CHAPTER-III

18s rRNA gene polymorphisms of *P. homarus* populations from different geographic regions

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INTRODUCTION

The spiny lobster *P. homarus* has long been of interest to evolutionary biologists because of its high level of species diversity, its wide geographic distribution, and the importance of many species to commercial fisheries. Among genera in the family Palinuridae, *Panulirus* has been the most successful in terms of species diversity; 19 species have been described to date, three of which are divided into seven recognized subspecies (Holthuis 1991; George 1997; Sarver *et al.,* 1998). The key to this successful radiation is thought to be the invasion by species of *Panulirus* of shallow-water, tropical environments, which permits the occupation of varied habitats not accessible to congeners (George and Main 1967; George 1997). Because of morphological, ecological, and behavioural diversity, both within and among species of *Panulirus*, the phylogenetic history of this group has not been well understood.

The 16S rRNA sequences (16S) have been one of the most common segments of the mitochondrial genomes used in phylogenetic analysis. For crustaceans they have been used in the analysis of penaeid prawns (Palumbi and Benzie, 1991; Bouchon *et al.,* 1994), freshwater crayfishes (Grandjean *et al.,* 1998; Crandall *et al.,* 1999), marine lobsters (Harding *et al.,* 1997; Ovendon *et al.,* 1997; Sarver *et al.,* 1998; Machordom and Macpherson, 2004), fairy shrimps (Daniels *et al.,* 2004), Caribbean sponge-dwelling snapping shrimps (Morrison et al., 2004), and freshwater prawns (Murphy and Austin, 2002,

2003, 2004, 2005; Liu *et al.,* 2007). A few recent studies indicated that the 16S produced poor resolution in reconstructed phylogenies for insects, perhaps due to its fast evolutionary rate (Misof *et al.,* 2001; Hasegawa and Kasuya, 2006). The poor resolution was also found for phylogenies of freshwater prawns. Murphy and Austin (2005) made the phylogenetic analysis of 30 species of Macrobrachium, but it resulted in a poor resolution; their phylogenetic tree had a very high degree of polytomy represented by a single multifurcating node with the clades of 28 taxa, 93% of the total taxa under the study. Liu *et al.,* (2007) increased the number of taxa to 38 but also obtained a poor resolution; their phylogenetic tree also had a high degree of polytomy represented by a multifurcating node with the clades of 37 taxa, 97% of the total taxa under the study.

Recently, nuclear 18S rRNA sequences (18S) have been used in phylogenetic studies of crustaceans (Crandall *et al.,* 2000; Jarman *et al.,* 2000). The 18S gene of nuclear DNA was found to have an evolutionary rate similar to or slower than that of the 16S gene (Fetzner and Crandall, 2001). It was able to clarify the relationships among distantly related taxa for water striders (Muraji and Tachikawa, 2000) and Odonata (Hasegawa and Kasuya, 2006), and also among closely related taxa for insects in Ichneumonoidea (Belshaw *et al.,* 1998) and the hominoids (Gonzalez *et al.,* 1990).

This study is intended to determine whether the 18S is a better tool than the 16S in phylogenetic analysis of spiny lobsters collected from five different geographical regions, by comparing their characters and evaluating topological resolution and phylogenetic signals of their reconstructed phylogenetic trees.

Materials and Methods

DNA extraction and Primer design

Total genomic DNA was extracted using a modified CTAB protocol as described in Chapter I. The 18S lobster primer sequences and their GC contents were given in table: 6. 1 μM concentration of primer was used in PCR as well as sequencing. The protocols were followed according to the manufacturer's directions.

Table: 6. 18S rRNA Sequence primer.

Primer	Sequence	Tm	%GC	Length(bp)
Forward 5'	CGCACGAGAATGAGCAATAA	59	45	20
Reverse 3'	GTACAAAGGGCAGGGACGTA	59	55	20

The portion of the 18S gene was amplified by PCR (polymerase chain reaction) with above primer. Double-stranded PCR products were obtained in a total reaction volume of 50 μL, containing 5 μL of 103 ExTaq buffer (Mg2+ added; TaKaRa), 5 μL of dNTP mixture (2.5 mM of each), 1 μM of each primer, 1 unit of Taq polymerase (TaKaRa Ex TaqTM; BIOGENO, INDIA), and 5 μL of DNA extract (100 ng). For the 18S sequences, the target segment was amplified by PCR in the following temperature regime: the initial denaturizing step at 94^{O}C for 5 min, followed by 35 cycles of denaturizing at 94^{O}C for 1 min, an annealing temperature of 48–55^{O}C for 1 min, an extension temperature of 72^{O}C for 1 min, and then an additional extension of 72^{O}C for 5 min.

For the 18S sequences, PCR products were purified using Geneaid Genomic DNA Purification Kit (ORCUTIS SCIENTIFIC). All sequencing reactions followed the Perkin Elmer protocol, using a BigDye Terminator v3.1 Cycle Sequencing Kit with a DNA Analyzer (APPLIED BIOSYSTEMS). The sequencing was performed in both directions. The forward and reverse sequence chromatograms were checked and edited manually using the programs of SeqMan 5.01 (DNASTAR) and BioEdit version 7.0.4.1 (Hall, 1999). Multiple alignments were performed using Clustal W (Thompson *et al.,* 1994) with default parameter values (gap open penalty 10, gap extension penalty 0.1 in pairwise and 0.05 in multiple alignments) and all alignments were manually checked. All sequences of the 18S extracted in this study were deposited at the GenBank database (Table 7).

Sequence Character Analysis

Characters of nucleotide sequences were examined with the exploratory data analysis, using MEGA version 3.1 (Kumar *et al.,* 2004), DAMBE version 50.7 (Xia and Xie, 2001) and DnaSP 4.00 (Rozas *et al.,* 2003). The genetic divergent distances among taxa were calculated using the best appropriated DNA substitution model obtained from Modeltest version 3.7 (Posada and Crandall, 1998). The distribution of pair-wise sequence divergences among the *P. homarus* sequences was established using SigmaPlot version 8.0.

True evolutionary relationships would be obscured in DNA sequence data sets if sites have become saturated by multiple substitutions (Swofford *et al.,* 1996; Jesus *et al.,* 2007). In order to assess the level of saturation in 18S rRNA gene, pair-wise sequences comparisons were performed by plotting the number of nucleotide substitutions per site for transitions and transversions, respectively, against the genetic divergent distances calculated from the best

appropriated DNA substitution model, using PAUP* 4.0b10 (Swofford, 2002) and SigmaPlot version 8.0.

RESULTS

Characters of the extracted 18S gene sequence of five different sites are shown in Plate: 1. The data has more or less same alignment length with 1798 nucleotide bases and were 0.01% in both variable and parsimoniously informative sites. Also, there was a significant difference in the percentage composition of nucleotide contents (adenine, thymine, cytosine, and guanine) between the collection sites. A significant difference was also found in relative frequencies of nucleotide substitutions (adenine-guanine, cytosine-thymine, adenine-cytosine, adenine-thymine, cytosine- guanine, and guanine-thymine).

The 18s rRNA gene was amplified and sequenced by using primer Forward 5'CGCACGAGAATGAGCAATAA and reverse 3'GTACAAAGGGCAGGGACGTA. The sequenced 18S rRNA genes were deposited on Genbank (Table: 7).

Table: 7. Genbank Accession Number and Length of Gene Sequence.

Species	Location	Gene	Genbank Accession No.	Gene sequence Length.
P.homarus	Vizhinjam	18S	GQ411528	1799 bp.
P.homarus	Cochin	18S	GQ411529	1798 bp.
P.homarus	Chinnamuttom	18S	GQ411530	1797 bp.
P.homarus	Tuticorin	18S	GQ411531	1800 bp.

Plate: 1

a) 18s rRNA sequence of *Panulirus homarus* from Vizhinjam

ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATT
CTAGAGCTAATACATGCATCACGTCTCTGACCGCAAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCAC
CCACCTGTGGTGAATCTGAATAACTTCTCGCTGTAGCCAGTGTCTACGCACCGGCGCCGAGTCTTTCAAGTGTCTGCCTTATCAGC
TTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCTAAGGGAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAC
TCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAATAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTTCA
CCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACGGCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGC
ACGGTTATTTTGAAAAAATTAGAGTGCTCAGAGCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCT
CGGTTCTATTTTGTTGGTTTGCGGAACCCGAGGTAATGACTAATAGGAATTGGCGGGGTCATTCGTATTGCGACGCTAGAGGTGAA
ATTCTTGGACCGTCGCAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTCATTAATCAAGAACGAAAGTTAGAGGTTCGAA
GGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGACCCGGCGGGCAGCT
TCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGG
AGTGGAGCCTGCGGCTTAATTTGACTCAACTCGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAGCTCTTT
CTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTAG
CCTATTAACTAGTCGACGGATCTCCAGCATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCC
GCACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAAGGGATCAACGTGTTCT
CCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATT
CCCAGTAAGCGCAAGTCATCAGCTTGCGTTGATTTCGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGATT
TAGTGAGGCCTTCGGACTGGCGCTCTTGGATGTTCTACCCCATCCCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCC
TCGAGCTGACGGAAAGATGTCCAAACTTGATCATCTAGAGGAAGTAAAGTCGTACCAAGGTTTCCGTAGGTGAACCTGC

b) 18s rRNA sequence of *Panulirus homarus* from Cochin

ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATT
CTAGAGCTAATACATGCATCACGTCTCTGACCGCAAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCAC
CCACCTGTGGTGAATCTGAATAACTTCTCGCTGAGCGCAGCGTTTACGCACCGGCGCCGAGTCTTTCAAGTGTCTGCCTTATCAGC
TTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAC
TCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTTCA
CCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACTTCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGC
ACGTTTACTTTGAAAAAATTAGAGTGCTCAGAGCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCT
CGGTTCTATTTTGTTGGTTTGCGGAACCCGAGGTAATGACTAATAGGAACAGGCGGGGGCATTCGTATTGCGACGCTAGAGGTGAA
ATTCTTGGACCGTCGCAAGACAACTACTGCGAAAGCATTTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGAGGTTCGAA
GGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGACCCGGCGGGCAGCT
TCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGG
AGTGGAGCCTGCGGCTTAATTTGACTCAACCGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAGCTCTTTC
TCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTAGC
CTATTAACTAGTCGACGGATCTCCAGCATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCG
CACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAAGGGATCAACGTGTTCTC
CCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTC
CCAGTAAGCGCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGATTT
AGTGAGGCCTTCGGACTGGCGCTCTTGGATGTTCTACCCCATCCCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCT
CGAGCTGACGGAAAGATGTCCAAACTTGATCATTTAGAGGAAGTTAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGC

c) 18s rRNA sequence of *Panulirus homarus* from Muttom

ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATT
CTAGAGCTAATACATGCATCACGTCTCTGACCGCAAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCAC
CCACCTGTGGTGAATCTGAATAACTTCTCGCTGAGCGCAGGGTCTACGCACCGGCGCCGAGTCTTTCAAGTGTCTGCCTTATCAGC
TTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAC
TCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTTCA
CCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACCTCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGC
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CGGTTCTATTTTGTTGGTTTACGGAACCCGAGGTAATGACTAATAGGAACAGGCGGGGGCATTCGTATTGCGACGCTAGAGGTGAA
ATTCTTGGACCGTCGCAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTCATTAATCAAGAACGAAAGTTAGAGGTTCGAA
GGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGACCCGGCGGGCAGCT
TCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGG
AGTGGAGCCTGCGGCTTAATTTGACTCAACTCGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAGCTCTTT
CTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTAG
CCTATTAACTAGTCGACGGATCTCCAGCATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCC
GCACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAAGGGATCAACGTGTTCT
CCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATT
CCCAGTAAGCGCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGATT
TAGTGAGGCCTTCGGACTGGCGCTCTTGGTTGTTCTACCCCATCCCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCC
TCGAGCTGACGGAAAGATGTCCAAACTTGATCATTTAGAGGAAGGAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGC

d) 18s rRNA sequence of *Panulirus homarus* from Chinnamuttom

ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATT
CTAGAGCTAATACATGCATCACGTCTCTGACCGCAAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCAC
CCACCTGTGGTGAATCTGAATAACTTCTCGCTGAGCGCACGGTCTACGCACCGGCGCCGAGTCTTTCAAGTGTCTGCCTTATCAGC
TTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAC
TCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTTCA
CCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACCGCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGC
TCGTTTACTTTGAAAAAATTAGAGTGCTCAGAGCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCT
CGGTTCTATTTTGTTGGTTTACGGAACCCGAGGTAATGACTAATAGGAACAGGCGGGGTCATTCGTATTGCGACGCTAGAGGTGAA
ATTCTTGGACCGTCGCAAGACAACTACTGCGAAAGCATTTGCCAAGGATGTTTCATTAATCAAGAACGAAAGTTAGAGGTTCGAAG
GCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGACCCGGCGGGCAGCTT
CCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAAAGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGA
GTGGAGCCTGCGGCTTAATTTGACTCAACAGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAGCTCTTTCT
CGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTAGCC
TATTAACTAGTCGACGGATCTCCAGCATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCGC
ACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAAGGGATCAACGTGTTCTCC
CCCTCCGAGAGGAGCGGGTAACCCGATCAAAACCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTCC
CAGTAAGCGCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGATTTA
GTGAGGCCTTCGGACTGGCGCTCTTGGATGTTCTACCCCATCCCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCTC
GAGCTGACGGAAAGATGTCCAAACTTGATCATTTAGAGGAAGTAGAGTCGTAACAAGGTTTCCGTAGGTGAACCTGC

e) 18s rRNA sequence of *Palinurus homarus* from Tuticorin

ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACCCACTTACTTGGATAACTGTGGTAATT
CTAGAGCTAATACATGCATCACGTCTCTGACCGCAAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCAC
CCACCTGTGGTGAATCTGAATAACTTCTCGCTGAGCGCAGTGTCTACGCACCGGCGCCGAGTCTTTCAAGTGTCTGCCTTATCAGC
TTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTAACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACG
GCTACCACATCTAAGGAAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAACGATGCGAGAC
TCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGAGGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCG
CGGTAATTCCAGCTCCAATAGCGTATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTTCA
CCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACGGCCGGCTCGCACGGGGTGCTCTTCATCGAGTGTCCCGAGTGGCCGGC
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CGGTTCTATTTTGTTGGGTTGTCGGAACCCGAGGTAATGACTAATAGAAACAGGCGGGGGCATTCGTATTGCGACGCTAGAGGTGA
AATTCTTGGACCGTCGCAAGACGAACTACTGCGAAAGCATTTGCCAAGGATGTTTCATTAATCAAGAACGAAAGTTAGAGGTTCGA
AGGCGATCAGATACCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGACCCGGCGGGCAGC
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GAGTGGAGCCTGCGGCTTAATTTGACTCAAAACGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAGCTCTT
TCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGTCTGGTTAATTCCGATAACGAACGAGACTCTA
GCCTATTAACTAGTCGACGGATCTCCAGCATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGC
CGCACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCTACACTGAAGGGATCAACGTGTTC
TCCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACCCTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAAT
TCCCAGTAAGCGCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTACTACCGATTGAATGAT
TTAGTGAGGCCTTCGGACTGGCGCTCTTGAATGTTCTACCCCATCCCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGC
CTCGAGCTGACGGAAAGATGTCCAAACTTGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTGC

Plate: 2.

ClustalW 2.0 Multiple Sequence alignment and Phylogenetic distance tree using UPGMA method

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Muttom          ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACC
Chinnamuttom    ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACC
Cochin          ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACC
Tuticorin       ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACC
Vizhijam        ATTAAGGCGAAACCGCGAATGGCTCATTAAATCAGCTATGATTCATTGGATCTGTAAACC
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Muttom          CACTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGCATCACGTCTCTGACCGC
Chinnamuttom    CACTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGCATCACGTCTCTGACCGC
Cochin          CACTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGCATCACGTCTCTGACCGC
Tuticorin       CACTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGCATCACGTCTCTGACCGC
Vizhijam        CACTTACTTGGATAACTGTGGTAATTCTAGAGCTAATACATGCATCACGTCTCTGACCGC
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Muttom          AAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCACCCACCTGT
Chinnamuttom    AAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCACCCACCTGT
Cochin          AAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCACCCACCTGT
Tuticorin       AAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCACCCACCTGT
Vizhijam        AAGGGAAGAGCGCTTTTATTAGTTCAAAACCAGTCGGGCCTCGGTCCGTCACCCACCTGT
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Muttom          GGTGAATCTGAATAACTTCTCGCTGAGCGCAGGGTCTACGCACCGGCGCCGAGTCTTTCA
Chinnamuttom    GGTGAATCTGAATAACTTCTCGCTGAGCGCACGGTCTACGCACCGGCGCCGAGTCTTTCA
Cochin          GGTGAATCTGAATAACTTCTCGCTGAGCGCAGCGTTTACGCACCGGCGCCGAGTCTTTCA
Tuticorin       GGTGAATCTGAATAACTTCTCGCTGAGCGCAGTGTCTACGCACCGGCGCCGAGTCTTTCA
Vizhijam        GGTGAATCTGAATAACTTCTCGCTGTAGCCAGTGTCTACGCACCGGCGCCGAGTCTTTCA
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Muttom          AGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTA
Chinnamuttom    AGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTA
Cochin          AGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTA
Tuticorin       AGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTA
Vizhijam        AGTGTCTGCCTTATCAGCTTTCGATTGTAGGTTATGCGCCTACAATGGCTATAACGGGTA
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Muttom          ACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG
Chinnamuttom    ACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG
Cochin          ACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG
Tuticorin       ACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG
Vizhijam        ACGGGGAATCAGGGTTCGATTCCGGAGAGGGAGCCTGAGAAACGGCTACCACATCTAAGG
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Muttom          AAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAAC
Chinnamuttom    AAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAAC
Cochin          AAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAAC
Tuticorin       AAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAAC
Vizhijam        GAGGCAGCAGGCACGCAAATTACCCACTCCCGGCACGGGGAGGTAGTGACGAAAAATAAC
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Muttom          GATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGA
Chinnamuttom    GATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGA
Cochin          GATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGA
Tuticorin       GATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGA
Vizhijam        GATGCGAGACTCATCCGAGGCCTCGCAATCGGAATGAGTACACTTTAAATCCTTTAACGA
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Muttom          GGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGT
Chinnamuttom    GGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGT
Cochin          GGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGT
Tuticorin       GGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGT
Vizhijam        GGATCCATTGGAGGGCAAGTCTGGTGCCAGCAGCCGCGGTAATTCCAGCTCCAATAGCGT
                ************************************************************

Muttom          ATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTT
Chinnamuttom    ATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTT
Cochin          ATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTT
Tuticorin       ATATTAAAGTTGTTGCGGTTAAAAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTT
Vizhijam        ATATTAAAGTTGTTGCGGTTAATAAGCTCGTAGTTGGATTTCAGTTCCGGACTGACGGTT
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Muttom          CACCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACCTCCGGCTCGCACGGGGTGC
Chinnamuttom    CACCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACCGCCGGCTCGCACGGGGTGC
Cochin          CACCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACTTCCGGCTCGCACGGGGTGC
Tuticorin       CACCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACGGCCGGCTCGCACGGGGTGC
Vizhijam        CACCGCCCGGTGTCTACTGTCACGCTCCGAACAGCCGCACGGCCGGCTCGCACGGGGTGC
                ************************************  ******************

Muttom          TCTTCATCGAGTGTCCCGAGTGGCCGGCTCGTTTACTTTGAAAAAATTAGAGTGCTCAGA
Chinnamuttom    TCTTCATCGAGTGTCCCGAGTGGCCGGCTCGTTTACTTTGAAAAAATTAGAGTGCTCAGA
Cochin          TCTTCATCGAGTGTCCCGAGTGGCCGGCACGTTTACTTTGAAAAAATTAGAGTGCTCAGA
Tuticorin       TCTTCATCGAGTGTCCCGAGTGGCCGGCACGTTTACTTTGAAAAAATTAGAGTGCTCAGA
Vizhijam        TCTTCATCGAGTGTCCCGAGTGGCCGGCACGGTTATTTTGAAAAAATTAGAGTGCTCAGA
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Muttom          GCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCTCGGTTC
Chinnamuttom    GCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCTCGGTTC
Cochin          GCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCTCGGTTC
Tuticorin       GCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCTCGGTTC
Vizhijam        GCAGGCTCCTTGAATGGCCTGAATGTCTATGCATGGAATAATGGAATAGGACCTCGGTTC
                ************************************************************

Muttom          TATTTTGTTGGTTTA-CGGAACCCGAGGTAATGACTAATAGGAACAGGCGGGGGCATTCG
Chinnamuttom    TATTTTGTTGGTTTA-CGGAACCCGAGGTAATGACTAATAGGAACAGGCGGGGTCATTCG
Cochin          TATTTTGTTGGTTTG-CGGAACCCGAGGTAATGACTAATAGGAACAGGCGGGGGCATTCG
Tuticorin       TATTTTGTTGGGTTGTCGGAACCCGAGGTAATGACTAATAGAAACAGGCGGGGGCATTCG
Vizhijam        TATTTTGTTGGTTTG-CGGAACCCGAGGTAATGACTAATAGGAATTGGCGGGGTCATTCG
                *********** **  ************************ **  ******* ******

Muttom          TATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACTGCGAAAGCAT
Chinnamuttom    TATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGAC-AACTACTGCGAAAGCAT
Cochin          TATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGAC-AACTACTGCGAAAGCAT
Tuticorin       TATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACTGCGAAAGCAT
Vizhijam        TATTGCGACGCTAGAGGTGAAATTCTTGGACCGTCGCAAGACGAACTACTGCGAAAGCAT
                ****************************************** ****************

Muttom          TTGCCAAGGATGTTT-CATTAATCAAGAACGAAAGTTAGAGGTTCGAAGGCGATCAGATA
Chinnamuttom    TTGCCAAGGATGTTT-CATTAATCAAGAACGAAAGTTAGAGGTTCGAAGGCGATCAGATA
Cochin          TTGCCAAGGATGTTTTCATTAATCAAGAACGAAAGTTAGAGGTTCGAAGGCGATCAGATA
Tuticorin       TTGCCAAGGATGTTT-CATTAATCAAGAACGAAAGTTAGAGGTTCGAAGGCGATCAGATA
Vizhijam        TTGCCAAGGATGTTT-CATTAATCAAGAACGAAAGTTAGAGGTTCGAAGGCGATCAGATA
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Muttom          CCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGA
Chinnamuttom    CCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGA
Cochin          CCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGA
Tuticorin       CCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGA
Vizhijam        CCGCCCTAGTTCTAACCATAAACGATGCTGACTAGCGATCCGCCGGCGTTATTCCCATGA
                ************************************************************

Muttom          CCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAA
Chinnamuttom    CCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAA
Cochin          CCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAA
Tuticorin       CCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAA
Vizhijam        CCCGGCGGGCAGCTTCCGGGAAACCAAAGTCTTTGAGTTCCGGGGGAAGTATGGTTGCAA
                ************************************************************

Muttom          AGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT
Chinnamuttom    AGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT
Cochin          AGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT
Tuticorin       AGCTGAAACTTAAAGGAATTGACGGAAGGGCACCCCCAGGAGTGGAGCCTGCGGCTTAAT
Vizhijam        AGCTGAAACTTAAAGGAATTGACGGAAGGGCACCACCAGGAGTGGAGCCTGCGGCTTAAT
                ********************************** ************************

Muttom          TTGACTCAACTCGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAG
Chinnamuttom    TTGACTCAACA-GGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAG
Cochin          TTGACTCAACC-GGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAG
Tuticorin       TTGACTCAAAACGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAG
Vizhijam        TTGACTCAACTCGGGGAACCTCACCAGGCCCGGACACCGGAAGGATTGACAGATTGAGAG
                *********   ************************************************

Muttom          CTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGT
Chinnamuttom    CTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGT
Cochin          CTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGT
Tuticorin       CTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGT
Vizhijam        CTCTTTCTCGATTCGGTGGGTGGTGGTGCATGGCCGTTCTTAGTTGGTGGAGCGATTTGT
                ************************************************************

Muttom          CTGGTTAATTCCGATAACGAACGAGACTCTAGCCTATTAACTAGTCGACGGATCTCCAGC
Chinnamuttom    CTGGTTAATTCCGATAACGAACGAGACTCTAGCCTATTAACTAGTCGACGGATCTCCAGC
Cochin          CTGGTTAATTCCGATAACGAACGAGACTCTAGCCTATTAACTAGTCGACGGATCTCCAGC
Tuticorin       CTGGTTAATTCCGATAACGAACGAGACTCTAGCCTATTAACTAGTCGACGGATCTCCAGC
Vizhijam        CTGGTTAATTCCGATAACGAACGAGACTCTAGCCTATTAACTAGTCGACGGATCTCCAGC
                ************************************************************

Muttom          ATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCGC
Chinnamuttom    ATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCGC
Cochin          ATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCGC
Tuticorin       ATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCGC
Vizhijam        ATGCTCCGTGGTGTCCGTTCGCAACGTCTTCTTAGAGGGATAAGCGGCAATTCTAGCCGC
                ************************************************************

Muttom          ACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
Chinnamuttom    ACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
Cochin          ACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
Tuticorin       ACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
Vizhijam        ACGAGAATGAGCAATAACAGGTCTGTGATGCCCTTAGATGTTCTGGGCCGCACGCGCGCT
                ************************************************************

Muttom          ACACTGAAGGGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACC
Chinnamuttom    ACACTGAAGGGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACC
Cochin          ACACTGAAGGGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACC
Tuticorin       ACACTGAAGGGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACC
Vizhijam        ACACTGAAGGGATCAACGTGTTCTCCCCCTCCGAGAGGAGCGGGTAACCCGATCAAAACC
                ************************************************************

 Muttom          CTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTCCCAGTAAGC
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Chinnamuttom      CTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTCCCAGTAAGC
Cochin            CTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTCCCAGTAAGC
Tuticorin         CTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTCCCAGTAAGC
Vizhijam          CTTCATGATAGGGATTGGGGCTTGCAATTGTTTCCCATGAACGAGGAATTCCCAGTAAGC
                  ************************************************************

Muttom            GCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTAC
Chinnamuttom      GCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTAC
Cochin            GCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTAC
Tuticorin         GCAAGTCATCAGCTTGCGTTGATTACGTCCCTGCCCTTTGTACACACCGCCCGTCGCTAC
Vizhijam          GCAAGTCATCAGCTTGCGTTGATTTCGTCCCTGCCCTTTGTACACACCGCCCGTCGCTAC
                  ************************ ***********************************

Muttom            TACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTGGTTGTTCTACCCCATC
Chinnamuttom      TACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTGGATGTTCTACCCCATC
Cochin            TACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTGGATGTTCTACCCCATC
Tuticorin         TACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTGAATGTTCTACCCCATC
Vizhijam          TACCGATTGAATGATTTAGTGAGGCCTTCGGACTGGCGCTCTTGGATGTTCTACCCCATC
                  ******************************************  **************

Muttom            CCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCTCGAGCTGACGGAAAGAT
Chinnamuttom      CCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCTCGAGCTGACGGAAAGAT
Cochin            CCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCTCGAGCTGACGGAAAGAT
Tuticorin         CCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCTCGAGCTGACGGAAAGAT
Vizhijam          CCTTCTATTCTCGCAAGGGTCCTGGAGGTTGGGGTTCTCGCCTCGAGCTGACGGAAAGAT
                  ************************************************************

Muttom            GTCCAAACTTGATCATTTAGAGGAAGGAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTG
Chinnamuttom      GTCCAAACTTGATCATTTAGAGGAAGTAGAGTCGTAACAAGGTTTCCGTAGGTGAACCTG
Cochin            GTCCAAACTTGATCATTTAGAGGAAGTTAAGTCGTAACAAGGTTTCCGTAGGTGAACCTG
Tuticorin         GTCCAAACTTGATCATTTAGAGGAAGTAAAGTCGTAACAAGGTTTCCGTAGGTGAACCTG
Vizhijam          GTCCAAACTTGATCATCTAGAGGAAGTAAAGTCGTACCAAGGTTTCCGTAGGTGAACCTG
                  **************** *********   ******* ************************

Muttom            C
Chinnamuttom      C
Cochin            C
Tuticorin         C
Vizhijam          C
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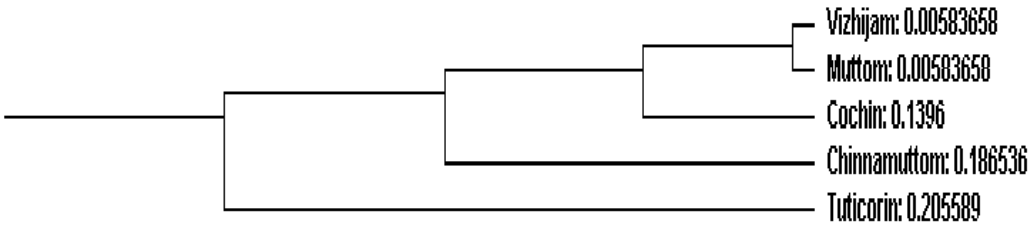
Fig: 12
Phylogenetic tree and genetic distance based on 18S rRNA Sequence.



Discussion

Characters of DNA sequences have been known to affect topological resolution in phylogenetic analysis. The most common such character is site saturation by multiple substitutions that may cause obscurity in true relationships of taxa in evolution (Swofford *et al.,* 1996; Jesus *et al.,* 2007). However, there is the lack of saturation of transitions for the 16S of the freshwater prawns (Murphy and Austin, 2005; Liu *et al.,* 2007). In our study, there was deceleration in transitions in 18S rRNA sequences. Increasing a sequence length increases the number of phylogenetic informative sites that improves the resolution of phylogenetic analysis (Miyamoto *et al.,* 1990; Chang *et al.,* 2005). It is correctly said that increasing a sequence length provides more data in the analysis, the better the chance to obtain a better phylogeny. Liu *et al.,* (2007) combined the 16S sequences and the COI sequences to make the combined sequences 37% larger in the length and 47% more in the total parsimonious information sites than those of the 28S. The combined sequences improved the resolution of the phylogenetic tree derived from the 16S, but many of the internal multifurcating nodes were unresolved and the phylogenic relationships of many taxa remained obscure. Obviously, transitional saturation and sequence length were not the primary factors for the 16S to produce poor resolution in the phylogenetic analysis for the freshwater prawns.

Murphy and Austin (2005) attributed this poor resolution in phylogenetic analysis of freshwater prawns from the 16S to a rapid and explosive radiation in their early evolutionary history, which might have occurred during the Oligocene or early Miocene. They speculated

that was a period with the proper environments for rapid dispersion and diversification of amphidromous prawns in the Southeast Asia and farther to Australia, the western Pacific islands, India, Africa, and even Atlantic coasts of Tropical America (Chen *et al.,* 2009). The rapid radiation created numerous taxa within a short time period. The 18S exhibits small genetic distance range, resulting in a severe convergence of the taxa within the range so that any bifurcating branching patterns could not be determined unambiguously, as expressed by the presence of severe polytomies in the phylogenetic trees.

Phylogenetic information in DNA sequences is built up by nucleotide substitutions over time. Due to the limited number of character states, phylogenetic signals also erode by similar process. Several evolutionary factors can potentially mislead phylogeny estimations (Conant and Lewis, 2001), such as heterogeneity in substitution rates among lineages (Felsenstein, 1978) and sites (Navidi *et al.,* 1991; Sidow and Steel, 1992; Yang, 1993), transition/transversion bias (Kimura, 1980; Wakeley, 1993), nonindependence of sites within a gene (Goldman and Yang, 1994; Schoniger and Von Haeseler, 1995; Muse, 1995, 1996), and non-stationarity of nucleotide frequencies across lineages (Loomis and Smith, 1990; Burggraf *et al.,* 1992; Hasegawa and Hashimoto, 1993; Lockhart *et al.,* 1994; Galtier and Gouy, 1995, 1998).

The gamma distribution has been proposed to model variable rates at sites (Jin and Nei, 1990; Li *et al.,* 1990; Tamura and Nei, 1993). The distribution involves a shape parameter of which the value is inversely related to the extent of rate variation at sites. Sullivan *et al.,*(1995) and Misof *et al.,*(2001) demonstrate that there is extreme among-site variation in mitochondrial 12S sequences, which has a highly skewed distribution of the rates

and makes the sequences particularly susceptible to the misleading effects of non-independency and other nonrandom noise in phylogenetic analysis.

The 18S had a-value less than 0.5, the presence of extremely high among-site rate variation (Tateno *et al.,* 1994; Sullivan *et al.,* 1995), whereas the 28S had the value higher than 0.5, indicating very low among-site rate variation. The lower a-value indicated higher among-site variation rates (Page and Holmes, 1998). The higher among site rate variation of the 16S as indicated by its low value suggested that most sites of its sequences were invariable, but a few had very high substitution rates. Consequently, it was less informative, and thus the divergences among sequences were smaller (Yang, 1996).

On the basis of Phylogenic tree highest genetic distance was found in Tuticorin population (0.205589) and less genetic distance found in Vizhinjam population (0.00583658). Phylogenic tree produced mainly two clusters. First cluster contains Tuticorin populations and the second cluster contains remain four populations. The results of multiple sequence alignment of 18S gene sequence in five different collection spots were showed that the base pairs varied in all populations.

The combined analysis of different DNA segments has been often tested, but the results are controversial (Brower *et al.,* 1996; Wiens, 1998; Lecointre and Deleporte, 2005; Hasegawa and Kasuya, 2006). A combined analysis is effective if the partitioned components of the data sets are congruent with consistent phylogenetic signals (Chavarria and Carpenter, 1995; Hasagawa and Kasuya, 2006). In this study, the partition-homogeneity

test showed that the 18S data sets were congruent; nevertheless, the combined analysis with ML, MP and MENJ showed no improvement from the 28S, but with BI produced mixed results. Obviously, in this instance, the combined analysis is not the first choice in this phylogenetic analysis; the separate analyses should be done first (Lecointre and Deleporte, 2005; Hasegawa and Kasuya, 2006). To find the most plausible phylogenetic hypothesis, a mutual reference should be made among the results of the separate and combined analyses (Hasegawa and Kasuya, 2006).