. 26th-31st Oct. 2014, IUI, Eilat, Israel. (Oral, Invited speaker.)

④ Sinniger F, Do different trenches share communities ? Hadal diversity viewed through environmental DNA. 3rd World Conference on Marine Biodiversity. 14th Oct. 2014 Qingdao, China (Poster)

⑤ Sinniger F, Metazoan Environmental DNA as a tool in deep-sea environmental impact assessment. 3rd World Conference on Marine Biodiversity. 14th Oct. 2014 Qingdao, China (Oral)

⑥ Sinniger F, Environmental DNA survey of metazoan biodiversity : A case study in hydrothermal vent sediments. Unraveling Biodiversity from DNA International Symposium. 9th Sep. 2014 NIES, Tsukuba (Poster)

６．研究組織
(1)研究代表者
フレデリック　シニゲル
（Frederic Sinniger）
琉球大学熱帯生物圏研究センター・
ポスドク研究員
研究者番号：10625940