Asian Fisheries Science **20**(2007):205-216
ISSN: 0116-6514
https://doi.org/10.33997/j.afs.2007.20.2.006

Asian Fisheries Society, Manila, Philippines

Sequence Comparison of Mitochondrial 16s rRNA Gene Segment in Penaeids

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Abstract

A partial fragment of 16s rRNA mitochondrial gene was amplified by PCR from *Penaeus monodon* samples collected from the west and east coast regions of India. The amplified 520 bp of 16s rRNA mitochondrial gene segment of *P. monodon* from different locations on sequencing revealed similar sequence identity and no apparent sequence variation could be observed in this amplified segment. Amplification of 16s rRNA mitochondrial gene segment was also carried out from *Marsupenaeus japonicus* and *Fenneropenaeus indicus*. The sequence identity of 16s rRNA mitochondrial gene segments between *P. monodon* and *M. japonicus*, between *P. monodon* and *F. indicus* and between *M. japonicus* and *F. indicus* was found to be 91%, 92% and 91% respectively. The high level of sequence identity observed in the amplified PCR products of 16s rRNA mitochondrial gene segment of *P. monodon* isolated from the east and west coast regions of India, suggests that it will be more useful to focus on other variable mitochondrial DNA regions which may be more informative for population studies.

Introduction

Penaeus constitutes an economically important brackishwater aquaculture commodity worldwide. Growth in the shrimp aquaculture industry

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is hampered to some extent as domestication in some of the species cultured has not been achieved completely. Selection of animals for improved traits such as growth rate is highly desirable, but strong environmental influence plays a major role on the phenotypes (Hedgecock et al. 1982). For effective genetic improvement breeding programs for the domestication of shrimps, studies relating to its population structure, genetic diversity and establishment of molecular markers for species level identification are very important. Mitochondrial DNA (mtDNA) has been extensively used in finding the existence of genetic variability in crustaceans (Chu et al. 2003a; Haye et al. 2002). Use of mtDNA has its own advantage in population studies because of its maternal mode of inheritance and rapid evolution rate (Avise and Lansman 1983). Penaeid conserved mtDNA genes like 16s and 12s ribosomal RNA (Palumbi and Benzie 1991; Garcia-Machado et al. 1993; Millan et al. 2002) and Cytochrome oxidase subunit I (COI) (Palumbi and Benzie 1991; Baldwin et al. 1998; Gusmao et al. 2000) have been widely used to study the phylogeny and population genetic analysis of penaeid shrimps. However, the ribosomal mitochondrial gene segments are found to be highly conserved in some regions and variable in other regions (Tong et al. 2000). Therefore, the regions selected in 16s rRNA mitochondrial gene have an important role to obtain sequence variability for population analysis studies.

In the present study, sequence analysis of mitochondrial 16s rRNA gene segment was carried out using the primers reported by Bouchon et al. (1994). These primers which are reported to be universal in insects were used to investigate the genetic variation in the *P. monodon* samples collected from the east and west coast regions of India. Sequence comparison of mitochondrial 16s rRNA gene segment was also carried out between *P. monodon*, *M. japonicus* and *F. indicus* as an approach to find the usefulness of this gene segment for phylogenetic analysis in penaeids.

Materials and Methods

Shrimp samples

P. monodon shrimp samples were collected from the east coast (Kakdwip, Kakinada, Chennai, Chilka) and west coast (Cochin, Goa) of India. Samples of *F. indicus* and *M. japonicus* were collected from the Chennai region. Fresh samples were either collected in ice and stored frozen or the muscle tissues were preserved in alcohol till further use.

Mitochondrial DNA extraction

Mitochondrial DNA was extracted from shrimps *P. monodon*, *F. indicus* and *M. japonicus* following the procedure of Bouchon et al. (1994). The muscle tissues (100 mg) were washed in TE buffer (10mM Tris-HCl, 1mM EDTA, pH 8.0) and homogenized in 1.5 ml Eppendorf tube with 500 µl of homogenization buffer (30mM Tris-HCl, 30mM EDTA, 15 mM NaCl, pH 7.8) containing 100 µg ml^{-1} proteinase K. The homogenized solution was mixed with two volumes of SDS 1%, NaOH 0.2 N. The samples were stored in ice for 5 min and gently mixed with 1.5 volumes of potassium acetate (3M potassium, 5M acetate). After incubating in ice for 5 min the samples were centrifuged for 10 min at 12000 X g. The supernatant was phenol chloroform extracted before ethanol precipitation of DNA.

Amplification of mitochondrial 16s rRNA genes

Polymerase chain reaction was carried out using primers for mitochondrial 16s rRNA genes as described by Bouchon et al. (1994). The sequence of the primers used were, 16s rRNA I: 5'-CGCCTGTTTAACAAAAACAT 3' and 16s rRNA II: 5'-CCGGTCTGAACTCAGATCATGT 3'.

The PCR reaction mixture included all 4 dNTPs (200 µM), 30 pmoles concentration of each primer, 1 unit of Taq polymerase and 1X polymerase buffer containing 1.5 mM $MgCl_2$. All the PCR reagents and the primers were procured from Bangalore Genei, India. The thermal program followed was: 93°C for 1 min followed by 30 cycles of 93°C for 1 min, 50°C for 30s, 72°C for 1 min and 72°C for 7 min as final extension cycle.

Sequencing and sequence analysis

The PCR products were sequenced using both 16s rRNA forward and reverse primers by Bangalore Genei Ltd (India). Sequence homology search was carried out using BLAST algorithm (www. ncbi. nlm. nih.gov). Sequence alignment was done using Clustal W program at (http://www.ebi.ac.uk) web site. Base composition analysis was done at (http://molbiol-tools.ca) web site.

Results

Approximately 520 bp of PCR amplified product was obtained from *P. monodon*, *F. indicus* and *M. japonicus* using 16s rRNA mtDNA primers. The nucleotide sequence comparison of PCR amplified 16s rRNA gene segment among the *P. monodon* samples collected from different locations of the east and west coasts of India is shown in figure1. The aligned nucleotide sequence showed similar sequence identity among the *P. monodon* samples of different locations. The 16s rRNA mitochondrial gene segment of Kakinada region showed a replacement of guanine with adenine at nucleotide position 135. Guanine at position 135 was found to be conserved in the 16s rRNA mitochondrial gene segment of all samples collected from other locations. The other difference which was observed in the sequence comparison was deletions at positions 416 and 424 for the Chilka sample and at 430 position for Kakdwip, Kakinada and Goa regions.

The 16s rRNA mitochondrial sequence of *P. monodon* of Chennai region was compared for sequence identity by BLAST analysis with other reported 16s rRNA mitochondrial sequence of *P. monodon* (Table 1). The sequence identity was found 100% and 99 % identical to the 16s rRNA mitochondrial gene sequence of *P. monodon* from Mumbai and Gopalpur regions of India respectively, whereas 97 % identity was observed with Australia, China, Fiji and Hong Kong regions.

Table 1. Sequence identity of 16s rRNA mitochondrial gene segment of *P. monodon* (Chennai region) with other reported *P.monodon* partial sequences of 16s rRNA mitochondrial gene

GenBank Accession No.	Percentage Identity	Location
AY744272	100	India (Mumbai)
AY744273	99	India (Gopalpur)
AF217843	97	Australia
AF279829	97	China
AF105039	97	Hong Kong
AY751800	97	Hainan
AJ388113	97	Fiji

Figure 1.

```
Kakdwip
AATTCAACATCGAGGTCGCAAACCTTCTTGTCGATGAGGACTCTCAAAGAAGATTACGCT 60
Kakinada
AATTCAACATCGAGGTCGCAAACCTTCTTGTCGATGAGGACTCTCAAAGAAGATTACGCT 60
Goa
AATTCAACATCGAGGTCGCAAACCTTCTTGTCGATGAGGACTCTCAAAGAAGATTACGCT 60
Cochin
AATTCAACATCGAGGTCGCAAACCTTCTTGTCGATGAGGACTCTCAAAGAAGATTACGCT 60
Chennai
AATTCAACATCGAGGTCGCAAACCTTCTTGTCGATGAGGACTCTCAAAGAAGATTACGCT 60
Chilka
AATTCAACATCGAGGTCGCAAACCTTCTTGTCGATGAGGACTCTCAAAGAAGATTACGCT 60
************************************************************

Kakdwip
GTTATCCCTAAAGTAACTTAATCTTTTAATCTTTAATAAAGGATCAATTATTTTTCAATT 120
Kakinada
GTTATCCCTAAAGTAACTTAATCTTTTAATCTTTAATAAAGGATCAATTATTTTTCAATT 120
Goa
GTTATCCCTAAAGTAACTTAATCTTTTAATCTTTAATAAAGGATCAATTATTTTTCAATT 120
Cochin
GTTATCCCTAAAGTAACTTAATCTTTTAATCTTTAATAAAGGATCAATTATTTTTCAATT 120
Chennai
GTTATCCCTAAAGTAACTTAATCTTTTAATCTTTAATAAAGGATCAATTATTTTTCAATT 120
Chilka
GTTATCCCTAAAGTAACTTAATCTTTTAATCTTTAATAAAGGATCAATTATTTTTCAATT 120
************************************************************
Kakdwip
ATACTTGTTAATAAGTATTTAAGAACAGTTACTAATTATATTCCCGTCGCCCCAACGCAA 180
Kakinada
ATACTTGTTAATAAATATTTAAGAACAGTTACTAATTATATTCCCGTCGCCCCAACGCAA 180
Goa
ATACTTGTTAATAAGTATTTAAGAACAGTTACTAATTATATTCCCGTCGCCCCAACGCAA 180
Cochin
ATACTTGTTAATAAGTATTTAAGAACAGTTACTAATTATATTCCCGTCGCCCCAACGCAA 180
Chennai
ATACTTGTTAATAAGTATTTAAGAACAGTTACTAATTATATTCCCGTCGCCCCAACGCAA 180
Chilka
ATACTTGTTAATAAGTATTTAAGAACAGTTACTAATTATATTCCCGTCGCCCCAACGCAA 180
************* ********************************************

Kakdwip
CAAACATTAATTAAGATCAAGTTATACTAACAATTTATAATTTAACTAATTTATTGTCAA 240
Kakinada
CAAACATTAATTAAGATCAAGTTATACTAACAATTTATAATTTAACTAATTTATTGTCAA 240
Goa
CAAACATTAATTAAGATCAAGTTATACTAACAATTTATAATTTAACTAATTTATTGTCAA 240
Cochin
CAAACATTAATTAAGATCAAGTTATACTAACAATTTATAATTTAACTAATTTATTGTCAA 240
Chennai
CAAACATTAATTAAGATCAAGTTATACTAACAATTTATAATTTAACTAATTTATTGTCAA 240
Chilka
CAAACATTAATTAAGATCAAGTTATACTAACAATTTATAATTTAACTAATTTATTGTCAA 240
************************************************************

Kakdwip
GTTTTATAGGGTCTTATCGTCCCCTTAAAGTATTTAAGCCTTTTCACTTAAAAGTTAAGT 300
Kakinada
GTTTTATAGGGTCTTATCGTCCCCTTAAAGTATTTAAGCCTTTTCACTTAAAAGTTAAGT 300
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Figure 1 (continued):

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Goa
GTTTTATAGGGTCTTATCGTCCCCTTAAAGTATTTAAGCCTTTTCACTTAAAAGTTAAGT 300
Cochin
GTTTTATAGGGTCTTATCGTCCCCTTAAAGTATTTAAGCCTTTTCACTTAAAAGTTAAGT 300
Chennai
GTTTTATAGGGTCTTATCGTCCCCTTAAAGTATTTAAGCCTTTTCACTTAAAAGTTAAGT 300
Chilka
GTTTTATAGGGTCTTATCGTCCCCTTAAAGTATTTAAGCCTTTTCACTTAAAAGTTAAGT 300
************************************************************

Kakdwip
TCAACTATTACAATTGAGACAGATTACTTTTTGTCCAACCATTCATACAAGCCTTCAATT 360
Kakinada
TCAACTATTACAATTGAGACAGATTACTTTTTGTCCAACCATTCATACAAGCCTTCAATT 360
Goa
TCAACTATTACAATTGAGACAGATTACTTTTTGTCCAACCATTCATACAAGCCTTCAATT 360
Cochin
TCAACTATTACAATTGAGACAGATTACTTTTTGTCCAACCATTCATACAAGCCTTCAATT 360
Chennai
TCAACTATTACAATTGAGACAGATTACTTTTTGTCCAACCATTCATACAAGCCTTCAATT 360
Chilka
TCAACTATTACAATTGAGACAGATTACTTTTTGTCCAACCATTCATACAAGCCTTCAATT 360
************************************************************

Kakdwip
AAAAGACTAATGATTATGCTACCTTCGCACGGTCAGTATACCGCGGCCCTTTAAAAATAA 420
Kakinada
AAAAGACTAATGATTATGCTACCTTCGCACGGTCAGTATACCGCGGCCCTTTAAAAATAA 420
Goa
AAAAGACTAATGATTATGCTACCTTCGCACGGTCAGTATACCGCGGCCCTTTAAAAATAA 420
Cochin
AAAAGACTAATGATTATGCTACCTTCGCACGGTCAGTATACCGCGGCCCTTTAAAAATAA 420
Chennai
AAAAGACTAATGATTATGCTACCTTCGCACGGTCAGTATACCGCGGCCCTTTAAAAATAA 420
Chilka
AAAAGACTAATGATTATGCTACCTTCGCACGGTCAGTATACCGCGGCCCTTTAAAA-TAA 419
************************************************** ***
Kakdwip        TTCAGTGGG-CAGGCTAGACTTTAT 444
Kakinada       TTCAGTGGG-CAGGCTAGACTTTAT 444
Goa            TTCAGTGGG-CAGGCTAGACTTTAT 444
Cochin         TTCAGTGGGGCAGGCTAGACTTTAT 445
Chennai        TTCAGTGGGGCAGGCTAGACTTTAT 445
Chilka         TTCA-TGGGGCAGGCTAGACTTTAT 443
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Figure 1. Multiple nucleotide sequence alignment of 16s rRNA mitochondrial gene segment of *P. monodon* collected from East and West coasts regions of India. The running total number of nucleotides are shown on the right. The degree of similarity is illustrated underneath the alignments with a series of consensus symbols. '*' represents residues in that column which are identical in all sequences in the alignment. Gaps are represented by '-' symbol.

Multiple nucleotide sequence alignment of 16s rRNA mitochondrial gene segment of *P. monodon*, *F.indicus* and *M. japonicus* is shown in figure 2. The sequence identity of 16s rRNA mitochondrial gene segment between *P. monodon* and *M. japonicus*, between *P. monodon* and *F. indicus* and between *M. japonicus* and *F. indicus* were 91%, 92% and 91%

respectively. The G+C base composition was in the same range in all the three penaeids, *P. monodon* (32.3%), *F. indicus* (32.4%), *M. japonicus* (34.0%) (Table 2).

Table 2. Base composition of 16s rRNA mitochondrial gene segment of *P. monodon*, *F. indicus* and *M. japonicus*

Species	Size (bp)	Base composition					
		A	C	G	T	A+T	G+C
P. monodon	509	33.6	13.0	19.3	34.1	67.7	32.3
F.indicus	491	33.9	12.4	20.0	33.7	67.6	32.4
M.japonicus	488	32.5	12.9	21.1	33.5	66.0	34.0

Discussion

Some of the early studies related to genetic characterization of aquatic species based on RFLP of mtDNA describes isolation of whole mtDNA involving tedious mtDNA extraction procedures and use of radio-labeled probes for restriction pattern analysis in *P. monodon* (Klinbunga et al. 1998), *P. vannamei* (Bagshaw and Quiel 1998), trout (*Salmo trutta*) (Moran et al. 1996). The first report by Bouchon et al. (1994), on the use of PCR for amplification and RFLP of mtDNA 12s and 16s ribosomal genes in *P. monodon* has simplified the technique which is faster as compared to conventional whole mtDNA isolation procedures in shrimps. A 520 bp amplified product obtained for 16s rRNA gene segment in the present study was in the expected size range using the primers reported by Bouchon et al. (1994), who obtained similar size of the amplified product of 16s rRNA mitochondrial gene segment in *P. monodon* and *M. japonicus.* Using these primers which are reported to be universal in insects, our previous study has shown the successful amplification of 16s rRNA mitochondrial gene segment from across the taxonomically different brackish-water species in addition to shrimps such as grey mullet, tilapia, Asian sea bass and mud crabs. Shrimps and mudcrabs showed amplified product of 520 bp whereas, finfishes showed amplified product of 700 bp using the same 16s rRNA mitochondrial primers (Shekhar et al. 2005).

Sequence alignment of *P. monodon* 16s rRNA gene segment obtained from different regions of India showed similar sequence with no apparent variation. The observation of very minor sequence variation in the 16s rRNA mitochondrial gene segment of Kakinada region which showed

only one nucleotide replacement of guanine with adenine is not very significant. The other deletions observed were present at positions 416 and 424 for the Chilka sample and at 430 position for Kakdwip, Kakinada and Goa regions as shown in figure 1. These deletions are located at the far 3' end region of 16s rRNA mitochondrial gene sequence obtained in the present study. However, other than these minor sequence variations, the overall sequence of 16s rRNA gene segment was observed to be the same for all the samples of *P.monodon* analysed. In addition, a 100% sequence similarity of *P. monodon* of Chennai region with Mumbai region (GenBank accession no. AY744272), indicates that the 16s rRNA gene segment amplified in the present study is highly conserved and does not show any sequence variation in the *P. monodon* samples between the east and the west coast regions of India. The conserved nature of 16s rRNA mitochondrial gene segment, suggests that the use of some other DNA genetic markers such as microsatellites, would be more appropriate to investigate the genetic diversity of natural *P. monodon* population of India.

Figure 2.

```
P.monodon
CATGTCTATATGATTGTTATATAAAGTCTAGCCTGCCCCACTGAATTATTTTTAAAGGGC 60
F.indicus
--------------TGTTATATAAAGTCTAGCCTGCCC-ACTGATTTAGTTT-AAAGGGC 44
M.japonicus
--------------TGTTATATAAAGTCTAGCCTGCCC-ACTGATTTGTTTT-AAAGGGC 44
              ************************ ***** **  *** *******

P.monodon
CGCGGTATACTGACCGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTA 120
F.indicus
CGCGGTATATTGACCGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGAAGGCTTGTA 104
M.japonicus
CGCGGTATATTGACCGTGCGAAGGTAGCATAATCATTAGTCTTTTAATTGGAGGCTTGTA 104
********* ****************************************** *********

P.monodon
TGAATGGTTGGACAAAAAGTAATCTGTCTCAATTGTAATAGTTGAACTTAACTTTTAAGT 180
F.indicus
TGAATGGTTGGACAAAAAGTAAGCTGTCTCAATTATAATAATTGAATTTAACTTTTAAGT 164
M.japonicus
TGAATGGTTGGACAAAAAGTAAGCTGTCTCGGTTATAATAATTGAACTTAACTTTTAAGT 164
********************** *******  ** ***** ***** *************

P.monodon
GAAAAGGCTTAAATACTTTAAGGGGACGATAAGACCCTATAAAACTTGACAATAAATTAG 240
F.indicus
GAAAAGGCTTAAATAAATTAAGGGGACGATAAGACCCTATAAAGCTTGACAATAATTTAA 224
M.japonicus
GAAAAGGCTTAAATGTTTCAGGGGGACGATAAGACCCTATAAAGCTTGACAATAATTTCG 224
**************   * * ********************** *********** **

P.monodon
TTAAATTATAAATTGTTAGTATAACTTGATCTTAATTAATGTTTGTTGCGTTGGGGCGAC 300
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Figure 2 (continued):

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F.indicus
TTATACTATCAATTGTTAGTATAACTTGGTTTTAATTAATATTTGTTGCGTTGGGGCGAC 284
M.japonicus
TTATATTATAAATTGTTAGTATAACTTGATTTTAACGGGAGTTTGTTTCGTTGGGGCGAC 284
*** * *** ****************** * ****      ****** ************

P.monodon
GGGAATATAATTAGTAACTGTTCTTAAATACTTATTAACAAGTATAATTGAAAAATAAT- 359
F.indicus
GAGAATATAATAGGTAACTGTTCTTAAATATTTAATAACAAATATAATTGGAAATTAGTA 344
M.japonicus
GGGAATATAATAAATAACTGTTCTTTTAAATATAATTACAAAAATACTTGGTAAATAAT- 343
* *********   ***********  * *  ** * ****  *** ***  ** ** *

P.monodon
TGATCCTTTATTAAAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTAATCTTCTT 419
F.indicus
TGATCCTCTATTAGCGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTAATCTTCTT 404
M.japonicus
TGATCCTCTATTAGAGATTAAAAGATTAAGTTACTTTAGGGATAACAGCGTAATCTTCTT 403
******* *****  ********************************************

P.monodon
TGAGAGTCCTCATCGACAAGAAGGTTTGCGACCTCGATGTTGAATTAAGGTATCCTTATA 479
F.indicus
TGAGAGTCCCTATCGACAAGAAGGTTTGCGACCTCGATGTTGAATTAAGGTATCCTTATG 464
M.japonicus
TGAGAGTCCACATCGACAAGAAGGTTTGCGACCTCGATGTTGAATTAAGGTATCCTTATA 463
*********  **********************************************

P.monodon       ATGCAGCAGTTATAAAGGAAGGTCTGTTCG 509
F.indicus       ATGCAGCAGTTATAAAGGAAGGTCTGT--- 491
M.japonicus     ATGCAGCAGTTATAAAGGAGGGTCT----- 488
                ******************* *****
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Figure 2. Multiple nucleotide sequence alignment of 16s rRNA mitochondrial gene segment of *P. monodon*, *F.indicus* and *M.japonicus*. The running total number of nucleotides are shown on the right. The degree of similarity is illustrated underneath the alignments with a series of consensus symbols. '*' represents residues in that column which are identical in all sequences in the alignment. Gaps are represented by '-' symbol.

For example, polymorphic microsatellites have been successfully developed for studying genetic diversity of *P. monodon* wild populations of Thailand and Taiwan (Pongsomboom et al. 2000; Pan et al. 2004). However, a 97 % sequence identity in 16s rRNA mitochondrial gene segment of *P. monodon* (Chennai region) was observed in the present study when compared to other reported 16s rRNA mitochondrial gene sequence of *P. monodon* from Australia, China, Fiji and Hong Kong regions. A similar significant intraspecific genetic differentiation (1.7 %) has been demonstrated between the Fiji population and the Malaysia and Australia populations of *P. monodon* by RFLP analysis of 12s and 16s rRNA mtDNA genes (Bouchon et al. 1994).

The sequence variation observed in the present study between *P. monodon* and *M. japonicus* is in close agreement with the reported mean genetic divergence of 13.9 ± 3.4 % between the same two species by RFLP analysis of 12s rRNA and 16s rRNA mtDNA gene segments (Bouchon et al. 1994). The sequence divergence is reported to be less in 16s rRNA than COI in *Metapenaeopsis*. Phylogenetic analysis of *Metapenaeopsis* based on partial sequences of 16s rRNA and COI mtDNA genes showed 6.4 % and 15.8 % sequence divergence respectively for the two mtDNA genes (Tong et al. 2000). Genetic difference of 11 % and 9.6 % in COI sequence and 12s rRNA mtDNA gene respectively has been observed between *P. stylirotris* and *P. vannamei* (Palumbi and Benzie 1991). A similar range of 11% nucleotide divergence was observed in 16s rRNA partial sequences between *P. notialis* and *P. schmitti* (Garcia-Machado et al. 1993). The G+C base composition in the present study was found to be in the same range in all the three penaeids, *P. monodon* , *F. indicus* and *M. japonicus* (Table 2). Similar observation of equal range G+C content in approximately 1300 bp fragment size of 12-16s ribosomal mtDNA has been reported in the case of *P. californiensis* (28.52 %), *P. vannamei* (28.79 %), *P. stylirostris* (28.7 %) and *P. monodon* (23.5 %) (Millan et al. 2002).

The high level of sequence identity observed in the amplified PCR products of 16s rRNA mitochondrial gene segment suggests that it will be more useful to focus on other highly variable mtDNA regions for population genetic survey. Since different regions of mtDNA evolve at different rates, therefore the primers designed based on the regions selected in 16s rRNA mtDNA gene have a critical role to obtain sequence variability. Similar observations have been reported by Tong et al. (2000), as the 16s rRNA gene sequences on alignment were found to be highly conserved in some regions and moderately variable and phylogenetically informative in other. In contrast, the variable sites in COI gene were found to be evenly distributed. Comparison in the mitochondrial gene fragments of *P. merguiensis* showed less number of variables and phylogenetically informative sites in 16s rRNA than COI fragment (Chu et al. 2003b). However, very low levels of sequence variation of COI gene have been observed in *Metapenaeopsis* species (Tong et al. 2000), *Penaeus* species (Baldwin et al. 1998) and between *Metapenaeopsis* and *Penaeus* species (Palumbi and Benzie 1991) suggesting highly overall conserved sequence of COI among different genera of the Penaeidae. In case of tautog (*Tautoga onitis*), DNA sequence analysis in several genes of mitochondrial genome showed

polymorphisms in ND2 and control region fragments but not in the COI, ATPase 6 or cyt b fragments (Orbacz and Gaffney 2000).

Although in the present study, sequence variation observed between the species was much higher as compared within the species still, more information is required on the sequence composition of other mitochondrial genes for phylogenetic analysis. Molecular phylogenetic tree construction based on one or two genes analysis may give misleading results due to insufficient informative characters (Wilson et al. 2000). The phylogenetic analysis therefore should not be dependent on single gene sequence analysis but should be a study of combination of genes or preferably should involve highly variable mtDNA regions such as control regions which may be more phylogenetically informative.

Conclusion

The sequence analysis of 16s rRNA mitochondrial gene segment did not show any apparent sequence variation in *P.monodon* samples collected from the east and west coast regions of India. It is suggested that primers design should be focused on more variable regions in mtDNA to get sufficient sequence variability for population analysis.

Acknowledgement

The authors wish to thank Dr. P. Ravichandran, Director, Central Institute of Brackishwater Aquaculture, Chennai, India, for providing the necessary facilities to carry out this study.

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Received March 30, 2005; Accepted December 19, 2006