

Role of 12 S Mitochondrial Gene on Dimorphism and Coiling Direction in Benthic Foraminiferal Species *Pararotalia nipponica*

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ABSTRACT

Despite the extensive use of foraminiferal proxies for paleoclimatic reconstructions, the question how and why morphologic variations of foraminifera are regulated by climatic changes remains a mystery. The answer to such changes probably lies in the intricacies of the molecular properties of the protoplasm. However, molecular systematic analyses of foraminifera are yet to start in India. Here, beginning has been made in this direction, by establishing foraminiferal culture program. For that, live specimens (without staining) of benthic foraminiferal species *Pararotalia nipponica* were collected from the waters off Goa. Based on coiling direction and proloculus size, these live specimens were then sorted into four different groups viz., dextral -megalospheric, dextral - microspheric, sinistral - megalospheric and sinistral -microspheric. The specimens of these four groups were then subjected to molecular systematic analysis utilizing 12 S mitochondrial gene, using Polymerase Chain Reaction (PCR) technique. After the amplification of 12 S gene it was resolved on 1.2% agarose gel containing ethidium bromide. To make the results clear, re-amplification was carried out by taking the amplified DNA of the first set. The result shows more or less identical 12 S mitochondrial gene amplicon for each group. This result does show that as far as 12 S mitochondrial gene is concerned, all the morphologically different specimens, at the level of present experiment may be clubbed together as one species but have limitation in assigning any reason for the morphological differences.

INTRODUCTION

In order to predict imminent climatic changes, the need to know paleoclimate is widely felt these days. Various tools are being devised and tested for this purpose. Foraminifera, by virtue of possessing several attributes like small unicellular body, hard test etc., act as an excellent tool for paleoclimatic reconstructions. For such studies, the most frequently used properties of foraminifera, include the variation in total foraminiferal number, variation in foraminiferal assemblages, benthic to planktic ratio (Nigam and Henriques, 1992), mean proloculus size and dimorphic ratio (Nigam, 1986, Nigam and Rao, 1987 Saraswati *et al*, 2000), frequency of coiling direction (Nigam and Khare, 1992), isotopic

and chemical composition of the test (Lee *et al*, 2002), etc. Despite the emphasis on confirming many of the above techniques through culture experiments (Sen Gupta, 1999), there have been only few attempts in this direction, especially from Indian waters. But, during the last few years, beginning has been made by undertaking climatic application specific culture studies of foraminifera (Nigam *et al*, 1996, Nigam and Caron, 2000). With the help of such studies, properties used as proxies can be confirmed and used in a far better and confident way. Foraminiferal culturing provides another opportunity to attempt molecular systematic analysis of foraminifera, which is the most recent approach to evaluate climatic proxies and solve taxonomic

intricacies. The molecular systematic technique has widespread applications in foraminiferal studies (Pawloski, 2000). In recent years, it has mostly been applied to look into evolutionary aspects (Pawloski *et al.*, 1996, De Vargas and Pawloski, 1998, Stewart *et al.*, 2001) of foraminifera.

Micropaleontologists often noticed that slightly different individuals were placed under different species (Bhalla, 1972), genera or sometimes altogether different families, leading to confusion, thus distracting new workers to venture in this field. Molecular systematic technique is found extremely useful to settle these taxonomic problems (Pawloski *et al.*, 1995, Holzmann, 2000). In our opinion, this technique can also be used to know how and why climate brings about changes in various morphological parameters of foraminiferal tests, which are extensively used for paleoclimatic reconstructions. In the present work an attempt has been made to make a beginning in this direction. The molecular systematic analysis utilizing mitochondrial DNA has been done for benthic foraminiferal, trochospiral species *Pararotalia nipponica* (Asano). The objectives for selecting this particular species are, (i) to study the role of genetics on a species which exhibits different coiling (dextral-sinistral) direction and dimorphism (megalospheric-microspheric) - because these properties have earlier been used as paleoclimatic proxies and (ii) to evaluate its taxonomic status through genetics as based on peripheral spines, size of test and number of chambers, three different species reported earlier *viz-* *P. nipponica* (Asano), *P. taiwanica* (Nakamura) and *P. ozawai* (Asano), were later clubbed under one species (Bhalla, 1972), namely *P. nipponica* following taxonomic procedures.

MATERIALS AND METHODS

As for this work live specimens are required, a foraminiferal culture program was established at National Institute of Oceanography, Goa. Live specimens (without staining) of *P. nipponica* were picked from both surface sediment samples as well as marine alga *Sargassum*, collected from the waters off Goa. Based on proloculus size and coiling

direction, they were sorted into four categories *viz.* dextral-megalospheric, dextral-microspheric, sinistral-megalospheric and sinistral-microspheric (Fig. 1) with each group having 10 to 15 individuals. While sorting the specimens on these bases it was noticed that specimens in each group belonged to one of the earlier defined separate species *viz.*, *P. nipponica* (Asano), *P. taiwanica* (Nakamura) and *P. ozawai* (Asano), distinguished on the basis of spines and test periphery. These were then washed thoroughly with filtered marine water so as to remove any other marine microorganism and subsequently subjected to molecular systematic analysis following the protocol as outlined by Langer *et al.*, (1993).

The foraminifera were washed twice with sterile milliQ water and then homogenized in 10 μ l milliQ water. The stock solutions, PCR buffer (10X10 μ l), MgCl₂(25mM 6 μ l), Primer 12 SAL, and 12 SBH (20 μ M 1 μ l), (forward as well as reverse primer for amplification) and dNTP (10mM 4 μ l), were added in steps. Water was added to make up the volume up to 100 μ l. So in the final mixture PCR buffer is present in IX concentration, MgCl₂ in 1.5 mM, primer in 0.2 nM and dNTP in 0.4mM concentration. (All the reagents were supplied by Genei Bangalore). The PCR amplification was performed in a Labline thermal cycler. The steps used for amplification include, hot start (94°C, 5 minutes), heat denaturation (94°C, 30 seconds), annealing (42°C, 30 seconds) and amplification (72°C, 1 minutes) for 34 cycles. Taq polymerase was added after heat denaturation. As the results after first amplification were not clear, so, reamplification of amplified PCR product was performed by taking 1 μ l of amplified product, following the same procedure.

After performing PCR amplification and reamplification, the products were resolved on 1.2% agarose gel, impregnated with ethidium bromide. The gel was run with 0.5X TBE buffer. A molecular weight marker (DNA Ladder) of 100 - 1000 bp size was also ran with the sample. After running the gel, products were observed under an Electronic UV Trans-illuminator (AAB). The photograph was taken by ID advanced image editor (AAB software).



Fig.-1. UV-Transilluminator photograph of PCR product: A. Sinistral-Microspheric, B. Sinistral-Megalospheric, C. Dextral-Microspheric, D. Dextral-Megalospheric, E. Negative Control (No DNA), M. Molecular Weight Standard (DNA Marker).

RESULT

In present experiment, amplicons of all four sets express themselves at equal distance, the most prominent band, implying that the 12 S mitochondrial gene in all four sets comprise of equal number of base-pairs. viz.. ~400 bp, as inferred from the equivalence of DNA ladder run with the amplicons (Fig. 1). Some more fainter bands are also observed, which might be because of partial amplification of some other gene, due to the universal nature of the primer used for amplification of the 12 S mitochondrial gene.

DISCUSSION

The organisms including foraminifera adopt themselves to be in tandem with their surrounding environment. Often, these adaptations of organisms are reflected in the change in frequency of various forms (dextral-sinistral, megalospheric microspheric) of single species and considered to be related to

certain climatic parameters. With all the probabilities, the climatic parameters affect the morphology through genetic pathways. 12 S mitochondrial gene has earlier been applied to construct phylogenetic tree, based on the degree of differences in it in various organisms, and has also been supposed to be responsible for bringing out intraspecific morphologic variations. But, at the level of present experiment, the 12 S mitochondrial gene of all the four sets was found to be identical as far as number of base-pairs are concerned.

Our result does show that at the level of present experiment, all forms with morphological differences probably belong to same species and also provides a supporting evidence for clubbing together of *P. nipponica*, *P. taiwanica* and *P. ozawai* in favour of *P. nipponica*. However, the similarity in number of base-pairs in 12 S mitochondrial gene for all the four sets indicates that the morphological variations in *P. nipponica* are not reflected at the level of number of base-pairs in 12 S mitochondrial gene. The differences probably lie somewhere in the different type of proteins synthesized by the same gene at different time of the year, depending upon the type of environment prevailing at that time, or in some other gene. These possibilities constitute the future course of the work to be done in this direction. It is hoped that this modest beginning will pave the way for further work to decipher how a particular change in climate triggers the genetic changes responsible for the mode of reproduction and shaping the morphology of the individual.

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