The Molecular Characterization and Phylogenetic Reconstruction of Penaeid Shrimps from Mumbai Coast, Maharashtra

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Abstract
The commercial Penaeid shrimp *Fenneropenaeus indicus* H. Milne-Edwards, 1837, *Parapenaeopsis stylifera* H. Milne-Edwards, 1837 and *Solenocera crassicornis* H. Milne-Edwards, 1837 were collected to estimate phylogenetic relationships and taxonomic status amongst other members of Family Penaeidae from Fishing Area 51. The present results suggest that *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* from different locations or countries from Fishing Area 51 belong to the same genetic population. This study revealed the identification of shrimp species based on the molecular approach using mitochondrial COI gene marker. Sequence of Penaeid shrimps, *Fenneropenaeus indicus* (MK488093), *Parapenaeopsis stylifera* (MH724294) and *Solenocera crassicornis* (MK511444) from Mumbai, western coast of Maharashtra were published in NCBI. This result will be useful for obtaining the intraspecific and interspecific genetic distance, genetic biodiversity of the population structure and for the conservation and management of these resources. *P. stylifera* and *S. crassicornis* shows close relation with each other and *F. indicus* form different clade.

Keywords: DNA barcoding, COI gene, penaeid shrimps, intraspecific and interspecific relationship, phylogenetic analysis

1. Introduction
The penaeid shrimps form varied group of marine decapods with more than 400 species globally. Their habitat is both shallow waters and abyssal zone under 5000 m. Family Penaeidae is a diversified, miscellaneous and worldwide scattered family of shrimps. Most of the shrimps from the family are served as delicacy in many countries which increases its economic status. This made family Penaeidae as the most important family amongst commercial crustaceans. As in most penaeid genera, species diversification is mainly based on morphological character such as rostrum structure, shapes of the genital organ i.e., thelycum in female and petasma in male. Identification of species by morphological structures many a times is insufficient and ambiguous may be because larval stages of some group cannot be assigned to the correct species [1]. Morphological identification becomes much difficult when specimens are damaged due to rough handling. As these are commercially important shrimps and can lead to fish fraud and economy loss. Shrimps have unique colour system and are capable to change body colour according to age, background and presence of sun or moon [2,3]. Thus, morphological classification of shrimp species almost leads to the unsuccessful and inconclusive assignment of correct species [1,4].
Molecular biology in recent years has become a tool to overcome the difficulties associated with morphological recognition, which requires knowledge to observe specific morphological characteristics. Molecular identification or DNA Barcoding technique was introduced for rapid, accurate and authenticate identification of biological specimens [1]. It also provides an opportunity to understand and evaluate genetic variability and diversity of species. In this technique, universally accepted mitochondrial cytochrome c oxidase subunit I (mt COI) gene is used as molecular marker. This gene is conserved as changes in its amino acid sequence happen comparative slowly and less subjected to external forces [5-7]. Molecular approaches are often predicted to offer a new and more specific method for species identification and generation of phylogenetic relationships among species.
Molecular evolutionary relationships between major penaeid shrimp lineages have been studied using mitochondrial gene sequences [8]. Molecular phylogenetics relationship of superfamily Penaeoidea was studied with respect to 16S rRNA gene [9-11]. The DNA barcoding technique was used to reveal genetic diversity of shrimps of Alaska, Turkish and Japanese waters [12-14]. The molecular phylogeny of the genus Penaeus of marine shrimp was reconstructed using mitochondrial DNA sequences [15].

Though there is economic significance, only few studies have been done from Indian waters. The marine penaeid shrimps and freshwater prawn species were morphologically described and molecularly identified from Tamil Nadu using mt COI gene [16, 17]. Kundu et al. (2018) had generated DNA barcode of morphologically identified six penaeid shrimps, *Penaeus monodon*, *Fenneropenaeus indicus*, *Litopenaeus vannamei*, *Metapenaeus ensis* and *Metapenaeus dobsoni* from Chilika Lake [18]. The sequences were phylogenetically compared with the database and the genetic variation describes different population with different collection sites. Purushothaman et al. 2019, studied the taxonomy of 14 commercially important deep water penaeid prawn from the south-eastern Arabian Sea and Bay of Bengal [19]. Karuppasamy et al. 2020 discloses the efficacy of mt COI genes in the reconstructing Penaeidean and Caridean phylogeny [20].

DNA barcode has become one of the most critical elements of molecular phylogeny and it is an upcoming branch of scientific research. In this study we used partial sequences of cytochrome c oxidase subunit I (COI) from mitochondrial genome to elucidate both taxonomy and phylogenetic relationships amongst almost all the taxa and forms in family Penaeidae. It can be further studied to define the grouping clades and to infer the origins, evolution and dispersal patterns of these commercially important shrimps from fishing area 51.

2. Materials and Method

2.1 Sample collection and morphological identification

Fresh samples of *Fenneropenaeus indicus* and *Parapenaeopsis stylifera* *Solenocera crassicornis* were collected from three major fishing centres of Mumbai, viz. New Ferry Wharf (Bhaucha Dhakka) located 18° 57' 22.97" N, 72° 50' 57.34" E from southeast Mumbai; Sassoon Dock, located 18° 54' 41.81" N, 72° 49' 34.11" E, the terminal point of the Mumbai suburban. They were morphologically identified with the help of field identification key (Fischer, Bianchi 1984) and later on authenticated by CMFRI, Mumbai [21].

2.2 Molecular Identification

DNA from the fresh muscle tissues was extracted using modified CTAB method [22, 23]. Agarose Gel Electrophoresis (AGE) technique was used to check the purity of extracted DNA. The polymerase chain reaction (PCR) technique was used to amplify mitochondrial COI gene by using forward primer LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAATATCA-3') in the GeneAmp 9700 Applied Biosystem thermal cycler [24]. The optimized PCR parameters was 5 min at 96°C, 35 cycles of 30 sec at 95 °C, 30 sec at 50 °C annealing, 30 sec at 72°C and final extension for 10 min at 72 °C [22, 25]. DNA Sequencing was carried out using Sanger’s Sequencing Method. The raw sequencing output data of *F. indicus* and *P. stylifera* were analysed and modified by various bioinformatic tools and software. Chromas Version 2.6.6 (http://technelysium.com.au/) software was used to trimmed, Multiple Alignment online software (http://multalin.toulouse.inra.fr/multalin/) used to align and merged by online tool Emboss Merger. The algorithm Basic Local Alignment Search Tool (BLAST) and BLASTx search were used to compare nucleotide and protein sequences from GenBank. The partial mitochondrial COI gene sequences of *F. indicus*, *P. stylifera* and *S. crassicornis* were deposited in International database NCBI BankIt/GenBank and allotted with Accession numbers MK488093 and MH724294 respectively and were published in NCBI.

2.3 Statistical Analyses

Multiple Alignment online software was applied to compare protein sequences of *F. indicus*, *P. stylifera* and *S. crassicornis* from various location from fishing area 51 to infer intraspecific relations.

To study interspecific relations, partial sequences of mt COI gene of 16 other family members of Penaeidae found in F.A.51, were downloaded from GenBank. The phylogenetic tree was built with the help of Pairwise Distance matrix. The cladogram was generated by protein sequence translated from DNA sequences in MEGA version 7.0.26 1993-2020 [26] using Neighbor-joining method [27]. The tree was built by bootstrap method [28] with 500 replications.

3. Results and Discussion

The extracted genomic DNA was analyzed on 0.8% agarose gel and it was found to be free of contaminants (Fig 1). This genomic DNA was used for PCR amplification. Amplified COI gene was visible at 700 bp on 1.5% agarose gel (Fig 2). DNA sequences of *F. indicus*, *P. stylifera* and *S. crassicornis* COI gene from various sites were successfully submitted to NCBI and published in GenBank with Accession No. MK488093, MH724294 and MK511444 respectively.

![Fig 1: Gel image represents pure genomic DNA. L1 signifies 1 kb DNA Ladder. L2, L3 and L4 stand for species *F. indicus*, *P. stylifera* and *S. crassicornis* respectively.](http://www.fisheriesjournal.com)
3.1 Intra specific relationship

Multiple alignment online tool compares protein sequences derived from GenBank database and depicts the relation between genetically isolated species. The homologous COI protein sequence of *F. indicus* from various places, MK488093 (Mumbai) India, KP688365 Mozambique, KU324636 Egypt were found to be from the same gene pool (Fig 3). The homologous COI protein sequence of *P. stylifera* from different places MH274294 Mumbai, India; MH171248 Ratnagiri, India; KU324661 Egypt and KR261594 Iran were found to be from the same gene pool (Fig 4). The homologous COI protein sequence of *S. crassicornis* from different places KP136603 Turkey, KJ879309 Spain, LC477205 Egypt, KX584723 Kerala India, MN40982 Gujarat India, AY264902 China, MK511444 Mumbai India, MT178734 Taiwan, MN205325 Pakistan were found to be from same gene pool (Fig 5).

The aligned species sequences were realized to be from the same population and genetic diversity was not seen with respect to mt COI gene. This is probably due to the reason that presently there is no isolation of water mass on the globe.

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**Fig 2:** Gel image signified an amplified COI gene at 700 bp. L1 denote 100 bp DNA Ladder. L2, L3 and L4 stand for species *F. indicus*, *P. stylifera* and *S. crassicornis* respectively.

**Fig 3:** Protein sequences alignment of *Fenneropenaeus indicus* KP688365.1 Mozambique, KU324636.1 Egypt, AF284431.1 Thailand, HM274161.1 Sri Lanka, MK488093.1 (Mumbai) India, MF614762.1 Bangladesh, AF279837.1 China, AY395239.1 South Africa.
2.2 Inter specific relationship
The phylogenetic tree, was built with the help of pairwise distance matrix of *F. indicus*, *P. stylifera* and *S. crassicornis* (Fig 6). Cladograms are tree like diagrams generated to study molecular phylogenetics which represents genetic evolutionary history of organisms. It expresses ancestries of organisms by using molecular data, i.e. DNA or protein sequences (Xiong 2006). Gene phylogenetic study expresses the evolution of that particular gene. The evolutionary cladogram was generated from DNA sequences in MEGA version 7.0.26 (Kumar et al., 2016). The constructed tree illustrates that *F. indicus*, *P. stylifera* and *S. crassicornis* are closely related to each other.
S. crassicornis found in different area belongs to their respective common genetic population and they make common clade with their groups. The sequence divergence of genera Parapenaeopsis and genera Solenocera is 0.04-2.0; genera Fenneropenaeus and Parapenaeopsis is 0.05-2.0 and genera Fenneropenaeus and Solenocera is 0.03-1.95. The pairwise distance of F. indicus from Mumbai and other areas is 0.02-1.9; P. stylifera from Mumbai and other areas is 0.01; S. crassicornis from Mumbai area and other areas is 0.04-1.9. Tree shows three major clades, one with group P. stylifera make close relation with group S. crassicornis whereas group F. indicus make outgroup.

![Phylogenetic tree](image)

**Fig 6:** Phylogenetic tree representing relationship between F. indicus, P. stylifera and S. crassicornis associated to COI gene protein sequences using NJ method.

| L   | 1          | 2          | 3          | 4          | 5          | 6          | 7          | 8          | 9          | 10         | 11         | 12         | 13         | 14         | 15         | 16         | 17         | 18         | 19         | 20         | 21         | 22         | 23         |
|-----|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|------------|
| 1   | KP688365.1 | 0.00       | AF279837.1 | 1.946      | MK488093.1 | 0.000      | KU324636.1 | 0.000      | AF284431.1 | 0.000      | HC417161.1 | 0.000      | MF614762.1 | 0.000      | KP688365.1 | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      |
| 2   | KP688365.1 | 0.00       | AF279837.1 | 1.946      | MK488093.1 | 0.000      | KU324636.1 | 0.000      | AF284431.1 | 0.000      | HC417161.1 | 0.000      | MF614762.1 | 0.000      | KP688365.1 | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      |
| 3   | AF279837.1 | 1.946      | AF279837.1 | 1.946      | MK488093.1 | 0.000      | KU324636.1 | 0.000      | AF284431.1 | 0.000      | HC417161.1 | 0.000      | MF614762.1 | 0.000      | KP688365.1 | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      | 0.029      |

**Table 1:** Phylogenetic tree between F. indicus, P. stylifera and S. crassicornis associated to COI gene protein sequences using NJ method.
According to Samadi et al. [29], P. stylifera and Metapenaeopsis striolata make monophyletic group showing close relation to Penaeus, *Fenneropenaeus merguiensis* and *Penaeus semisulcatus* using COI gene. But studies using both 16S rRNA and COI gene [15, 29-32] indicate that, *F. indicus* shows close relationship to sister group of *F. penicill* and *F. silasi*, *F. merguiensis* join as subclade and *F. chinensis* make outlying sister taxon. The present work shows similar results to Rajkumar et al. [16] stating that *S. crassicornis* and *P. stylifera* are monophyletic and *F. indicus* form different clade as evident from Neighbour-joining tree of Mt-COI gene sequence.

4. Conclusion

The taxonomy is usually considered with the support of morphological characters but morphological data is inadequate to attain phylogenetic relationship. This problem has been overcome by molecular technique i.e. DNA barcoding and its application on zoogeographical evolution. Producing DNA barcodes and submitting to GenBank is contributing for building of the gene library and modern world. Population study is generally studied concerning morphological characters but now a days it is supported by genetical records. Species from different areas are morphologically and genetically identical, zoogeographically isolated or not can be confirmed by molecular alignments (multiple alignments).

In this present research work, we were able to find intra and inter specific relations of *F. indicus*, *P. stylifera* and *S. crassicornis* using bioinformatic tools. *P. stylifera* and *S. crassicornis* are closely related to each other and *F. indicus* is recent common ancestor. Until now the doubtful evolutionary relation amongst Penaeidae genera not properly addressed, hence this inclusive evaluation of Penaeidae phylogenetics helped to look at a few elements of it. The phylogeny inferred in this study may be used in the future for taxonomic purposes and probably in a divergence time evaluation which may additionally make clear the origin and diversification of the family. More genes should be sequenced to make clear the taxonomic dilemmas of the family.

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