

Marine barcoding- how will it help Indian marine benthic studies?

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DNA barcoding has unraveled issues related to cryptic species complexes, assigning life history stages of unidentified organisms and also towards better understanding of evolutionary relationships between organismal groups. Given the advantages of DNA barcoding, one can strongly argue that barcodes may prove to be extremely useful for marine meiobenthic studies particularly for taxonomic groups such as free-living marine nematodes and polychaetes from the Indian subcontinent. An integrative taxonomic approach based on morphological traits based taxonomy and DNA barcoding would be ideal for studying the meiobenthic groups from Indian sedimentary environments.

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Introduction

Sound taxonomy underpins almost all biological research, nowhere more so than in ecology. Presently there is a serious biodiversity crisis with the disappearance of numerous taxa each day as a result of uncontrolled anthropogenic activities including global warming. The biodiversity crisis is accompanied by dwindling number of taxonomists throughout the scientific community resulting in the neglect of many highly diverse groups of organisms and more so in marine environments^{1,2}. Recruitment of new generation of taxonomists has been poor so far due to poor funding and added uncertainties in pursuing a career in taxonomy³.

DNA barcoding, based on the analysis of a small segment of the genome, is one potential way of simplifying and speeding up the evaluation and identification of taxonomically intractable groups. DNA barcoding promises fast accurate species identification by focusing on a short standardized segment of the genome^{4,5,6}. The main ambitions of DNA barcoding are to: (i) assign unknown specimens to species and (ii) enhance the discovery of new species and facilitate identification, particularly in cryptic microscopic and other organisms with complex or inaccessible morphology and to massively increase speed and processing larger data sets^{7,8,9}. Cytochrome c oxidase subunit I (COI) has been

proposed as the principle gene for barcoding organisms. Based on multiple studies, Hebert *et al* have suggested an approximately 650 bp of the COI gene which is relatively easy to amplify with standard primers¹⁰ and is sufficient enough to obtain resolution on all levels between species and phylum for majority of the groups albeit with some exceptions (e.g. nematodes). At gene level it provides, in many animal groups, strong species-level resolution, as for example for spiders¹¹, fishes¹², and moths¹³.

Application of DNA barcoding in marine meiobenthic studies

Biodiversity in the marine environment seems to be much less compared to the terrestrial environment and the valid species number is 184,850 or 350,607 taxa according to the World Register of Marine Species (WoRMS; <http://www.marinespecies.org>) (July, 2010). More thorough studies in oceanic realms might reveal that marine biodiversity is closer to terrestrial biodiversity. However, due to the impending taxonomic crisis there is a serious neglect in studying many marine invertebrate taxa, especially those from benthic sediments, which contain species rich communities of metazoans including large numbers of nematodes, polychaetes, crustaceans and molluscs^{14,15,16}. Majority of the species rich communities, especially those of nematodes and

polychaetes are believed to be of great importance ecologically and have proved to be highly sensitive indicators of anthropogenic stress in a range of situations^{17,18,19}. Despite such attributes, these groups have seen relatively limited use by marine ecologists largely as a result of taxonomic intractability. Also, the overwhelming number of individuals present in a square meter of sediment (for example nematodes $1 \times 10^{5-7}$) impedes attempts to describe communities in detail, even when such expertise is available. It is obvious that different organism groups are not equally well determined. Molluscs, annelids, echinoderms and crustaceans are examples of groups that are identified to a large part because they are well studied and thoroughly documented with description and identification keys. That may not be the case for under-studied groups such as the free-living marine nematodes. For example, British waters are amongst the most well-known in the world, but only 450 species of free-living nematodes have been recorded and described so far. This accounts for about 10% of the global described fauna. On the other hand it is extremely difficult to estimate marine nematode diversity and species richness from the Indian waters because of severe scarcity of datasets accompanied by limited taxonomic expertise. A common reason for not being able to determine the species fully is also that samples get damaged during collection, so that the diagnostic characters are lost. Individuals may also happen to be in a life history stage or of a sex that does have the diagnostic morphological characters to separate it from other species. For example, amongst the molluscs the aplacophorans are seldom, if ever, determined to species during inventories²⁰.

DNA barcoding has a lot to offer when it comes to the identification of organisms (e.g. nematodes, polychaetes) that are beyond the reach of traditional morphological demarcation and diagnostics. For example, a DNA barcoding approach based on the nuclear small subunit ribosomal DNA (18S rDNA) and large subunit ribosomal DNA (28S rDNA) have received great attention in terrestrial and marine nematology^{21,22,23}. The semi-conserved domains in 18S rDNA and 28S rDNA unraveled deep phylogenetic relationships within the phylum Nematoda, yet at the same time the more variable regions in the gene often works well for resolving relationships at different levels of classification^{24,25}. Both the loci are not known to significant levels of intraspecific polymorphism, and provide very good

separation of cryptic species in some taxonomic groups^{23,26}. Relative ease in PCR amplification and presence of several sets of robust PCR primers for these genes are an added advantage. Presently, a total of 600 plus sequences (18S and 28S) of free living marine nematodes have been deposited in GenBank and EMBL databases. These sequences can be assigned to 150 nominal species from 104 genera. In total, there are 41 species assigned to Enopleia and 109 species to Chromadorea. Within the second class, the most sequenced family is Chromadoridae (with 18 species), followed by Desmodoridae (9 species), Ethmolaimidae (9 species) and Xyalidae (9 species). However most of these sequences are either from the European or North American waters. Not a single marine nematode sequence from the Indian waters is represented in the GenBank and EMBL databases. While the COI has been proposed as a locus of choice for DNA barcoding, it is widely accepted that this gene is extremely difficult to amplify and unreliable in case of free-living marine nematodes. For polychaetes and other meiobenthic groups limited DNA barcoding efforts have been undertaken at a global scale. Therefore, it is extremely timely to initiate a coherent marine DNA barcoding programme with a focus on the marine meiobenthic communities from the Indian estuarine and coastal sedimentary environments. A national level workshop on DNA barcoding was held in Goa (December, 2008) under the auspices of Indian Ocean Census of Marine Life and similar views were also shared amongst the participants.

DNA characterization has given us new means to understand the morphological disparity among organisms. DNA barcoding has already made large impact in most fields of biology, not least in systematic, and many recent taxonomic revisions are based on insights from DNA studies. Hence a plea for DNA studies in taxonomy^{27,28} does not at all seem to be an undue recommendation without implying that DNA sequences are the only valid currency in taxonomy. The implementation of an integrative taxonomy approach (here we refer it to as a combination of taxonomy and DNA barcoding) will certainly provide new discoveries in Indian marine benthic study that could be pursued by research and requires a multitude of data about organisms and also wise taxonomic judgement. A coherent Indian DNA barcoding programme for marine meiobenthic groups would have far-reaching scientific advantages and

provide an excellent opportunity to contribute to the training of a new generation of taxonomists with molecular expertise. Once the reference DNA barcoding libraries for the Indian meiobenthic groups are completed, next generation sequencing like pyrosequencing (454 GS FLX platforms) will allow more reliable identification of environmental samples (e.g. deep-sea sediments, mangrove sediments) or species diet, with reverberations for studying the ecosystem level of biodiversity and may also help decision making to preserve and protect marine biodiversity now and into the future.

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