Molecular biodiversity of benthic communities in deep-sea sediments-
examples from the phylum Foraminifera and Nematoda

Punyasloke Bhadury

Integrative Taxonomy and Microbial Ecology Research Group, Department of Biological Sciences, Indian Institute of Science Education and Research-Kolkata (IISER-K), Mohanpur Campus, Nadia, West Bengal, India.

[E-Mail: pbhadury@iiserkol.ac.in]

Received 8 November 2012; revised 5 December 2012

Biodiversity in deep-sea environments is very high but often under-estimated to a large extent due to paucity in sampling and taxonomic intractability of certain faunal groups. In deep-sea benthic environments, two faunal groups namely Foraminifera and Nematoda often dominate in their abundance and contribute to major ecosystem processes. However, information at the species level and biogeographic patterns of these two major benthic faunal groups are largely unknown with some scattered studies undertaken so far based on the application of molecular techniques. This review highlights how application of molecular techniques, including next generation sequencing (NGS) methodologies have improved our understanding of biodiversity patterns of two major groups, namely Foraminifera and Nematoda from deep-sea benthic habitats.

[Keywords: Biodiversity, Landscape, Meiobenthic, Biodiversity, Biogeographic]

Introduction

One of the significant findings in biodiversity research has been the discovery of high species diversity in deep-sea benthos. Deep-sea biodiversity has been found to be rich at landscape and community levels. Benthic diversity varies widely within and among habitats and found to be strongly correlated with depths and latitudes in deep-sea ecosystem as reported from different studies. Although deep-sea represents one of the world’s largest habitats, there is scarcity in terms of understanding of the meiofaunal biodiversity and community interactions from this type of environment. Limited information exists on deep-sea benthic communities at the molecular level, particularly for meiofauna which constitute a major component of over all diversity. Thus fundamental questions pertaining to genetic structure of deep-sea metazoan taxa may have major effects towards understanding of global biodiversity.

Molecular techniques offer an alternate perspective for a better understanding of deep-sea benthic diversity. Next-generation sequencing (NGS) technology is a new molecular revolution that is increasingly applied to study biodiversity of different organisms including deep-sea benthic fauna. Next-generation sequencing using Roche 454, Illumina and ABI SOLiD platforms with capacities to process millions of sequences in parallel have revolutionized biological research in recent years. Individual read lengths in majority of next-generation platforms are usually limited but the depth of coverage per base pair and advanced sequence assembly software allow sequencing of giga base pairs (Gbp) in a single run. The progress in sequencing methodologies has resulted in advancement of bioinformatics tools and analytical pipelines that can deal with large NGS datasets. NGS has been applied in numerous studies including elucidating microbial communities from deep-sea environments.

In this review, molecular diversity trends for two of the most abundant and functionally important deep-sea benthic phyla, Foraminifera and Nematoda have been discussed with a special emphasis on how NGS methodologies has increased understanding of biodiversity of these two groups from deep-sea benthic habitats.
Foraminifera

Benthic foraminifera, shell-bearing protists are more widely distributed in deep abyssal depths compared to their coastal and sublittoral counterparts\(^{16}\). However, undescribed single-chambered forms are reported to be more extensive in deep-sea benthic environments\(^{16}\). Adding to that, paucity of sampling from deep-sea benthic habitats makes it extremely difficult to establish the ranges of less common foraminiferal species, and endemism may be more prevalent than currently realized. Several studies have been undertaken in recent years to investigate molecular diversity and biogeographic patterns of benthic foraminifera which constitute major component of deep-sea benthic fauna. A study undertaken by researchers based on the amplification and sequencing of nuclear ribosomal RNA markers (SSU and ITS) revealed high genetic similarity between Arctic and Antarctic populations of three common deep-sea foraminiferal species namely *Epistominella exigua*, *Cibicides wuellerstorfi* and *Oridorsalis umbonatus* separated by distances of up to 17000 km\(^{17}\). The results were in contrast to substantial level of cryptic diversity usually revealed by molecular studies, of shallow-water benthic and planktonic marine organisms. The authors also concluded that the broad range distribution of deep-sea foraminifera supported the hypothesis of global distribution of small eukaryotes and that deep-sea biodiversity may be more modest at global scales than present estimates suggest. In another study, researchers targeted the small-subunit (SSU) and internal transcribed spacer (ITS) rDNA region in the Pacific Ocean population of benthic foraminifera *Epistominella exigua* and showed that the population were almost identical to those reported previously from the Arctic, Atlantic and Antarctic oceanic realms\(^{18}\). Their result confirmed genetic homogeneity in case of *E. exigua*, which was in contrast with the prevalence of highly differentiated populations in planktonic and shallow-water benthic foraminifera.

Based on next generation sequencing metagenetic approach, a group of researchers examined the richness of benthic foraminifera from 31 unsieved deep-sea sediment samples collected across five distinct oceanic regions\(^{11}\). Authors obtained 495,978 unique sequences based on a short fragment of the small subunit ribosomal DNA hypervariable region which they subsequently grouped into 1,643 operational taxonomic units, of which about half (841) were reliably assigned to Foraminifera. It was found that vast majority of the operational taxonomic units (nearly 90%) were either assigned to ancient lineages of soft-walled, single-chambered (monothalamous) foraminifera or remain undetermined and most likely belonged to unknown early lineages. Their study for the first time deviated from the classical view of multi-chambered taxa dominating foraminiferal assemblage and reflected an unexpected diversity of monothalamous lineages that are as yet unknown based on conventional micropaleontological observations. The authors also concluded that high-throughput sequencing can significantly improve understanding of deep-sea benthic diversity considered until now as unknowable based on traditional taxonomic approach.

Nematoda

Free-living marine nematodes are highly abundant in deep sea sediments, comprising 85-96% of all benthic metazoan phyla\(^{5}\). It has been estimated that the nematode species diversity in deep sea environments could be as high as $1 \times 10^8$\(^{19}\). However, accurate identification and subsequent estimation of nematode species diversity, in addition to their biogeographic patterns, has been largely hindered due to taxonomic intractability, shortage of nematode taxonomists and logistical problems associated in sampling the deep-sea.

For the first time using near complete sequence for 18S and 28S rRNA it has been shown that deep-sea nematodes from Pacific and sub Antarctic sites belonging to the order Enoplida, support a high global species richness and the observed phylogeographic patterns suggested existence of closely related, globally distributed species complexes in the deep sea\(^{20}\). The authors highlighted that future nematode studies should incorporate an extended sample set
covering a wide depth range (shelf, bathyal, and abyssal sites), utilize additional genetic loci (e.g., mtDNA) that are informative at the species level, and apply high-throughput sequencing methods to fully assay community diversity.

Based on 454 sequencing approach, a study was undertaken to assess microbial eukaryotic communities across depth (shallow water to abyssal) and ocean basins (deep-sea Pacific and Atlantic). Within the 12 sites examined, the authors found that some taxa can maintain eurybathic ranges and cosmopolitan deep-sea distributions, but the majority of species appear to be regionally restricted. Contrary to previous observation of nematodes being the most abundant members in sediment, the authors found equal or more dominant role for other taxonomic groups in some of the deep-sea sites (e.g. unicellular eukaryotes in the Pacific) based on 454 sequencing. In addition, low genetic divergence between geographically disparate deep-sea sites suggested either a shorter coalescence time between deep-sea regions or slower rates of evolution across this vast oceanic ecosystem. In another study involving deep-sea sites in the Southern Ocean, the authors investigated the association between deep-sea nematodes and marine fungi and potential implications including food preference of nematodes based on capillary and 454 sequencing approaches.

Way forward

One of the major obstacles towards application of molecular techniques in deep-sea benthic research has been limited availability of eukaryotic molecular reference sequence in published databases. This has largely hindered the development of robust computational pipelines needed to process NGS datasets for representative marker or gene. For example, only selected 18S rRNA sequences are available for deep-sea nematodes in reference databases (e.g. GenBank and EMBL) and therefore these under-represented databases are largely preventing the use of tools required for taxonomic lineage information (e.g. RDP classifier) in case of groups such as Nematodes. Although handful of studies have used molecular techniques including NGS platforms in deep-sea benthic groups such as Foraminifera and Nematoda, nevertheless vital information pertaining to deep-sea benthic diversity have been unravelled in recent years. The flow diagram (see Fig. 1) provides an overview of approaches to be undertaken to facilitate and speed up deep-sea benthic diversity research in future studies.

![Flow diagram](image.png)
To ensure reliable and speedy benthic biodiversity surveys of deep-sea sites there is an urgent need to generate full-length eukaryotic reference sequences for lesser known benthic phyla such as Foraminifera and Nematoda. In addition to development of robust eukaryotic 18S rRNA databases, there is a need to cover alternate locus such as 28S rRNA or almost nonexistent markers such as mitochondrial cox1 for facilitating future high-throughput NGS studies.

Although some progress has been made for benthic groups based on application of molecular tools, however major challenges including technological constraints lie ahead. There is a strong need to initiate sampling activities encompassing deep sea sites along with enriching the sequence databases to make biodiversity surveys more robust. In future, large-scale application of high throughput sequencing methods based on NGS platforms along with high end computational pipelines will provide much needed information on benthic biodiversity from the deep-sea bathyal and abyssal environments.

Acknowledgements

Grant awarded by the Ministry of Earth Sciences, Govt. of India under the Marine Living Resources [MLR] Programme is gratefully acknowledged.

References


