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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 [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 monoceros*, *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 origin, 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′-TAAACTTCAGGGTGACCAAAAAATCA-3′) in the GeneAmp 9700 Applied Biosystem thermal cycler [24]. The optimized PCR parameters was 5 min at 96^{O}C, 35 cycles of 30 sec at 95 ^{O}C, 30 sec at 50 ^{O}C annealing, 30 sec at 72^{O}C and final extension for 10 min at 72 ^{O}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.

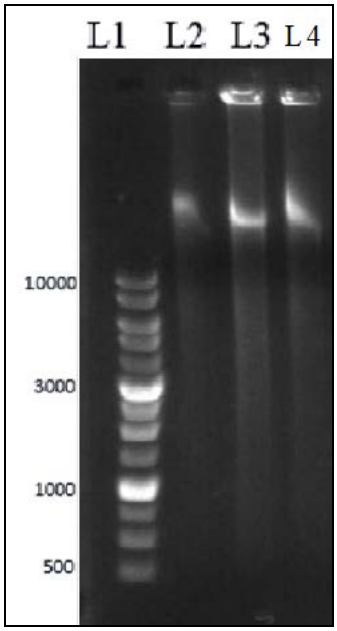

Fig 1: Gel image represents pure genomic DNA. L1 signifies 1 kb DNA Ladder. L2, L3 and L4 stand for species *F. indicus, P. stylifera* and *S. crassicornis* respectively.

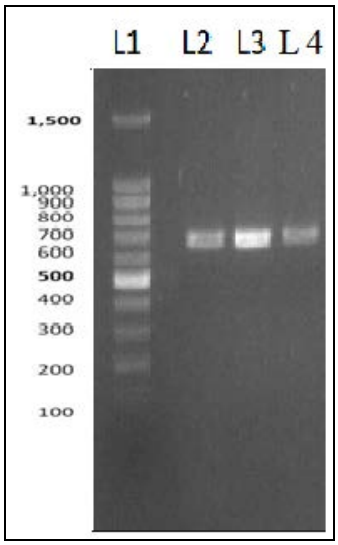

Fig 2: Gel image signified an amplified COI gene at 700 bp. L1 denote 100 bp DNA Ladder. L2, L3 and L4 stand for species *F. indicus*, *P. stylifera* and *S. crassicornis* respectively.

3.1 Intra specific relationship

Multiple alignment online tool compares protein sequences derived from GenBank database and depicts the relation between geographically 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 MH724294 Mumbai, India; MH712489 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, MN340982 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.

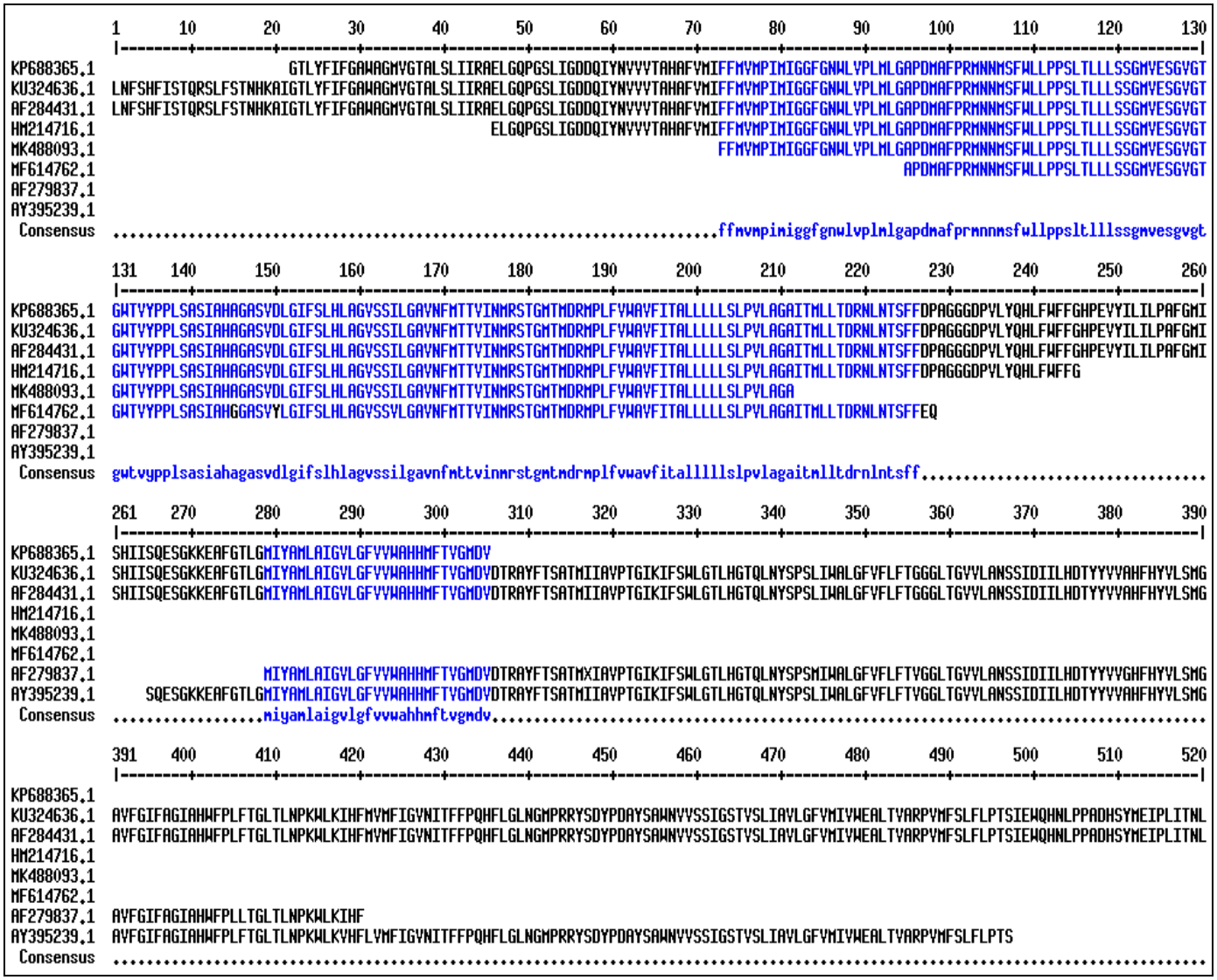

Fig 3: Protein sequences alignment of *Fenneropenaeus indicus* KP688365.1 Mozambique, KU324636.1 Egypt, AF284431.1 Thailand, HM214716.1 Sri Lanka, MK488093.1 (Mumbai) India, MF614762.1 Bangladesh, AF279837.1 China, AY395239.1 South Africa.

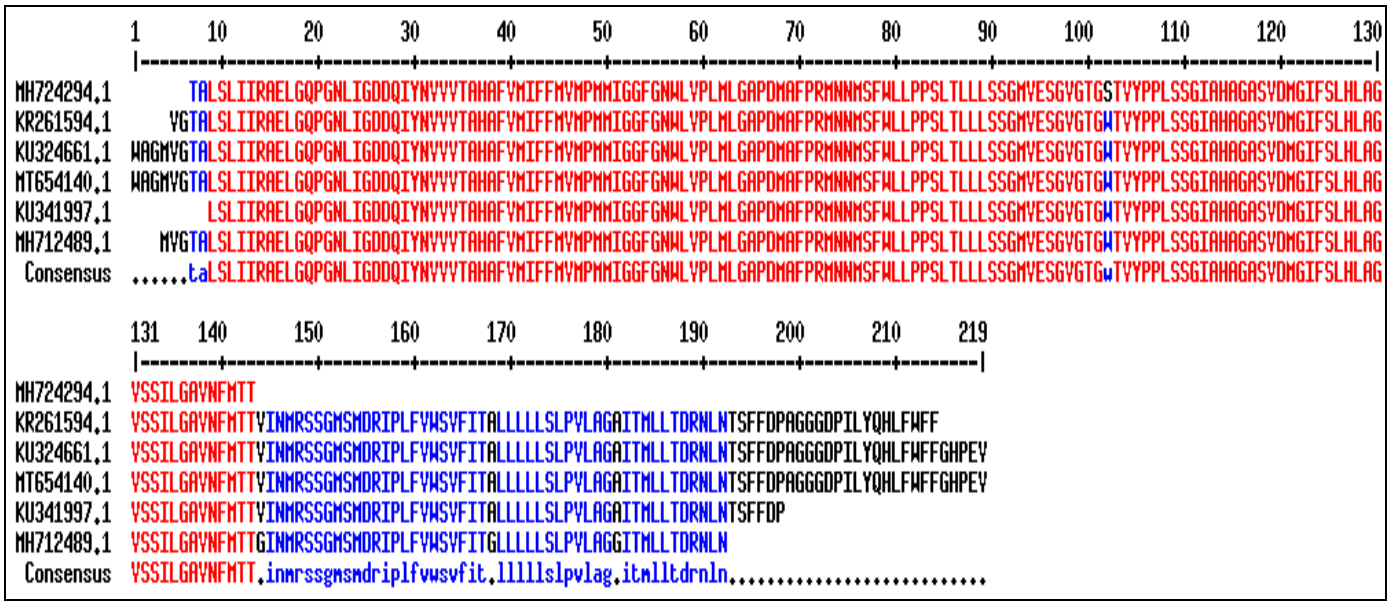

Fig 4: Protein sequences alignment of *Parapenaeopsis stylifera* found MH724294.1 (Mumbai) India, KR261594.1 Iran, KU324661.1 Egypt, MT654140.1 (Tamil Nadu) India, KU341997.1 (Kerala) India, MH712489.1 (Ratnagiri) India.

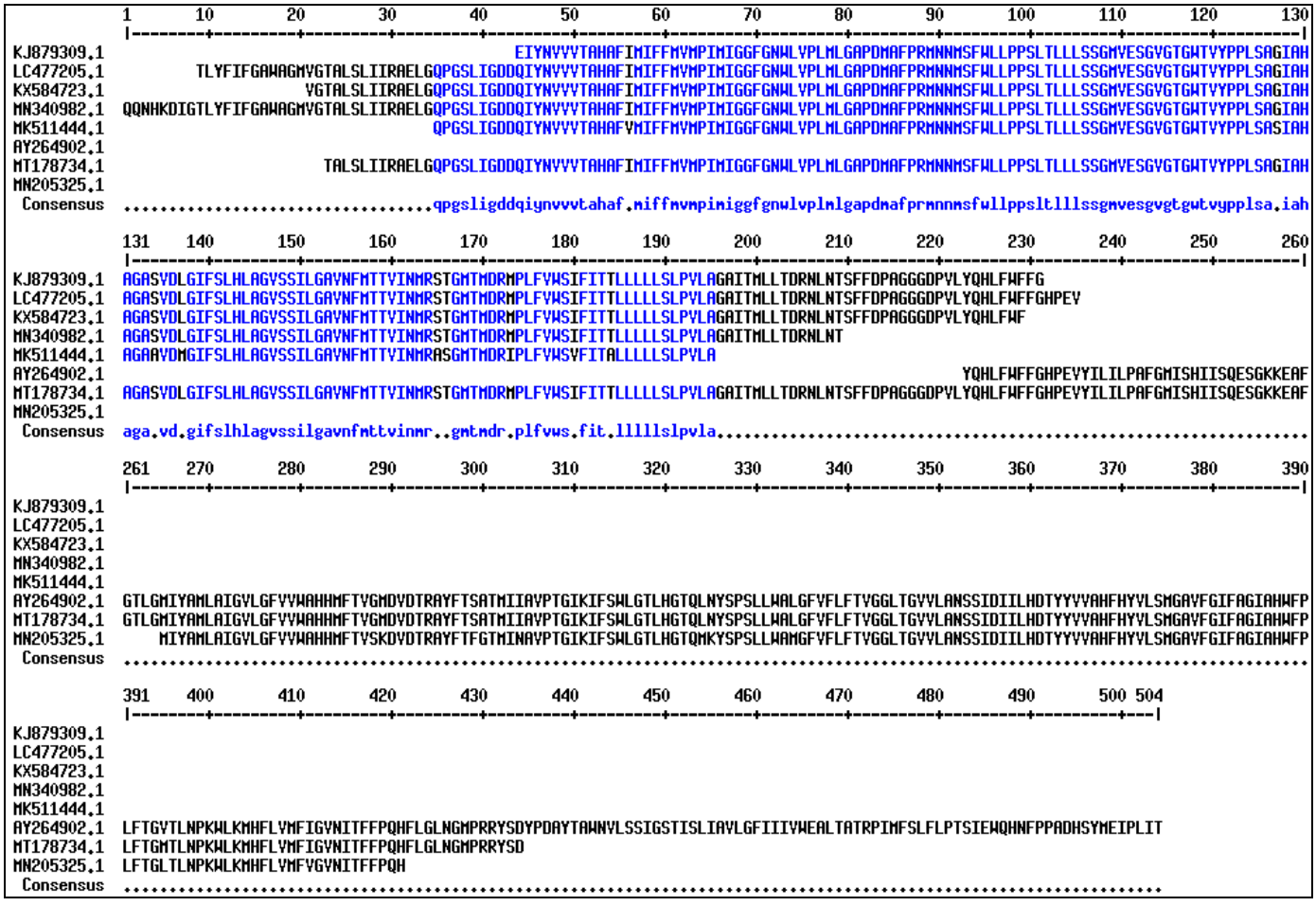

Fig 5: Represents the protein sequences alignment of *Solenocera crassicornis*, KJ879309.1 Spain, LC477205.1 Egypt, KX584723.1 (Kerala) India, MN340982.1 (Gujarat) India, AY264902.1 China, MK511444.1 (Mumbai) India, MT178734.1 Taiwan, MN205325.1 Pakistan.

3.2 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 acquired from the gene/protein sequences can be contradictory from species evolutionary history. Therefore, the species evolution may not link with the gene evolutionary line.

The cladogram was generated by protein sequence translated from DNA sequences in MEGA version 7.0.26 1993-2020. The constructed tree illustrates that *F. indicus*, *P. stylifera* and

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.

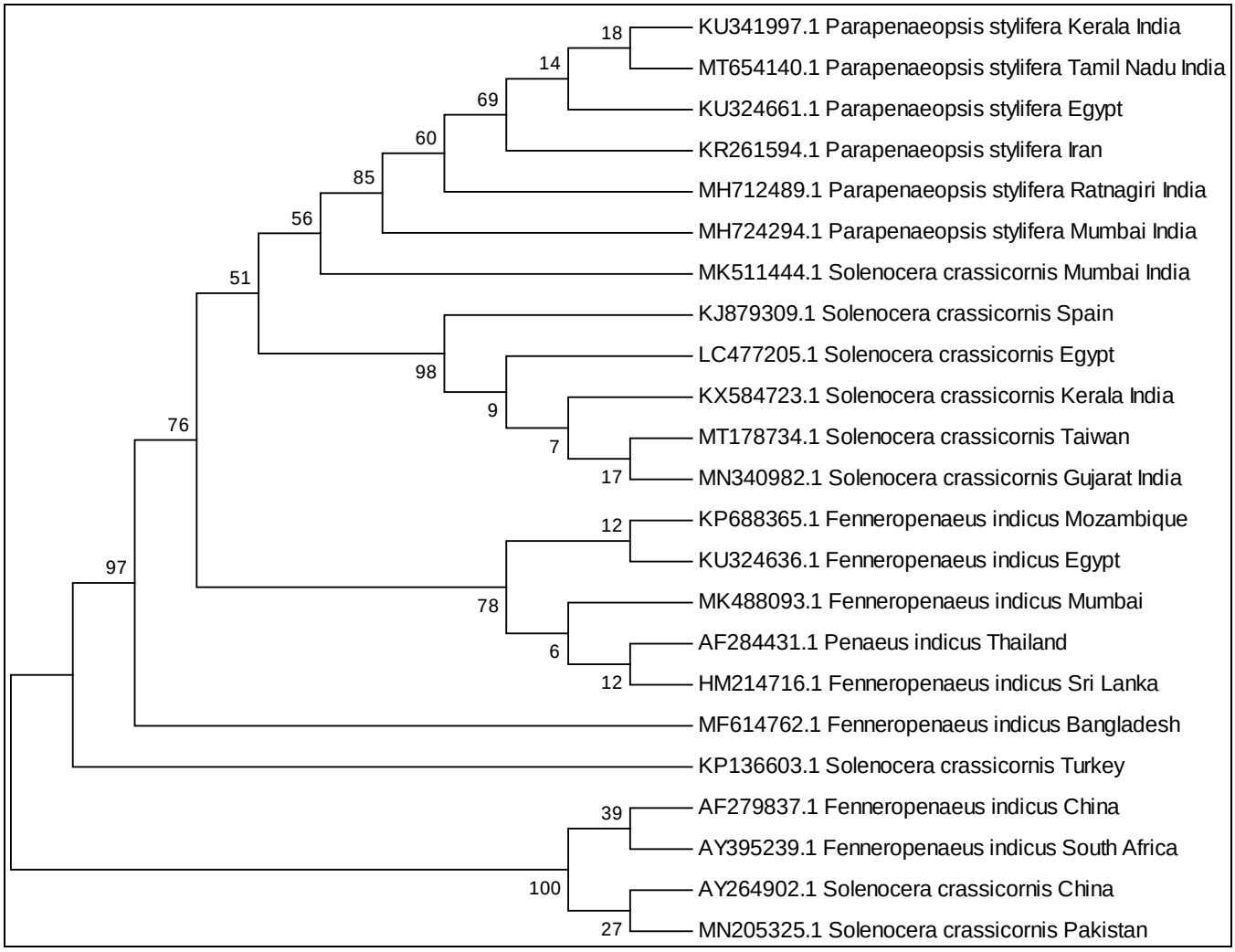

Fig 6: Phylogenetic tree representing relationship between *F. indicus P. stylifera* and *S. crassicornis* associated to COI gene protein sequences using NJ method.

Table 1: Phylogenetic tree between *F. indicus P. stylifera* and *S. crassicornis* associated to COI gene protein sequences using NJ method

.		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_*Fenneropenaeus_indicus* Mozambique																							
2	KU324636.1_*Fenneropenaeus_indicus*_Egypt	0.000																						
3	AF279837.1_*Fenneropenaeus_indicus*_China	1.946	1.946																					
4	MK488093.1_*Fenneropenaeus_indicus*_Mumbai	0.000	0.000	1.946																				
5	AF284431.1_*Penaeus_indicus*_Thailand	0.000	0.000	1.946	0.000																			
6	HM214716.1_*Fenneropenaeus_indicus*_Sri_Lanka	0.000	0.000	1.946	0.000	0.000																		
7	MF614762.1_*Fenneropenaeus_indicus*_Bangladesh	0.029	0.029	1.821	0.029	0.029	0.029																	
8	AY395239.1_*Fenneropenaeus_indicus*_South_Africa	1.900	1.900	0.032	1.900	1.900	1.900	1.773																
9	MH724294.1_*Parapenaeopsis_stylifera*_Mumbai_India	0.052	0.052	1.936	0.052	0.052	0.052	0.095	1.946															
10	KR261594.1_*Parapenaeopsis_stylifera*_Iran	0.065	0.065	1.946	0.065	0.065	0.065	0.099	1.900	0.010														
11	KU324661.1_*Parapenaeopsis_stylifera*_Egypt	0.065	0.065	1.946	0.065	0.065	0.065	0.099	1.900	0.010	0.000													
12	MH712489.1_*Parapenaeopsis_stylifera*_Ratnagiri_India	0.082	0.082	2.003	0.082	0.082	0.082	0.120	1.954	0.010	0.016	0.016												

13	KU341997.1_*Parapenaeopsis _stylifera*_Kerala_India	0.065	0.065	1.946	0.065	0.065	0.065	0.099	1.900	0.010	0.000	0.000	0.016											
14	MT654140.1_*Parapenaeopsis _stylifera*_Tamil_Nadu_India	0.065	0.065	1.946	0.065	0.065	0.065	0.099	1.900	0.010	0.000	0.000	0.016	0.000										
15	KP136603.1_*Solenocera _crassicornis*_Turkey	0.669	0.669	1.930	0.669	0.669	0.669	0.674	1.995	0.714	0.717	0.717	0.734	0.717	0.717									
16	KJ879309.1_*Solenocera _crassicornis*_Spain	0.032	0.032	1.946	0.032	0.032	0.032	0.068	1.900	0.042	0.065	0.065	0.074	0.065	0.065	0.685								
17	AY264902.1_Solenocera _crassicornis_China	1.900	1.900	0.049	1.900	1.900	1.900	1.773	0.024	1.946	1.900	1.900	1.954	1.900	1.900	1.995	1.900							
18	MN205325.1_*Solenocera _crassicornis*_Pakistan	1.954	1.954	0.100	1.954	1.954	1.954	1.830	0.074	2.020	1.954	1.954	2.011	1.954	1.954	2.056	1.954	0.065						
19	LC477205.1_*Solenocera _crassicornis*_Egypt	0.032	0.032	1.946	0.032	0.032	0.032	0.068	1.900	0.042	0.065	0.065	0.074	0.065	0.065	0.685	0.000	1.900	1.954					
20	KX584723.1_*Solenocera _crassicornis*_Kerala_India	0.032	0.032	1.946	0.032	0.032	0.032	0.068	1.900	0.042	0.065	0.065	0.074	0.065	0.065	0.685	0.000	1.900	1.954	0.000				
21	MT178734.1_*Solenocera _crassicornis*_Taiwan	0.032	0.032	1.946	0.032	0.032	0.032	0.068	1.900	0.042	0.065	0.065	0.074	0.065	0.065	0.685	0.000	1.900	1.954	0.000	0.000			
22	MN340982.1_*Solenocera_ crassicornis*_Gujarat_India	0.032	0.032	1.946	0.032	0.032	0.032	0.068	1.900	0.042	0.065	0.065	0.074	0.065	0.065	0.685	0.000	1.900	1.954	0.000	0.000	0.000		
23	MK511444.1_*Solenocera_ crassicornis*_Mumbai_India	0.048	0.048	1.892	0.048	0.048	0.048	0.089	1.848	0.052	0.048	0.048	0.065	0.048	0.048	0.701	0.065	1.848	1.900	0.065	0.065	0.065	0.065	

According to Samadi *et al.* [29], *P. stylifera* and *Metapenaeopsis stridulans* 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.

5. Acknowledgement

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6. References

1. Hebert PDN, Cywinska A, Ball SL, deWaard JR. Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B: Biological Sciences. 2003;270(1512):313-321.
2. CSIRO. Crustaceans unique colour control system. Location: Eco. Sciences Precinct-Dutton Park, 41 Boggo Road, Dutton Park QLD 4102, Australia, 2013.
3. Montgomery SS. Biology and life cycles of prawns. Fisheries Research Centre: Sydney, NSW, Australia, 2010.
4. Rajkumar G, Bhavan PS, Udayasuriyan R, Vadivalagan C. Molecular identification of shrimp species, *Penaeus semisulcatus*, *Metapenaeus dobsoni*, *Metapenaeus brevicornis*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* inhabiting in the coromandel coast (Tamil Nadu, India) using MT-COI gene. International Journal of Fisheries and Aquatic Studies. 2015;2(4):96-106.
5. Cox AJ, Hebert PD. Colonization, extinction, and phylogeographic patterning in a freshwater crustacean. Molecular Ecology. 2001;10(2):371-386.
6. Knowlton N, Weigt LA. New dates and new rates for divergence across the Isthmus of Panama. Proceedings of the Royal Society of London. Series B: Biological Sciences. 1998;265(1412):2257-2263.
7. Wares JP, Cunningham CW. Phylogeography and historical ecology of the North Atlantic intertidal. Evolution. 2001;55(12):2455-2469.
8. Voloch CM, Freire PR, Russo CAM. Molecular phylogeny and divergence time estimates of Penaeid Shrimp Lineages (Decapoda: Penaeidae). Zootaxa. 2009;2107;(1):41-52.
9. Vazquez-Bader AR, Carrero JC, Garcia-Varela M, Gracia A, Laclette JP. Molecular phylogeny of Superfamily Penaeoidea Rafinesque-Schmaltz, 1815, based on mitochondrial 16S partial sequence analysis. Journal of Shellfish Research. 2004;23(3):911-918.
10. Quan J, Zhuang Z, Deng J, Dai J, Zhang Y. Phylogenetic Relationships of 12 Penaeoidea Shrimp Species Deduced from Mitochondrial DNA Sequences. Biochemical Genetics. 2004;42(9, 10):331-345.
11. Chan TY, Tong J, Tam YK, Chu KH. Phylogenetic relationships among the genera of the Penaeidae (Crustacea: Decapoda) revealed by mitochondrial 16S rRNA gene sequences. Zootaxa. 2008;1694(1):38.
12. Kubo I. Studies on penaeids of Japanese and its adjacent waters. Journal of the Tokyo College of Fisheries.

1949;36:1-467.
13. Drumm DT, Maslenikov KP, Van Syoc R, Orr JW, Lauth RR, Stevenson DE *et al*. DNA Barcoding Analysis of Marine Caridean Shrimps from Alaska. An annotated checklist of the marine macroinvertebrates of Alaska, 2016.
14. Bilgin R, Utkan MA, Kalkan E, Karhan SU, Bekbölet M. DNA barcoding of twelve shrimp species (Crustacea: Decapoda) from Turkish seas reveals cryptic diversity. Mediterranean Marine Science. 2015;16(1):36-45.
15. Lavery S, Chan TY, Tam YK, Chu KH. Phylogenetic relationships and evolutionary history of the shrimp genus Penaeus sl derived from mitochondrial DNA. Molecular Phylogenetics and Evolution. 2004;31(1):39-49.
16. Rajkumar G, Bhavan PS, Udayasuriyan R, Vadivalagan C. Molecular identification of shrimp species, *Penaeus semisulcatus*, *Metapenaeus dobsoni*, *Metapenaeus brevicornis*, *Fenneropenaeus indicus*, *Parapenaeopsis stylifera* and *Solenocera crassicornis* inhabiting in the coromandel coast (Tamil Nadu, India) using MT-COI gene. International Journal of Fisheries and Aquatic Studies. 2015;2(4):96-106.
17. Udayasuriyan R, Bhavan PS, Vadivalagan C, Rajkumar G. Efficiency of different COI markers in DNA barcoding of freshwater prawn species. J Entomol Zool Stud. 2015;3(3):98-110.
18. Kundu S, Rath S, Tyagi K, Chakraborty R, Kumar V. Identification of penaeid shrimp from Chilika Lake through DNA barcoding. Mitochondrial DNA Part B. 2018;3(1):161-165.
19. Purushothaman P, Chakraborty RD, Kuberan G, Maheswarudu G. Integrative taxonomy of commercially important deep water penaeoid shrimps from India. Journal of Genetics. 2019;98(1):12.
20. Karuppasamy PK, Logeshwaran V, Priyadarshini RSS, Ramamoorthy N. Molecular and Systematic Identification of Food Marine Shrimps Using mtCOI Marker from Southeast Coast of India. Thalassas: An International Journal of Marine Sciences, 2020, 1-9.
21. Fischer W, Bianchi G. FAO species identification sheets for fishery purposes: Western Indian Ocean (Fishing Area 51). V. 1: Introductory material. Bony fishes, families: Acanthuridae to Clupeidae.-v. 2: Bony fishes, families: Congiopodidae to Lophotidae.-v. 3:... Families: Lutjanidae to Scaridae.-v. 4:... Families: Scatophagidae to Trichiuridae.-v. 5: Bony fishes, families: Triglidae to Zeidae. Chimaeras. Sharks. Lobsters. Shrimps and prawns. Sea turtles. V. 6: Alphabetical index of scientific names and vernacular names, 1984.
22. Doyle JJ, Doyle JL. A rapid DNA isolation procedure for small quantities of fresh leaf tissue (No. Research), 1987.
23. Sawant TM, Kaur R, Uppal GJ. The use of DNA barcoding in identification & conservation of *Nematopalaemon tenuipes*, non-penaeid shrimp from Mumbai coast, Maharashtra, India. Journal of Emerging Technologies and Innovative Research (JETIR). 2019;6(1):727-732.
24. Vrijenhoek R. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol Mar Biol Biotechnol. 1994;3(5):294-299.
25. Sawant TM, Kaur R, Uppal GK. Molecular phylogeny and population structure of *Exhippolysmata ensirostris* from west coast of Maharashtra using mitochondrial COI gene. International Journal of Multidisciplinary Educational Research. 2020;9(2-5):235-246.
26. Kumar S, Stecher G, Tamura K. MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. Molecular Biology and Evolution. 2016;33(7):1870-1874.
27. Saitou N, Nei M. The neighbor-joining method: A new method for reconstructing phylogenetic trees. Molecular Biology and Evolution. 1987;4(4):406-425.
28. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. Evolution. 1985;39(4):783-791.
29. Samadi S, Ghavam Mostafavi P, Rezvani Gilkolaii S, Fatemi M, Fazli H. Phylogenetic relationships of the commercial marine shrimp family Penaeidae from Persian Gulf. Iranian Journal of Fisheries Sciences. 2016;15(1):333-346.
30. Katneni VK, Shekhar MS, Jangam AK, Paran BC, Selvaraj A, Krishnan K *et al*. Phylogenetic relations and mitogenome-wide similarity metrics reveal monophyly of Penaeus sensu lato. Ecology and evolution. 2021;11(5):2040-2049.
31. Voloch CM, Freire PR, Russo CA. Molecular phylogeny of penaeid shrimps inferred from two mitochondrial markers. Genetics and Molecular Research. 2005;4(4):668-674.
32. Hurzaid A, Chan TY, Mohd Nor SA, Muchlisin ZA, Chen WJ. Molecular phylogeny and diversity of penaeid shrimps (Crustacea: Decapoda) from South-East Asian waters. Zoologica Scripta. 2020;49(5):596-613.