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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

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Abstract
DNA barcoding is a vital tool for assessing non described and cryptic morphological species. Literature revealed that a number of marine shrimp species have morphologically been described. However, it has lots of misleading due to cryptic characters, such as dimorphism, larval and adult variation etc. In the present study, fourteen marine shrimp species (*Penaeus semisulcatus*, *Penaeus monodon*, *Metapenaeus affinis, Metapenaeus lysianasa, Metapenaeus dobsoni, Metapenaeus brevicornis, Metapenaeopsis stridulans, Fenneropenaeus indicus*, *Fenneropenaeus merguiensis*, *Parapenaeopsis stylifera*, *Parapenaeopsis maxillipedo*, *Marsupenaeus japonicas, Solenocera crassicornis* and *Alpheus paludicola*) were collected from Coromandel Coast of Tamil Nadu, India, and they were identified morphologically. Among these, six species (*P. semisulcatus, M. dobsoni, M. brevicornis, F. indicus, P. stylifera* and *S. crassicornis*) were taken for molecular identification by adopting DNA barcoding of mitochondrial cytochrome c oxidase subunit I (COI) gene. 650 bp sequences were obtained when universal primers (LCO1490 and HCO2198) were used. Their similarity was checked with BLAST-NCBI, and each and every species showed > 80% identity. Maximum intra specific divergence was calculated as 0.8% in *F. indicus* of India, Thailand and China. Inter specific divergence was found to be maximum, 3.95% between *S. crassicornis* and *S. koelbeli* of India and China. Minimum divergence (0.00%) was observed between two Indian haplotypes, *M. dobsoni* and *M. brevicornis*, which overlaps each other. Further studies at population levels of these two species are required to rule out this ambiguity. On the whole, the phylogenetic tree revealed the polyphyletic clade.

Keywords: DNA barcoding, MT-COI gene, marine shrimps, divergence, haplotype, phylogeny.

1. Introduction
The shrimps and prawns have great economical value as they earn valuable foreign exchange. Generally, more than 10 million tons of crustaceans are produced annually for human consumption. Crustacean often referred to as "Insect of Sea" as the range of morphological characteristics for taxonomical identification exceeds than that of the insecta. There are approximately 50,000 - 67,000 crustaceans have been estimated worldwide. They show an enormous diversity and different range of sizes. Morphological identification of crustaceans is very critical because, this group has different larval stages, sexual dimorphism, plasticity, trading etc.

Species identification by morphological features is sometime ineffective and misleading, because, larval stages of some species groups often cannot be assigned to the correct species [1]. The morphological identification is more complicated when the species are damaged due to rough handling, and there may have chances for fish fraud [2]. The unique colour system in crustacean often plays an important role in aquaculture because their colour affects the quality and market price [2]. Prawns, like most other crustaceans are able to change colour depending upon growth, background coloration and time of day due to chromatophores [3]. These problems can be overcome by molecular identification or DNA Barcoding.

For ensuring rapid and accurate identification of a broad range of biological specimens, [1] proposed "DNA Barcoding" technique, in which a primer set of DNA was used to amplify a

648 base pair mitochondrial cytochrome c oxidase subunit I (MT COI) gene, because, its mutation rate is often fast enough to distinguish closely related species and also its sequence is conserved among conspecifics. Therefore, DNA barcoding provides an opportunity to identify, invent, and study specimens in order to understand the diversity of species within an ecosystem, and also to evaluate the genetic variability within species. In the present study, DNA barcoding technique based on MT-COI gene was adopted to identify few marine shrimp species inhabiting in the Coromandel coastal region (Cape Comorin to False Divi Point) in the Bay of Bengal, Tamil Nadu, India. MT-COI (COX I) gene has been employed as a possible DNA marker for species identification. This gene has two important advantages, (i). Universal primers are very robust for this gene, enabling recovery of its 5 primer end from the representatives [4-6], and, (ii). COI likely possesses a greater range of phylogenetic signal than any other MT gene. In common with other protein-coding genes, its third position nucleotides show a high incidence of base substitutions. However, changes in its amino acid sequence occur more slowly than those in any other mitochondrial gene [7-9]. Therefore, this gene is conserved and less subjected to external forces**.**

Even now a day, we are relied on classical taxonomy for validating shrimp and prawn species. Therefore, in the present study it was aimed to validate few marine prawn species through molecular identification based on DNA barcoding of MT-COI gene. Further, it was aimed to determine the nucleotide divergence or the barcoding gap (and similarity as well), and possibility for occurrence of distinct heplotypic variations among them. Furthermore, it was aimed to construct a possible molecular phylogenetic tree with selected shrimp species for understanding their evolutionary relationship/ significance.

2. Materials and Methods

2.1. Species collection and identification

A total of fourteen shrimp species was collected from two different sites, Nagapattinam (10°.76 N, 79°.83 E) and Mallipattinam (10°.28 N, 79°.31 E) situated in the Coromandel Coast of Tamil Nadu, India. These species were identified by using taxonomic keys described by [10] "Edible Penaeid Shrimps in India" in the Training Manual "GIS and Marine Biodiversity" edited by John Milton (2008). Finally, these species were confirmed by Mr. M. Kathirvel, Former Principal Scientist, Central Institute of Brackish water Aquaculture, ICAR, Chennai.

2.2. Molecular analysis

Genomic DNA was isolated from the muscle tissue by using Qiagen Dneasy Blood and Tissue Kit (Germany). 1% Agarose Gel Electrophoresis (GENEI, Bangalore) was performed to detect the genomic DNA using Gel documentation (Mediccare, India). DNA amplification of MT-COI gene was carried out in Eppendorf Thermo Cycler by using the forward (LCO1490: 5'-GGTCAACAAATCATAAAGATATTG-3') and reverses (HCO2198: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3') primers [4]. Amplification was performed in a total volume of 50 µl containing 4 µl of DNA template, 20 p.mol of each primers, 400 µM of dNTP and 0.4 µl of Taq DNA polymerase (Qiagen). Thermo cycler conditions were as follows: 5 min at 95°C for pre-running, then 35 cycles of 60 s at 95°C for denaturation, 60 s at 49-52 °C for annealing, and 90 s at 72 °C for extension followed by 5 min at 72 °C for a final extension. The final product was stored at -20 °C for further usage. The amplified product was resolved with 2% AGE. Sequencing was done by using ABI 3500 XL Genetic Analyzer with manufacturer's protocol of Chromos Biotech, Pvt. Ltd., Bangalore, India.

2.3. Sequence statistical analysis

The sequences were aligned pair wise by using EMBL-ABI. Stop codons were removed by using BLAST, and the reading frame shift was deducted by ORF finder. The trimmed sequence was authenticated with GenBank. The similarity between sequences was identified by BLAST. The multiple sequence alignment was done by using T- Coffee and the aligned sequence was highlighted with multiple align show (MAS). Nucleotide divergence was calculated by adopting both DnaSP v5 (Intraspecific) and K2P (Interspecific). The evolutionary relationship was analyzed by using MEGA v6.

3. Results and discussion

In this study, a total of fourteen species of Penaeid shrimps were identified by morphologically. Among these, twelve species belong to the family Penaeidae (*P. semisulcatus*, *P. monodon*, *M. affinis, M. lysianasa, M. dobsoni, M. brevicornis, M. stridulans, F. indicus*, *F. merguiensis*, *P. stylifera*, *P. maxillipedo*, and *M. japonicas*), one species (*S. crassicornis*) to the family, Solenoceridae and another one species (*A. paludicola*) to the family, Alpheidae. Thus, species belong to the family, Penaeidae was dominant, and most of them are commercially important. According to [11], the Penaeid shrimps family comprises of 13 genera and 105 species. The photographs of these species and their morphological differences are presented in Figure 1 and Table 1 respectively.

Among the fourteen species identified morphologically, only six species were subjected to DNA barcoding (*P. semisulcatus*, *M. dobsoni, M. brevicornis, F. indicus, P. stylifera* and *S. crassicornis*). The remaining eight species could not be included in barcode analysis, because, they were not successfully amplified with the universal decapods primer (LCO1490 and HCO2198) used in this study. This may be due to several reasons right from the handling of tissue samples to the steps involved in PCR amplification, since the same primer has produced successful amplification in *P. monodon* [12]. The isolated genomic DNA showed greater than 10 Kb nucleotides (Figure 2). The amplified products showed approximately 700 bp (Table 2 and Figure 3). Several studies have been reported that sequence diversity in a ~650 bp region near the 5' end of the MT-COI gene provides strong species-level resolution for varied animal groups, including birds [13, 14], fishes [15, 16], springtails [17, 18], spiders [19, 20] and moths [21, 22]. It has been reported that the primer pair, LCO1490 and HCO2198 was not so ''universal'' as thought before, as it would still fail to amplify some taxa [23, 24].

While performing BLAST, the sequences of six shrimp species showed varied degrees of similarity with existing data in the NCBI database (Table 3). Both *P. semisulcatus, P. stylifera* showed 100% similarity, the *F. indicus* with 99% and the remaining three species *M. dobsoni M. brevicornis, S. crassicornis* with an identity of >83%. The GenBank accession numbers of all six species and other marine prawn species retrieved are presented in Table 4. The multiple sequence analysis using T- coffee with MAS showed 223 identical amino acid residues, 44 similar, identical amino acid residues and 384 variations in amino acid sites. The results obtained in MAS with high similarity positions are also shown in Figure 4.

The nucleotide divergence calculated between Penaeid shrimp

species with the data acquired from NCBI for Penaeidae super family is presented in Figure 5. The mean divergence within the Super family, Penaeidae was 1.49%. The mean nucleotide divergence calculated between the genus, *Penaeus*, *Metapenaeus*, *Fenneropenaeus*, *Parapenaeopsis* and *Solenocera* were 1.62%, 2.01%, 3.70%, 1.43% and 2.40% respectively. The maximum interspecies variation was observed between *S. crassicornis* (Indian haplotype) and *S. koelbeli* (Chinese haplotype) as 3.95%, and the minimum interspecies divergence of 0.00% was observed between two Indian species, *M. dobsoni* and *M. brevicornis*. Which was overlapped each other and thus, considered as the same species. However, this can be checked further by studying each specific population separately for species discrimination.

An inter species variation ranged between 0.24-1.2% has been reported in 12 species of the Penaeidae family [25]. Similarly, an inter species variation of 0-3% has been reported in 13 species of the genus, *Penaeus* [26]. According to [27], pair-wise level of base divergence (*p* distances) between *Penaeus* species varied from 20.22% (between *P. indicus* and *P. kerathurus*) to 0.72% (between the very closely related Western Atlantic species, *P. duorarum* and *P. notialis*). The sequence divergence between *P. merguiensis* and *P. monodon*, between *P. monodon* and *P. japonicus*, and between *P. merguiensis* and *P. japonicas* were 34.0%, 42.1% and 38.9% respectively has been reported [28].

The sequence divergence ranged from 0-1.20% (within species, *Penaeus penicillatus*), 6.5% (between species, *Penaeus canaliculatus* and *Penaeus japonicus*), 21.09% (between species, *P. canaliculatus* and *P. penicillatus*), 18.67% (between genera, *Parapenaeus fissuroides* and *Metapenaeopsis barbata*), 25.39% (between genera, *Parapenaeopsis hardwickii* and *P. penicillatus*), 18.70% (between genera, *S. crassicornis* and *P. fissuroides*), 22.88% (between genera, *S. crassicornis* and *P. penicillatus*), 26.38% (between genera, *Portunus trituberculatus* (crab) and *S. crassicornis*), and 31.87% (between genera, *P. trituberculatus* and *Metapenaeus ensis*) have been reported [25]. In the genus *Penaeus*, large genetic variations of COI gene have been reported among 15 species [26, 29]. In the present study, the nucleotide divergence for the selected shrimp species was calculated as between 0.00 - 3.95% with the average interspecies nucleotide divergent value of 1.49%, which is less than the significant 3% threshold value as per the 10X rule of [30]. Therefore, all the six species studied are closely related from each other, and also with the retrieved species.

Figure 6 depicts the details of distinct haplotypes available in the data base for the selected Penaeid shrimp species. While searching for species haplotypes only one sequence was retrieved from the GenBank databases, (i.e. available for *F. indicus* from two different geographical regions, Thailand and China), they showed only 0.8% variation when compare with Indian haplotype. About 0-3% intra specific variation has been reported for the COI gene in 13 *Penaeus* species [26].

General Time Reversible (GTR+G) model's maximum likelihood phylogenetic tree showed polyphyletic clades (Figure 7). The non-linear tree exhibited two major clades among the subjected and retrieved species. This may be due to the differences in primers used. Similar opinion has been postulated by [25], the mean AT content was 63.3% in the COI gene and 66.25% in the 16s rRNA gene. The rarest base was G (average 16.2%) in the COI gene and C (average 12.63%) in the 16s rRNA gene. These patterns of base composition are consistent with the descriptions of other arthropod mtDNA sequences [31-36] as well as other marine crustacean mtDNA sequences [26, 37-39]. In a study, [40], reported that COI gene sequence analysis indicated that the differences recorded among the species *P. merguiensis, P. silasi*, and *P. indicus.* Among these *P. silasi* and *P. indicus* was formed monophyletic, but these species showed paraphyletic with *P. merguiensis* [41] has been reported paraphyletic clade among several genera in the subfamily Palaemoninae, such as *Macrobrachium, Cryphiops, Palaemon, Palaemonetes,* and *Pseudopalaemon*. According to [42] *Macrobrachium* formed a paraphyletic group with the monophyletic out a group of genera, *Palaemonetes*, *Palaemon* and *Exopalaemon*. According to [43], a partial sequence of about 300 bp of the 16S mitochondrial gene support monophyly of the superfamily, Penaeoidea but they showed paraphyletic with regard to the closely related families, Solenoceridae and Penaeidae. In this study, it is polyphyletic with COI gene >500 bp at species level.

Table 1: Morphological differences identified in marine shrimp species

Species	Common Name	Rostral Teeth: Upper/ Lower	Body Colour	Uropod Colour	Appearance of Telson	Antennal Colour
P. semisulcatus	Green Tiger Shrimp	6-8/3	Reddish brown to pale brown	Reddish brown with red margin	Without lateral spines	White and brown bands
P. monodon	Tiger Shrimp	6-8/3	Grayish, greenish or darkgreenish blue; Reddish brown in large adults.	Reddish with black margin	---	Brownish red
M. affinis	Jinga Shrimp	7-8/0	Brownish yellow	White with red margin	No distal fixed pair of spines on the telson	Reddish brown
M. lysianassa	---	6-7/0	Pale whitish	Pale white with red margin	Telson armed only with spinules	Grey
M. dobsoni	Kadal Shrimp	5-8/0	Pale yellow to brownish red	Reddish brown and are distributed at the distal region.	---	Red
M. brevicornis	Yellow Shrimp	5-8/0	Yellowish red	Red	With a pair of distal spine and series of minute	Very long; Yellowish red

					spinules	
M. stridulans	---	5-7/0	Red to dark	Red to dark	---	Brownish red
F. indicus	Indian White Shrimp	7-9/3-6	Yellowish white	Yellowish red margin	---	Pale yellowish
F. merguiensis	Banana Shrimp	7-9/4-6	Bright yellow	Yellowish white	---	Yellowish white
P. stylifera	Kiddi Shrimp	7-9/0	Yellowish red with black spots	Reddish	Without fixed sub-apical spines	Red
P. maxillipedo	Torpedo Shrimp	8-10/0	Brownish yellow with blackish green lines	Greenish black with brown margin	Telson without sub apical spines	Greenish yellow
M. japonicus	Kuruma Shrimp	9-10/1	Pale yellowish and crossed with dark brown transverse bands	Bright yellow	Telson with three pairs of movable lateral spines	Yellowish brown
S. crassicornis	Coastal Mud shrimp	8-10/0	Reddish	Reddish with black margin	Telson simple	Yellowish red
A. paladicola	Kemp's Pistel	---	Greenish white	Greenish black	Two pairs of dorsal spines	Brownish red

Table 2: MT-COI gene sequences of selected marine shrimp species

Name of the species	**Sequences**	**Base pair length**
P. semisulcatus	CTTGAGCTGGAATAGTAGGTACAGCTCTTAGACTTATTATTCGTGCTGAATTAGGTC AACCTGGTAGACTTATTGGAGATGATCAAATTTATAATGTGGTTGTAACAGCTCACG CTTTTGTTATAATTTTCTTCATAGTTATACCTATCATGATTGGAGGATTTGGTAACTG ACTAGTTCCTCTAATATTAGGAGCTCCAGATATAGCTTTCCCTCGTATAAATAATAT AAGCTTCTGGCTTTTACCTCCTTCACTAACCTTACTTTTATCTAGAGGTATAGTAGAA AGAGGAGTAGGAACAGGTTGAACAGTATACCCTCCTTTATCTGCCAGAATTGCTCAC GCAGGTGCTTCAGTAGACTTAGGGATCTTCTCACTTCATCTAGCAGGTGTATCATCT ATTTTAGGTGCCGTAAATTTTATAACAACCGTTATTAATATACGATCTACTGGAATA ACTATAGACCGAATACCTCTGTTCGTTTGAGCGGTATTTATTACTGCCCTGCTTCTAC TTCTATCTTTACCAGTACTAGCAGGAGCTATTACAATGCTTCTAACAGACCGAAATC TAAATACATCCTTCTTCGACCCTGCCGGTGGAGGAGACCCTGTACTATATCAACACT TATTTTGATTTTTTGGTCACCCTGAAGTTTA	661
M. dobsoni	CTGGATAGTAGGTACTGCTTTAAGTTTAATTATCCGAGCCGAACTTGGTCAACCAGG TAGACTTATTGGAGACGATCAAATTTATAATGTTGTAGTTACCGCCCACGCTTTTGTT ATAATTTTCTTTATAGTTATACCAATTATGATTGGTGGATTTGGTAATTGACTTGTCC CTCTTATACTCGGAGCACCCGATATAGCATTCCCACGAATAAACAATATGAGTTTTT GACTACTTCCACCATCCTTAACACTCCTTCTTTCTAGTGGAATAGTAGAAAGAGGTG TAGGAACAGGATGAACGGTTTATCCTCCCTTAGCAGCTGGAATTGCCCACGCAGGA GCTTCAGTTGATATAGGAATTTTTTCTCTACATCTTGCTGGAGTTTCATCTATTTTAG GAGCAGTTAATTTCATAACAACAGTCATTAACATACGCCCTGCTGGAATAACTATAG ACCGTATACCACTTTTTGTATGGGCCGTATTTATTACAGCCTTACTTCTTTTATTATC ACTACCAGTTTTAGCTGGGGCTATCACTATGCTTTTAACAGACCGAAACCTTAATAC ATCCTTTTTCGATCCCGCTGGAGGAGGAGATCCAATTCTATACCAGCATTTATTTTGA TTTTTTGGTCACCCTTGAAGTTTAAA	657
M. brevicornis	CTGGATAGTAGGTACTGCTTTAAGTTTAATTATCCGAGCCGAACTTGGTCAACCAGG TAGACTTATTGGAGACGATCAAATTTATAATGTTGTAGTTACCGCCCACGCTTTTGTT ATAATTTTCTTTATAGTTATACCAATTATGATTGGTGGATTTGGTAATTGACTTGTCC CTCTTATACTAGGAGCACCCGATATAGCATTCCCACGAATAAACAATATGAGTTTTT GACTACTTCCACCATCCTTAACACTCCTTCTTTCTAGTGGAATAGTAGAAAGAGGTG TAGGAACAGGATGAACGGTTTATCCTCCCTTAGCAGCTGGAATTGCCCACGCAGGA GCTTCAGTTGATATAGGAATTTTTTCTCTACATCTTGCTGGAGTTTCATCTATTTTAG GAGCAGTTAATTTCATAACAACAGTCATTAACATACGCCCTGCTGGAATAACTATAG ACCGTATACCACTTTTTGTATGGGCCGTATTTATTACAGCCTTACTTCTTTTATTATC ACTACCAGTTTTAGCTGGGGCTATCACTATGCTTTTAACAGACCGAAACCTTAATAC ATCCTTTTTCGATCCCGCTGGAGGAGGAGATCCAATTCTATACCAGCATTTATTTTGA TTTTTTGGTCACCCTGGAAGTTTAAA	657
F. indicus	GCTGGAATAGTAGGGACTGCCCTTAGACTTATTATTCGTGCCGAATTAGGTCAACCG GGAAGCCTTATTGGAGATGACCAAATTTATAATGTAGTAGTTACAGCCCACGCTTTT GTTATAATTTTCTTTATAGTTATGCCTATTATAATTGGGGGATTTGGAAATTGACTAG TACCTTTAATGTTAGGTGCTCCTGATATGGCTTTTCCACGAATAAACAATATGAGTTT CTGGCTCCTACCTCCTTCACTAACACTACTTCTTTCTAGAGGTATAGTTGAAAGAGG AGTAGGAACAGGATGAACTGTTTACCCTCCTTTATCAGCCAGTATTGCTCATGCTGG GGCTTCGGTAGATTTAGGAATTTTCTCCCTACACTTGGCAGGTGTTTCTTCAATTTTA GGAGCTGTAAATTTTATGACATCTTTTTTTAACATACG	440

P. stylifera	TTGAGCTGGAATAGTTGGTACTGCTCTCAGCCTTATTATCCGGGCCGAATTAGGTCA ACCAGGAAACCTTATTGGAGATGATCAAATTTATAATGTAGTGGTCACCGCCCACGC TTTTGTAATAATTTTCTTTATGGTTATACCTATGATAATTGGTGGGTTTGGAAACTGA TTAGTTCCACTAATATTAGGAGCCCCTGATATAGCATTTCCACGAATAAATAATATA AGATTTTGACTCCTCCCTCCCTCCTTAACCCTTCTCCTCTCAAGTGGAATAGTAGAAA GTGGAGTAGGAACCGGTTGAACTGTTTATCCTCCATTATCAAGAGGTATTGCTCACG CAGGAGCCTCTGTAGACATAGGAATCTTCTCCCTTCATTTAGCCGGAGTTTCCTCCAT TTTAGGGGCCGTTAATTTTATAACAACAGTTATCAACATACGATCTTCGGGAATATC AATAGACCGTATCCCCTTGTTTGTATGATCAGTTTTCATTACAGCCCTCCTCCTTCTC CTTTCCCTTCCAGTTCTAGCCGGAGCTATTACAATATTATTAACAGATCGAAACTTA AATACCTCTTTCTTTGACCCAGCTGGAGGAGGAGACCCAATTTTGTATCAACATCTA TTCTGATTTTTTGGTCACCCTGAAGTTTAAA	662
S. crassicornis	TTCTTTGTTGGTGGCGCAATGGCGATGGTGATCCGTGCTGAATTATTCCAGCCTGGA TTACAGCTTGTTGAGCCTAATTTCTTTAATCAAATGACCACGGTACACGGTTTGATC ATGGTGTTTGGGGCGGTGATGCCTGCCTTTACTGGGCTTGCGAATTGGATGATCCCA ATGATGATTGGGGCGCCTGATATGGCACTGCCAAGAATGAATAACTGGAGCTTTTG GATCTTACCTTTCGCATTTTCTTTATTGTTGGCATCTCTTTTTATGGAAGGGGGCGGT CCTAACTTTGGTTGGACTTTCTATGCGCCGCTTTCAACTACGTATAGCCCAGCCAGTA CAGGTTTATTCGTCTTTGCTATTCATATTATGGGGATCAGCTCCATTATGGGGGCGAT TAACGTTGTTGTGACCATTGTAAATATGCGCGCACCGGGTATGACGTATATGAAAAT GCCACTGTTTGTTTGGACATGGTTGATCACAGCATTTTTATTAATTGCGGTGATGCCA GTACTTGCAGGGGCCGTAACCATGGTACTGACTGATAAATACTTTGGTACCAGCTTT TTTGATGCAGCTGGTGGTGGTGATCCGGTCATGTTCCAGCATATTTTCTGATTTTTTG GTCACCCTGAAGTTTAATAA	651

Table 3: BLAST identification of sequences for selected marine shrimp species in NCBI database

Species Name	Score	Expected	Identities	Gaps	Strand
P. semisulcatus	1218 bits (659)	0.0	659/659 (100%)	0/659 (0%)	Plus/Plus
M. dobsoni	584 bits (316)	3e-163	529/635 (83%)	2/635 (0%)	Plus/Plus
M. brevicornis	584 bits (316)	3e-163	529/635 (83%)	2/635 (0%)	Plus/Plus
F. indicus	780 bits (422)	0.0	434/440 (99%)	0/440 (0%)	Plus/Plus
P. stylifera	1218 bits (659)	0.0	659/659 (100%)	0/659 (0%)	Plus/Plus
S. crassicornis	669 bits (362)	0.0	550/644 (85%)	0/644 (0%)	Plus/Plus

Table 4: Species collection information and GenBank accession number for MT-COI gene sequences of selected marine shrimp genus

Species	Country	Reference and Year	GenBank Accession No.
P. semisulcatus	India	Paper authors, 2013	KF613002
M. dobsoni	India	Paper authors, 2013	KF540213
M. brevicornis	India	Paper authors, 2013	KF540212
F. indicus	India	Paper authors, 2013	KF649208
P. stylifera	India	Paper authors, 2013	KF613003
S. crassicornis	India	Paper authors, 2013	KF540214
Penaeus monodon	Thailand	Khamnamtong *et al.,* 2012	EF646261
Penaeus chinensis	China	Quan *et al.,* 2000	AF247771
Penaeus paulensis	USA	Baldwin *et al.,* 1998	AF029392
Penaeus kerathurus	USA	Baldwin *et al.,* 1998	AF029391
Metapenaeus moyebi	China	Mai and Hu 2009	FJ435653
Metapenaeus affinis	China	Mai and Hu, 2009	FJ435653
Metapenaeus ensis	China	Mai and Hu, 2009	FJ435651
Metapenaeus joyneri	China	Mai and Hu, 2009	FJ435650
Fenneropenaeus merguiensis	Thailand	Wanna *et al.,* 2010	HQ206436
Fenneropenaeus silasi	Thailand	Wanna *et al.,* 2010	HQ206442
Fenneropenaeus chinensis	China	Kong *et al.,* 2008	EU366250
Fenneropenaeus penicillatus	China	Mai and Hu, 2009	FJ435661
Parapenaeopsis coromandelica	Iceland	De Croos and Palsson, 2011	HQ180264
Parapenaeopsis hungerfordi	China	Mai and Hu, 2009	FJ435656
Parapenaeopsis tenella	China	Mai and Hu, 2009	FJ435655
Parapenaeopsis hardwickii	China	Mai and Hu, 2009	FJ435654
Solenocera membranacea	UK	Matzen da Silva, 2012	JQ305940
Solenocera koelbeli	China	Mai and Hu, 2009	FJ435663

Fig 1: Photographs of fourteen marine shrimp species collected from the Coromandel coastal regions of Tamil Nadu, India.

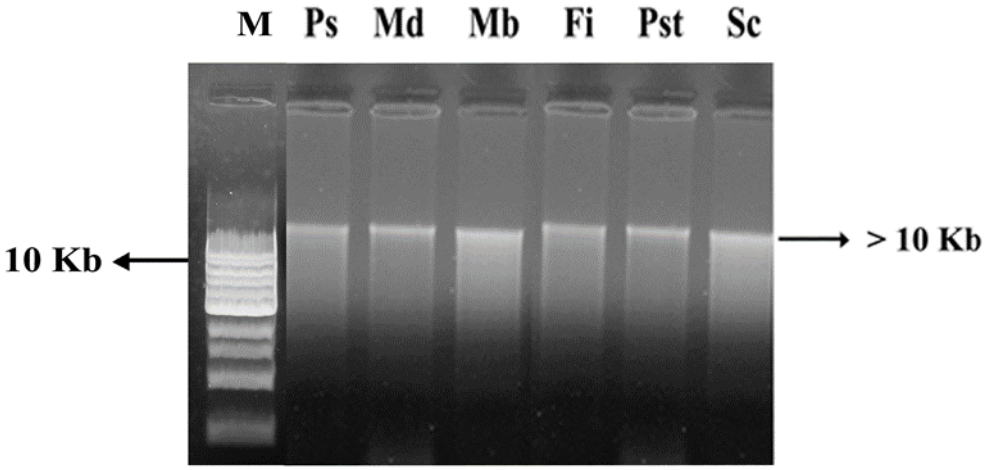

Fig 2: 1% AGE shows 10 kb genomic DNA. M, 1 Kb Marker ; Ps, *P. semisulcatus*; Md, *M. dobsoni*; Mb, *M. brevicornis*; Fi, *F. indicus;* Pst, *P. stylifera*; Sc, *S. crassicornis*.

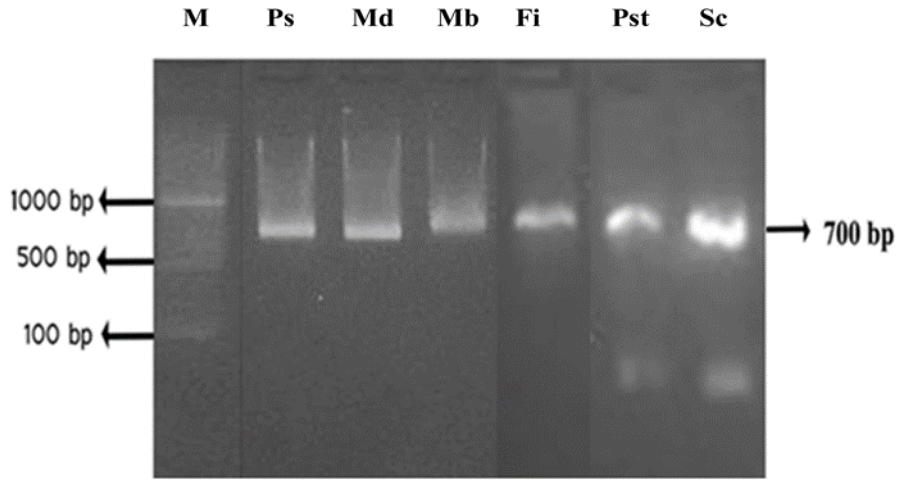

Fig 3: 2% AGE shows 700 bp PCR amplified product of MT-COI gene. M, 100 bp Marker; Ps, *P. semisulcatus*; Md, *M. dobsoni*; Mb, *M. brevicornis*; Fi, *F. indicus;* Pst, *P. stylifera*; Sc, *S. crassicornis*.

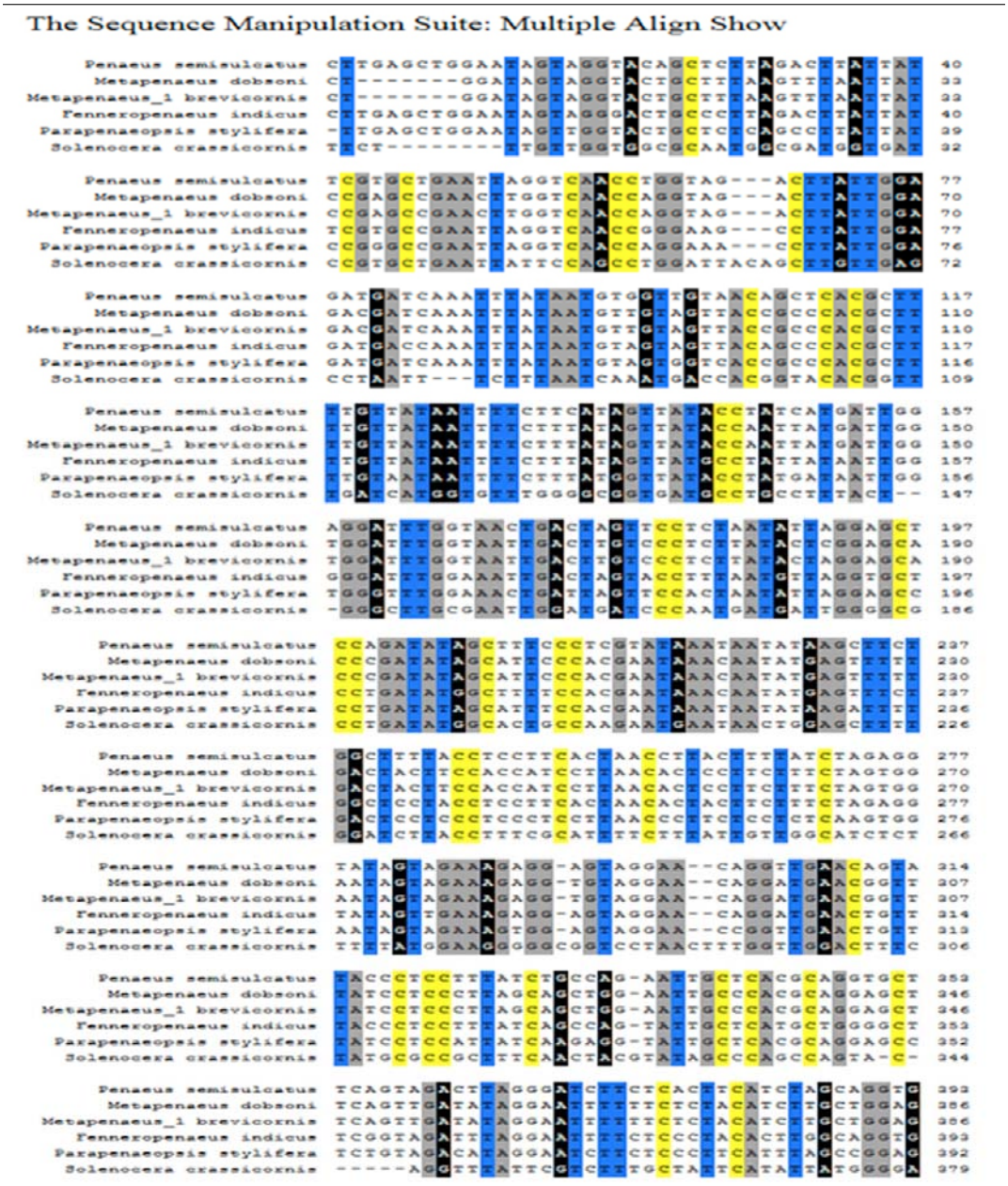

Fig 4: Multiple sequence analysis with multiple align show. An alignment formatted by multiple aligns show using colored backgrounds and a consensus setting of 100%. Identical residues were by amino acid colour and similar residues were black in colored. Gaps and other residues were given a white color background.

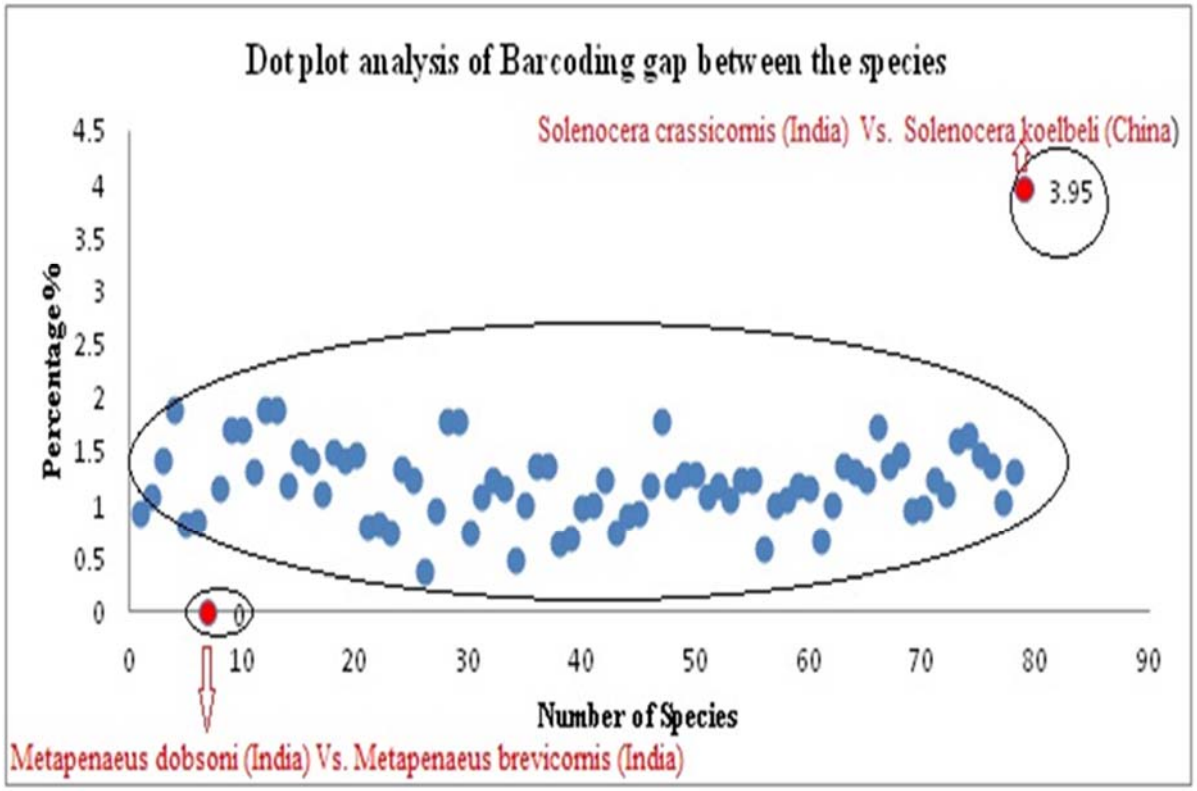

Fig 5: Interspecies nucleotide divergence between species levels: The mean divergence within the Super family Penaeoidae was 1.49%. The mean nucleotide divergence within the genus was maximum in *Fenneropenaeus* (3.70%), followed by *Solenocera* (2.40%), *Metapenaeus* (2.01%), *Penaeus* (1.62%), and *Parapenaeopsis* (1.43%). The maximum interspecies variation between heplotypes *S. crassicornis* (India) and *S. koelbeli* (China) was 3.95%. The minimum interspecies divergence was observed between Indian haplotypes, *M. dobsoni* and *M. brevicornis* was 0.00%, which was overlapping each other.

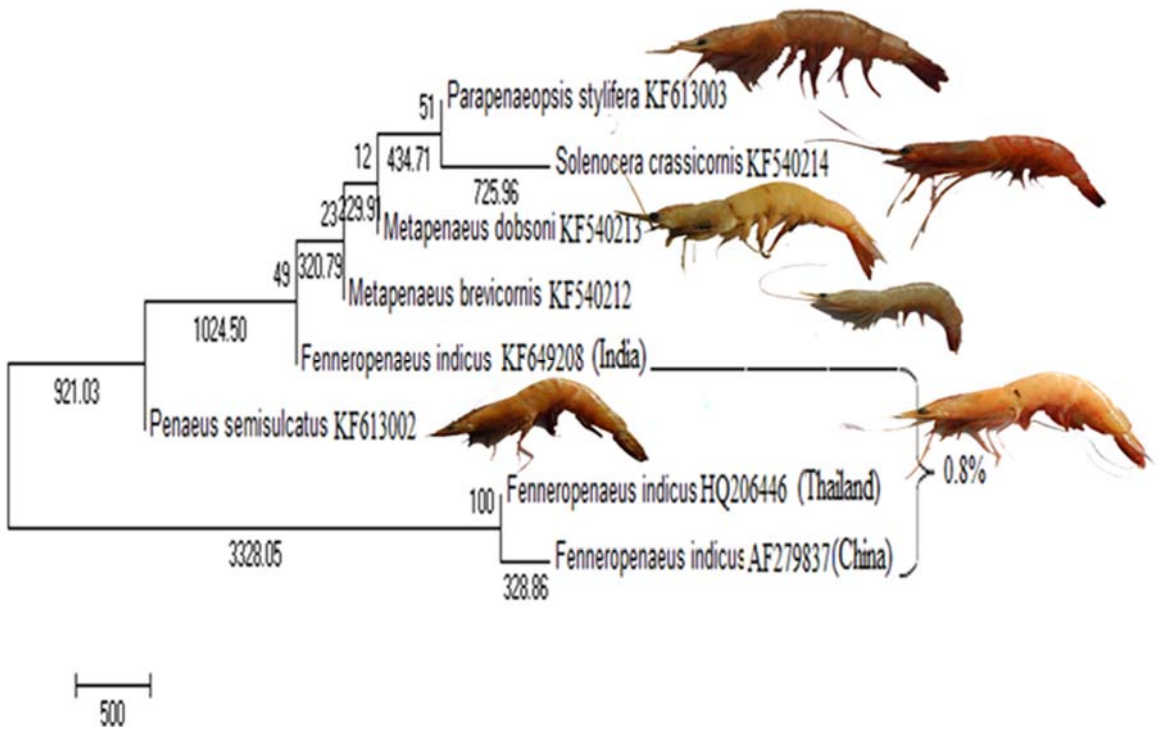

Fig 6: Distinct haplotypic phylogeny with available retrieved species: Neighbor-joining tree (Non-linear) of MT-COI sequence divergence (Jukes-Cantor method) for selected marine shrimp species. Intra species nucleotide divergence value of 0.8% between subjected species and acquired species of *F. Indicus* is shown.

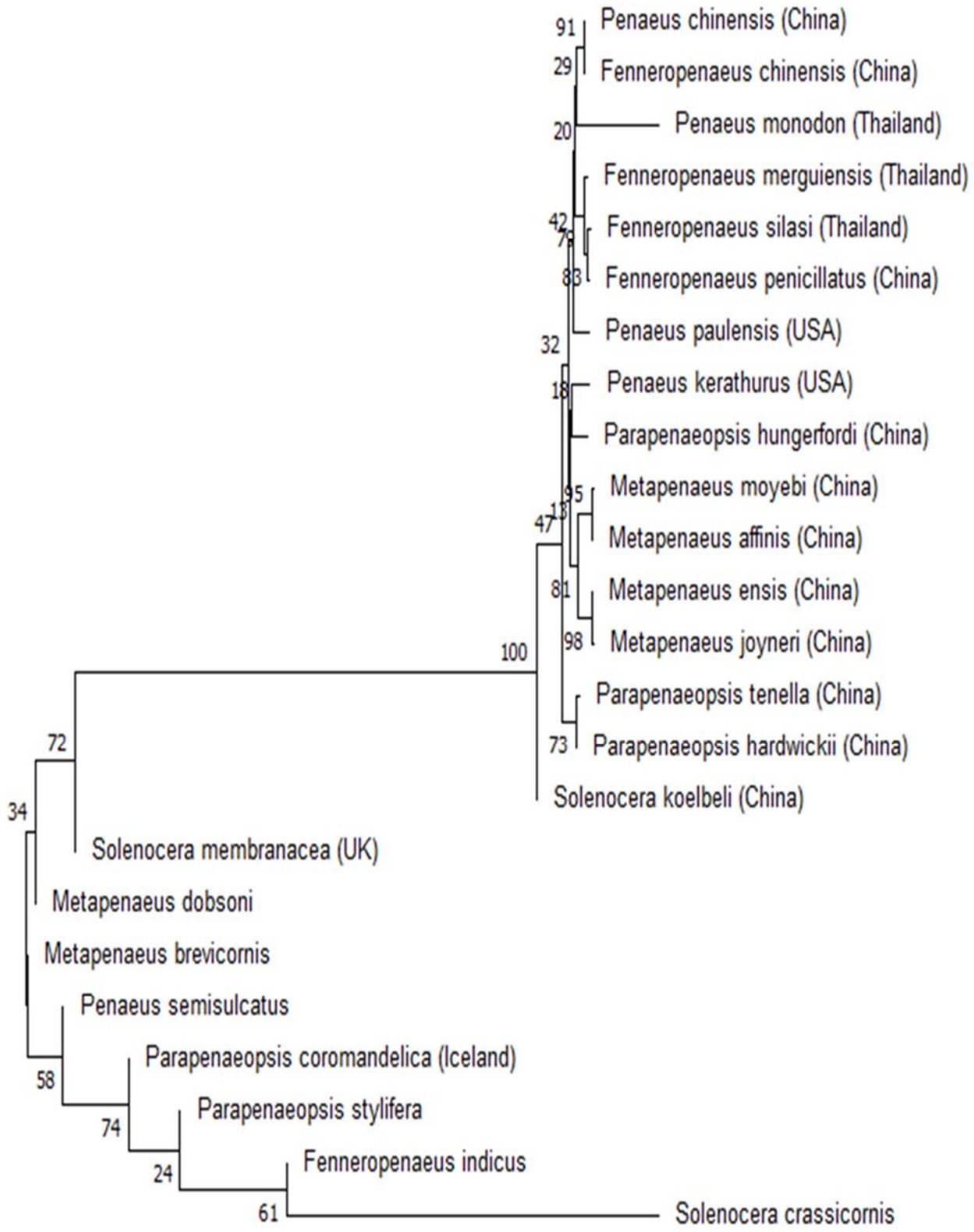

Fig 7: Phylogenetic relationship of General Time Reversible (GTR+G) model's maximum likelihood tree for marine shrimp species. The phylogenetic tree of Super family, Penaeoidae well resolved with the phylogenetic evolution of the species, which form the polyphyletic clade.

4. Conclusions

In this study, the universal decapods primer, LCO1490 and HCO2198 has worked well with six species (*P. semisulcatus*, *M. dobsoni, M. brevicornis, F. indicus, P. stylifera* and *S. crassicornis*). Further studies with other species (*P. monodon*, *M. affinis, M. lysianasa, M. stridulans, F. merguiensis*, *P. maxillipedo*, *M. japonicas*, and *A. paludicola*) are necessary to conclude that whether the primer used is species specific. The subjected species showed average nucleotide divergence of 1.49% from its closest relative, which was <3%. Therefore the species could not be discriminated. The minimum interspecies divergence, 0.00% was observed between two Indian

haplotypes *M. dobsoni* and *M. brevicornis*, which overlap each other. Therefore, further studies at population levels of these two species are required to rule out this ambiguity.

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