

# THE USE OF DNA BARCODING IN IDENTIFICATION & CONSERVATION OF *NEMATOPALAEMON TENUIPES*, NON-PENAEID SHRIMP FROM MUMBAI COAST, MAHARASHTRA, INDIA.

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**Abstract :** Crustaceans are one of the best studied group of marine invertebrates because of its immense diversity. *Nematopalaemon tenuipes* belonging to the family Palaemonidae, is commercially important non-penaeid shrimp from the Maharashtra coast. So far, maximum species of shrimps have been demarcated based on morphological characters only. The aim of this study was to apply DNA Barcoding method to support Molecular Taxonomy by generating gene database of cytochrome c oxidase subunit I (COI) gene for *Nematopalaemon tenuipes*. COI gene from mitochondria is considered to be standard barcode as it shows potential to construct revealing phylogenetic clarifications for taxonomy. We were the first to publish genetic information on NCBI GenBank for *N.tenuipes* nucleotide sequence (Accession Number KY776472) which will contribute to build the gene library. The existing study serves as a primary record to identify and differentiate species on bases of molecular taxonomy.

**Keywords** - DNA Barcoding, *Nematopalaemon tenuipes*, COI gene, DNA Barcode Library, Molecular Taxonomy.

## I. Introduction

DNA barcoding has become a most important aspect of Molecular phylogenetics and it is an upcoming branch of scientific research which implies combination of molecular and statistical techniques to infer evolutionary relationships among organisms or genes. In recent years Molecular Biology has become a tool to overcome problems related to morphological identification which requires expertise to study the detailed morphological characters. DNA barcoding provides accurate, precise and reliable method for identification of species, larval forms, damaged specimens and processed food.

In DNA barcoding, sequencing mitochondrial gene COI have been found useful for correlation between taxonomic ranks and molecular divergence. For animals, a 648-bp fragment of the mitochondrial gene cytochrome c oxidase subunit I (COI) has been chosen as the standard barcoding marker due to its high interspecific variation, low intraspecific variation, and relatively universal primers (Hebert et al. 2003a, 2003b). Approximately a 648 base pairs gene of cytochrome c oxidase I (COI) in the 5'end of mitochondrial DNA (mt 37 DNA) is sequenced and used as the barcode by majority of researchers. Crustaceans exhibits wide range of morphological diversity which is not seen in majority of animals group. They are ecologically and economically very important group of invertebrates which includes crabs, shrimps, lobsters and stomatopods majorly in fishing. Shrimps are economically very much important as it is exported with high price rate. Penaeids shrimps are usually exported because of their size whereas small size shrimps or non-penaeid shrimps are consumed by locals. Non-penaeid shrimps are an important component of the dol net fishery of Maharashtra and occurs in enormous abundance, have great value in local market as they are consumed fresh and also in dried form (Deshmukh 1995). Non-penaeids catch contributes not only in economy but also on ecology being an important factor in marine food chain. Non-penaeid shrimps represent one of the important fishery resources contributing to 10.6% of total marine fish production from Maharashtra in 2016 (<http://www.cmfri.org.in/fish-catch-estimates>).

A great diversity of organisms is seen from marine, freshwater and semi-terrestrial ecology from order Decapoda.

*Nematopalaemon* is the most primitive genus from family Palaemonidae, infraorder Caridea under the order Decapoda. There are three species under this genus *hastatus* Aurivillius 1898 *schittimii* Holthuis 1950 and *tenuipes* Henderson 1893 around the world.

These species are widely spread throughout the marine water bodies on earth whereas *Nematopalaemon tenuipes* (Figure 1) is found only in fishing area no. 51 (Fischer et al. 1984). This paper deals with generating DNA barcode database for

*Nematopalaemon tenuipes*.

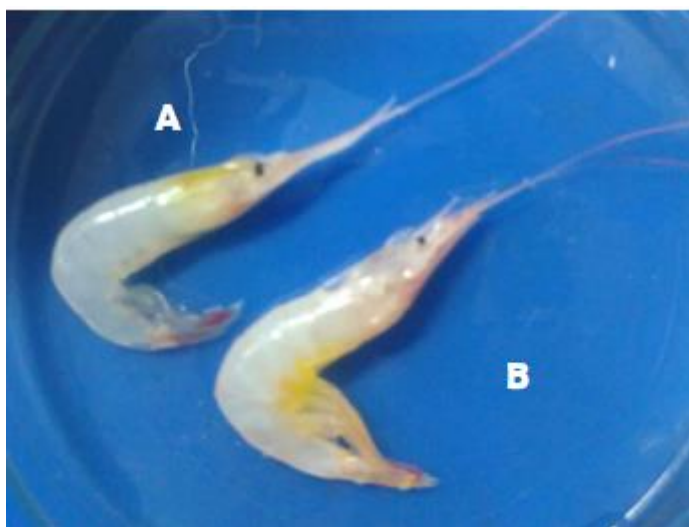


Figure 1. *Nematopalaemon tenuipes* (A) Above is the male specimen. (B) Below is female specimen carrying eggs.

## II. Material and methods

### Study area & collection site

Sassoon Dock is one of the oldest and major landing center of Mumbai, Maharashtra, India. It is the terminal point of Mumbai, (Figure 2) surrounded by Arabian Sea. Sassoon dock is the commercial landing center of non-mechanized boats operates dol nets in shallow waters i.e. 10-15 m depth (Sukumaran 1982). *N. tenuipes* is the main catch of dol net. Samples were collected from June to December 2016. Around 200 specimens were collected and were morphologically field identified using identification keys (Fischer et al. 1984).



Figure 2. Map showing Sassoon Dock from Mumbai City which is surrounded by Indian

### Morphological studies & Molecular analyses

The specimen of *N. tenuipes* were collected and submitted to Central Marine Fisheries Research Institute, Mumbai for authentication. Genomic DNA (gDNA) extraction method was standardized by modifying CTAB method (Doyle et al 1987, 1990). Muscle tissues from fresh samples were used for further treatment as it is perishable and affects the quality of gDNA. Quantification of gDNA was done with the help of NANO DROP 2000/200c at 260nm. The purity of gDNA was checked by Agarose Gel Electrophoresis (AGE) which reveals RNA contamination thus purification method was developed by treating DNA sample with RNase.

Amplification of mitochondrial COI gene was achieved using primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3' and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer et al 1994) by Polymerase Chain Reaction (PCR) technique. The reaction was carried out using GeneAmp 9700 Applied Biosystem thermal cycler. The reaction volume of 25  $\mu$ L containing 2.5  $\mu$ L of 10X buffer, 2  $\mu$ L of 10 mM dNTP, 1  $\mu$ L of Taq Polymerase, 10 p.mol of each primer and 100 ng concentration of DNA. Cycling parameter was optimized as, pre-running at 96°C for 5 minutes, followed by 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds and extension at 72°C for 30 seconds, and followed by final extension at 72°C for 10 minutes. Amplified product was resolved with 1.5% AGE and documented in GEL Documentation System. DNA Sequencing was performed by Sanger's Sequencing Method, from Eurofins, Bangalore, India. Further the raw sequences obtained from sequencing were statistically analyzed.

### III. Statistical analysis

The sequences were aligned using Multi Align (Corpet 1988) software online tool to verify the similarities between the sequences. Then they were trimmed by Chromas 2.6.4 version software and merged manually. Basic Local Alignment Search Tool (BLAST) which is an algorithm was performed to compare a sequence with a library or database of sequences. Identification and comparison of the similar protein sequences, BLASTx was carried out. The nucleotide sequence was translated into protein sequence or amino acid sequence using EXPASY (online) software.

### IV. Results:

**4.1. DNA extraction profiling:** (Figure 3) The pure genomic DNA isolated from *N. tenuipes* was detected by Agarose Gel Electrophoresis. The product from DNA extraction was run on 0.8% agarose gel containing Ethidium Bromide with 1Kb DNA Ladder. The gel shows good quality of gDNA which is further used for amplification of COI gene.

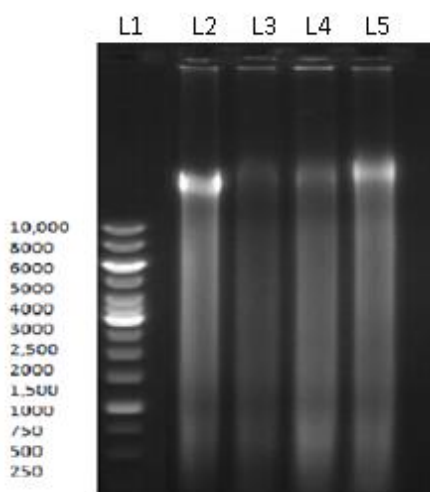


Figure 3. Lane 1 represents 1 KB DNA Ladder. Lane 2 to Lane 5 represents pure genomic DNA bands nearer to the well.

**4.2 COI gene amplification:** (Figure 4) PCR technique was used to amplify COI gene. To ensure amplification of interested gene, the PCR product was made to run on 1.5% agarose gel (containing Ethidium Bromide as staining dye) with 100bp for reference. The bands were observed around 700bp which means amplified gene is 700bp in length.

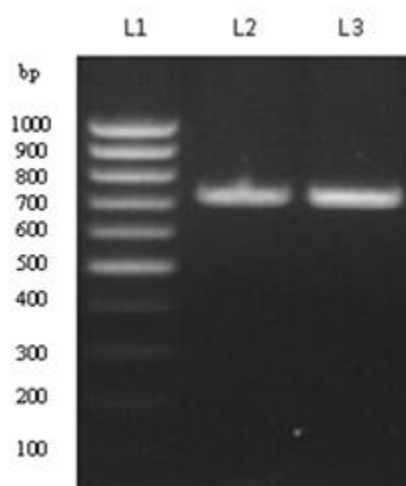


Figure 4. Lane 1 represents 100 base pairs DNA Ladder. Lane 2 & Lane 3 represents bands which shows COI gene amplification at approximately 700bp.

**4.3 DNA sequencing:** Sequencing of COI gene from *Nematopalaemon tenuipes* was carried out and submitted to BankIt, NCBI. It was allotted with Accession No. KY776472. This is the first sequenced data of *Nematopalaemon tenuipes* cytochrome oxidase subunit I gene to be published in gene bank. The published DNA sequence is as follows:

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TTAATCTGTGGGGCCTCAGCAGGTATAAGGGGAACATCCGTTAGGCTTTTAATCC
GAGCAGAGTTAGGACAACCAGGAAGACTCATCGGAAATGACCAGATCTACAACG
TTATTGTTACAGCTCATGCATTCATTATAATTTTCTTCATAGTTATACCAATTATA
ATTGGGGGCTTTGGGAACTGACTAGTACCGCTAATACTAGGGGCACCAGACATG
GCATTCCCTCGTATAAATAACATAAGGTTTTGACTCCTACCGCCATCTCTAACTCT
TCTCTTATCTAGAGGTATAGTGGAAGGGGAGTAGGAACTGGGTGAACTGTTTAC
CCCCGTTATCTGGAATATTGCTCATGCAGGGGCTTCAGTAGACCTAGGTATTT
TCTCACTTCACTTAGCAGGAGTTTCATCTATCCTAGGGGCTGTCAATTTTCATCACC
ACAGTAATTAATATACGATCAGCGGGCATAACTATAGACCGAATGCCTTTATTG
TGTGAGCAGTATTCTTGACCGCAATTCTTCTTCTCCTATCACTACCTGTCCTCGCA
CAGC
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## V. Discussion:

The studies related to DNA barcoding of crustaceans have been carried out by various researches around the world. Raupach et al. (2015) have barcoded marine crustaceans of North Sea; Biodiversities of decapoda crustaceans along coast of Brazil was studied by Manteatto et al. (2011) using DNA barcodes; The barcoding approach was also used to characterize the genetic diversity of shrimps of Alaska (Drumm et al. 2013) and Turkish waters (Bilgin et al. 2014). Central Institute of Fisheries Education, Mumbai have barcoded most of the Indian marine fishes. National Institute of Oceanography, regional center, Cochin have barcoded some penaeid prawns of Indian coast, of which samples were collected from Mumbai, Cochin (west coast) and Kakinada and Tutucorin (east coast); Rajkumar G. (2015) carried out molecular identification of shrimp species, *Penaeus semisulcatus*, *Metapenaeus*



*dobsoni*, *Metapenaeus revicornis*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* inhabiting Coromandel coast (Tamil Nadu, India) using mt-COI gene; R. Udayasuriyan from Tamil Nadu in 2015 carried out similar work on *Macrobrachium rosenbergii*, *Macrobrachium malcolmsonii*, *acrobrachium lamarrei*, *Macrobrachium lamarrei lamarroids* and *Caridina gracilipes*.

*N.tenuipes* is a caridean prawn found throughout the year caught from dol net and some particular period of year from trawl net. It has low fecundity as recorded by Sukumaran in year 1983 which led to the great danger of being overfished to the extent of total depletion. This will disturb the food chain as it is main food for many fishes. Shubhadeep Ghosh had mentioned *N. tenuipes* as the most important food item in the diet of *Harpadon nehereus* commonly known as Bombay duck. The species was studied in various aspects from 1979 till 2009 by various researchers. The topics like biology, mortality, population dynamics etc. had attracted their interest. (Sukumaran 1982, 1983; Deshmukh 1988, 1994; Kunju 1967, 1979; Kizhakudan et al 2009)

Till date there is no molecular level investigation carried out in support of morphological identification of *N. tenuipes*. Here, the application of DNA barcode shows great valuable role to define species which are important for studying molecular diversity. DNA barcoding serves as a basic tool to understand Molecular phylogenetics. It can be termed as the blind test for authenticity of species.

Fresh samples from Sassoon Dock were collected and process to get good quality gDNA. Pure gDNA was obtained by trying different extraction methods which was finally standardized to modified CTAB method. Molecular diagnostic instrument like NANO DROP was used to check gDNA quality and quantity. gDNA quality and quantity was analyzed by using NANO DROP. RNA contamination will interrupt in amplification; hence RNase treatment was carried out to get pure gDNA. PCR technique was used to produce multiple copies of COI gene by optimizing reaction and cycling parameters. Amplified DNA was sequenced by Sanger's Sequencing method. The barcode sequence was prepared for submission by applying various Bioinformatic tools. High quality DNA barcode was obtained for *Nematopalaemon tenuipes* COI gene, which served as primary information for barcode library. Gene Bank provides a database that could be used to identify unknown species by comparing with previous records. *N. tenuipes* is the only species from genus *Nematopalaemon* under family *Palaemonidae* found in Indian marine waters which comes under Fishing Area 51. BLAST reveals nucleotide sequence comparison which shows maximum similarity of this species with *Macrobrachium* species as both rests in same family. This information can be useful for further inter species and intra species molecular studies. Further this work will form a base line to work on molecular taxonomical studies to find geographically distant species, morphological characters compared with their molecular data and many more aspects related to it.

The research work was intended to determine the efficacy of mitochondrial cytochrome oxidase subunit I gene (mt COI) gene to verify the taxonomically identified *N. tenuipes*, a non-penaeid shrimp from Mumbai region, Maharashtra and also to appraise the genetic variability.

## VI. Acknowledgement:

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