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# Molecular Identification of Marine Calanoid Copepod *Paracalanus parvus* (Claus 1863) Using RFLP.

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**Abstract:** Correct identification of species is very essential to understand the zooplankton community structure and diversity. Owing to the similar morphological and restricted features of zooplanktonic copepods, to make correct identification is frequently complicated. Molecular techniques have the potential to provide definitive species identification, thereby overcoming the taxonomic ambiguity. Genomic DNA sequences, RAPD and RFLP are used to discriminate the closely related species. In the present study the genomic DNA of calanoid copepod *Paracalanus parvus* was isolated and digested with EcoR I and Hind III restriction enzymes. It produces their own fingerprints of 856, 691, 499, 285 and 090bp. This RFLP clearly distinguish the *Paracalanus parvus* from the other morphologically similar copepods.

**Key words:** Molecular identification % *Paracalanus Parvus* % Genomic DNA % RFLP

## INTRODUCTION

Copepods represent one of the orders of the phylum Arthropoda and are the most numerically abundant metazoans on the earth [1]. They comprise 11,500 nominal species placed in 200 families and 1,650 genera, although this figure may represent only 15% of the actual number of species [2]. Species identification is very essential to understand the zooplankton community structure and diversity. The usual approach to enumeration of the species in mixed samples is based on the slightly variable morphological characteristics like length of antenna, fifth walking leg and curvature coxa of the fifth pair of swimming legs.

Microscopic discrimination of copepods is possible only by experienced taxonomist at the late copepodite and adult stages. For a non-taxonomist, morphological method is both time consuming and ambiguous. Identification of the copepods at their larval stages is more intricated, because they show only limited variations in their morphology [3]. Copepod taxonomists with expertise in the identification of species in different groups of copepods are increasingly rare; consequently that species may be more frequently misidentified in ecological and oceanographic studies [4]. Therefore it is necessary to develop an effective and sensitive technique to identify the target species distributed in the plankton samples [5].

The introduction of molecular techniques into morphological ecology has greatly increased our knowledge by identifying the smaller aquatic organisms [6]. Molecular techniques have the potential to provide definite species identification, thereby overcoming the taxonomic ambiguity. Morphological similarities of the genus *Calanus* exhibit considerable base - sequence difference in their genomes [7] and thus it may be helpful to differentiate species. Genomic DNA sequences, Restriction Fragment Length Polymorphism (RFLP) and Random Amplified Polymorphic DNA (RAPD) techniques are mostly used to discriminate the closely related species [8]. The simple molecular technique of RFLP was used to differentiate the Calanoid copepods at any stages of its development [9]. In India, only very few works are available on molecular taxonomy/identification of marine copepods [10].

The Present study dealt with the extraction of genomic DNA from the calanoid copepod, *Paracalanus parvus* and its molecular characterization using RFLP.

## MATERIALS AND METHODS

**Collection and Preservation of the Samples:** Zooplankton samples were collected from the Vellar estuary (Lat.11°29' N and Long 79° 46' E ) using Indian Ocean Standard plankton net (Cloth No.10; Mesh size 158 µm; 0.35 m mouth diameter). The collected samples were first screened through 500 µm mesh to remove the fish and prawn larvae. After that the sample was rinsed with clear seawater and preserved in 95% ethanol

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at the rate of 100 individuals / ml. Adult specimens of *Paracalanus parvus* were identified and sorted out, using standard protocols of Kasturirangan [11], Newell and Newell [12] and Perumal *et al*. [13] and then preserved in 95 % ethanol to facilitate DNA isolation.

| Taxonomical position of *Paracalaus parvus* | |
|---|---|
| Phylum | : Arthropoda |
| Class | : Crustacea |
| Order | : Copepoda |
| Sub – order | : Calanoida |
| Family | : Paracalanidae |
| Genus | : *Paracalanus* |
| Species | : *parvus* (Claus, 1863) |

**Key to *Paracalanus parvus:*** It is a small calanoid copepod rather less than 1 mm in length. There are only three apparent thoracic segments because the first antenna is fused with the head and the fifth is fused with fourth segment. No plumose setae on the furcal rami. Urosome is segmented into five. The left foot is much longer than the right. A first antenna of the male is geniculate only in the right side. First antenna is not reaching beyond the caudal rami.

**Isolation of DNA:** The DNA was isolated by the Saline Citrate Solution (SCS) method. Two hundred milligram of copepod samples were suspended in 800µl of saline citrate solution (0.14M NaCl and 0.02M Trisodium citrate) and homogenized by ultra sonicator. The homogenate was transferred into a fresh centrifuge tubes and centrifuged at 3000 rpm for 10 min. After centrifugation, the supernatant was discarded and the pellet was resuspended in saline citrate solution and centrifuged at 3000 rpm for 5min. The pellet was again resuspended in 400 µl of 2M NaCl solution and centrifuged at 10,000 rpm for 15min at 4°C. Followed by centrifugation, the supernatant was collected in fresh microfuge tubes. To this double volume of ethanol was added and allowed for 6 min to precipitate the DNA. The purity of the isolated DNA was checked by an U.V Spectrophotometer at 260 nm and 280nm.

**Restriction Fragment Length Polymorphism (RFLP) Analysis:** Restriction digestion of the isolated DNA was done by using EcoR I and Hind III (Genei, India). Restriction digestions were performed on a 15 µl aliquot DNA from each stock by the addition of 0.5 µl 5M NaCl, 2 µl Bovin serum albumin (1mg $ml^{-1}$) and 2.5 Units of each restriction enzyme (EcoR I and Hind III). Incubations were performed at 37°C for 1 hr and the digested products were separated by electrophoresis through a 1.5% agarose gel impregnated with ethidium bromide. The gel was observed and the banding patterns were compared with molecular weight marker (100bp ladder). The fragments were analyzed using a gel documentation system (Lark, India).

## RESULTS

*Paracalanus parvus* was stored in 95% ethanol at the density rate of 30 individuals/ ml to acquire adequate quantity of DNA for digestion. The isolated DNA showed the absorbance of 1.78 at 260/280 nm. The DNA, was digested with the EcoR I and Hind III which produced five different fragments with the sizes of 856bp, 691bp, 499bp, 285bp and 090bp (Figs. 1-3).

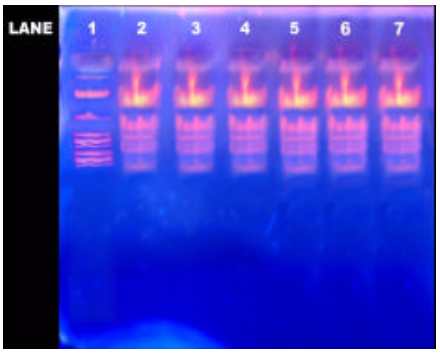


Fig. 1: Shows the RFLP banding patterns of Paracalanus parvus (Lane: 1 100bp marker, Lane 2-7 sample)

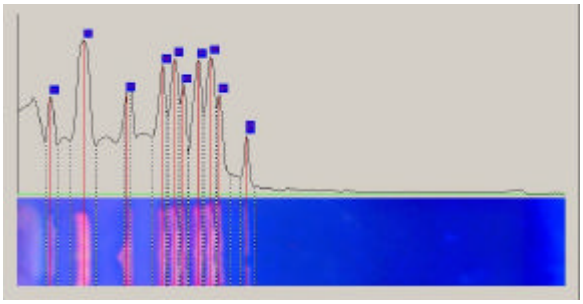

Fig. 2: Molecular weight marker.100- 1000 base pairs

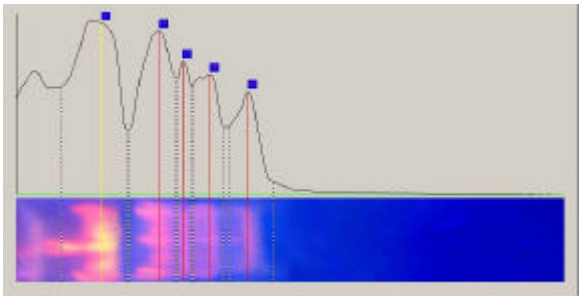

Fig. 3: Molecular weight of the RFLP bands. First (yellow) line- 856 bp, lines 2-5(Red): 691bp, 499bp, 285bp and 090bp respectively

## DISCUSSION

In this study the copepods were preserved in 95% ethanol to get adequate amount of DNA for digestion. The samples stored at higher density, debris are possible to be contaminating the samples [14]. So that in this study, the samples were initially preserved in higher density for the purpose of transportation and subsequently adult specimens of *Paracalanus parvus* were sorted out from the samples and stored in less density for DNA amplification. In this study the isolated DNA had the absorbance of 1.78 at 260/280 nm and thus showed very less contamination of protein.

RFLP represents a stretch of DNA that serves as a marker for mapping a specified gene. Restriction endonucleases are called "molecular scissors", as they can specifically recognize the base pairs of palindromic sequences and exactly cut the nucleotides at specific site. The EcoR I has 5'GAATTC 3' and 3'CTTAAG 5' recognition sequences and it cleaves at 5'G 9AATTC 3' and 3'CTTAA 9G 5' positions. Hind III has 5'-AAGCTT-3' and 3'-TTCGAA-5' recognition sequences and it cleaves at 5'-A9AGCTT-3' and 3'-TTCGA9A-5'positions. Sizes of the DNA Fragments (bands) produced by the RFLP are taken into genetic identification of the species [15]. RFLP is being employed to identify the individual calanoid copepods and this technique was first described by Lindeque *et al*.[9]. Presently *Paracalanus parvus* DNA was digested with EcoR I and Hind III, thus produced five fragments with the sizes of: 856bp, 691bp, 499bp, 285bp and 090bp respectively. Lack of studies related in this kind of the work make difficulties to compare the data's with others. Earlier Rajthilak [16] found that the copepod *Acrocalanus gracilis* was digested with EcoR I and Hind III produced only two bands (400 bp and 500 bp) and *Euterpina acutifrons* produced six fragments with the sizes of 700bp, 650bp, 600bp, 500bp, 400bp and 370bp. Poongothai [10]found that copepods like *Oithona rigida* and *Pontella danae* digested with EcoR I produced two fragments with the sizes of 1984bp, 846bp and 2752bp,1948bp respectively. Hind III also produced two bands with the sizes of 2313bp, 1708bp in *Oithona rigida* and 2249bp, 1437bp in *Pontella danae*.

The presence of specific palindromic sequences may be due to genomic structure of the individual. Bell *et al.* [17] have reported that RFLP was used to distinguish species within the family. Lindeque *et al.* [18] reported that RFLP was used to differentiate the calanoid copepods, even during their larval stages. Poongothai [10] pointed out that the RFLP was used to differentiate the morphologically similar but genetically varied (intra specifically varied) marine copepods. Beartriz diez *et al.* [15] also reported that the RFLP (Hae III), was easily differentiated the eukaryotic Pico plankton from their relative groups. The present investigation of RFLP can be used to easily discriminate the calanoid copepod *Paracalanus parvus* from their relatives by means of their own finger prints (856bp, 691bp, 499bp, 285bp and 090bp).Further studies are warranted along this line using many more copepod species.

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