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RESEARCH ARTICLE

Molecular phylogeny of commercially important lobster species from Indian coast inferred from mitochondrial and nuclear DNA sequences

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Abstract

Lobsters constitute low-volume high-value crustacean fishery resource along Indian coast. For the conservation and management of this declining resource, accurate identification of species and larvae is essential. The objectives of this work were to generate species-specific molecular signatures of 11 commercially important species of lobsters of families Palinuridae and Scyllaridae and to reconstruct a phylogeny to clarify the evolutionary relationships among genera and species included in this study. Partial sequences were generated for all the candidate species from sampling sites along the Indian coast using markers like Cytochrome oxidase I (*COI*), *16SrRNA, 12SrRNA*, and *18SrRNA* genes, and analyzed. The genetic identities of widely distributed *Thenus* species along the Indian coast to be *Thenus unimaculatus* and the sub-species of *Panulirus homarus* to be *P. homarus homarus* were confirmed. Phylogeny reconstruction using the individual gene and concatenated mtDNA data set were carried out. The overall results suggested independent monophyly of Scyllaridae and Stridentes of Palinuridae. The interspecific divergence was found to be highest for the 12SrRNA compared with other genes. Significant incongruence between mtDNA and nuclear 18SrRNA gene tree topologies was observed. The results hinted an earlier origin for Palinuridae compared with Scyllaridae. The DNA sequence data generated from this study will aid in the correct identification of lobster larvae and will find application in research related to larval transport and distribution.

Keywords

Mitochondrial markers, nuclear markers, lobsters

History

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Introduction

The lobster fishery is low volume but valuable and highly priced, which constituted 1410 MT (0.3%) of total marine crustaceans landed in India during 2013 (CMFRI, 2014). Even though the fishery was to the tune of 0.058% of total marine landings in 2009, its contribution was 0.254% in quantity and 1.01% in value of marine exports (MPEDA, 2009). Lobster fishery, distributed throughout the coastline of India, is considered to be of multi-species comprising 14 species of littoral and six species of deep sea forms. Among these, four littoral and one deep sea form are significant in commercial landings that is concentrated mainly on the northwest, southwest, and southeast coasts (Radhakrishnan & Manisseri, 2003). The annual landing of the lobsters in the country is on the decline as evident from catch data over the years (CMFRI, 2004–2014; Radhakrishnan et al., 2005).

The 11 commercially important lobster species in this study belong to two families, Palinuridae and Scyllaridae (Table 1). Spiny lobsters (Palinuridae) are one of the most commercially important groups of decapod crustaceans (Phillips, 2006). Slipper or shovel-nosed lobsters (Scyllaridae) are being targeted as a saleable by-product of spiny lobster or shrimp fisheries and is the focus of directed fisheries in India (Lavalli & Spanier, 2007; Vijayakumaran & Radhakrishnan, 2011). The shovel-nosed lobster genus *Thenus* Leach, 1815, long considered to contain only *Thenus orientalis* (Lund, 1793), was revised and five species were recognized (Burton & Davie, 2007). Earlier studies and reports of the genus *Thenus* in India were based on the single species – *Thenus orientalis* (Chacko, 1967; Vijayakumaran & Radhakrishnan, 2011). However, in view of the species revision, the species of *Thenus* widely distributed along the coast of India needed to be confirmed since it has been identified as a candidate species of aquaculture interest (Vijayakumaran & Radhakrishnan, 2011).

Accurate species and larval level identification is essential for the conservation and management of the dwindling lobster resource of the country. Globally mitochondrial cytochrome oxidase I gene (*COI*) is elected as the standardized tool for molecular taxonomy and identification (Ratnasingham & Hebert, 2007). The objective of the present study was to generate species-specific signatures for the selected lobster species from Indian coast using COI, additional mitochondrial (mtDNA) genes like *16SrRNA*, *12SrRNA*, as well as by the nuclear *18SrRNA* gene in order to make the species identifications more accurate and reliable. Molecular genetic data have become a customary tool for understanding the evolutionary history and relationships among species and DNA sequences have been extensively used for

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Table 1. Sampling location of commercially important lobsters along Indian Coast.

		Collection locality along the Indian Coast								
		West Coast				East Coast				
		1	2	3	4	5	6	7	8	
Location and code *(as in map)*		Veraval (VRL)	Mumbai (MUM)	Lakshadweep Island (LKDP)	Kollam (QLN)	Nagercoil (NGR)	Chennai (CHE)	Visakhapatnam (VSK)	Andaman & Nikobar Islands (A&N)	
State in India		Gujarat	Maharashtra	UT	Kerala	Tamil Nadu	Tamil Nadu	Andhra Pradesh	UT	
Geographic Location of sampling site	Latitude Longitude	20° 54′ N 70° 22′E	18° 56′ N 72° 45′E	8°&12° 13′ N 71° and 74°E	8° 94′ N 76°55′E	8°17′ N 77°43′E	13°06′ N 80°18′E	17°14′ N 83°17′ E	6° &14° N 92°& 94° E	Number of individuals collected
S. no.		Species								
Family Palinuridae										
1	*Panulirus homarus*				✓		✓	✓		180
2	*Panulirus versicolor*				✓		✓			6
3	*Panulirus ornatus*						✓			5
4	*P. longipes longipes*					✓				5
5	*P. polyphagus*	✓	✓							10
6	*P. penicillatus*			✓						5
7	*Peurulus sewelli*				✓					5
8	*Linuparus somniosus*								✓	2
Family Scyllaridae										
9	*Thenus unimaculatus*	✓			✓		✓	✓		240
10	*Thenus indicus*							✓		3
11	*Petrarctus rugosus*						✓			5

Large sample sizes for *P. homarus* and *T. unimaculatus* for population structure analysis.

phylogenetic reconstruction in a variety of invertebrates (Botello et al., 2013; Pedraza-Lara et al., 2012) including lobsters (Yang et al., 2012). The use of nuclear genes in addition to mitochondrial genes adds to the number of independent markers in a dataset, thus increasing the chances of reconstructing the true species phylogeny (Toon et al., 2009). In this study, we have attempted to reconstruct the phylogeny to infer evolutionary relationships of the lobster species using mtDNA and nuclear gene markers.

Materials and methods

Taxon sampling

The samples of 11 commercially important lobster species, eight of which (*Panulirus homarus homarus*, *Panulirus versicolor*, *Panulirus ornatus*, *Panulirus longipes longipes*, *Panulirus polyphagus*, *Panulirus penicillatus*, *Peurulus sewelli*, and *Linuparus somniosus*) belonged to family Palinuridae and three of Scyllaridae (*Thenus unimaculatus*, *Thenus indicus*, and *Petrarctus rugosus*) were collected from their places of abundance from Indian coast. Details of sample collection are provided in Table 1 and depicted in Figure 1. The species were identified as per Holthuis (1991) and Burton & Davie (2007). Tissue samples for extraction were taken from pleopods, using minimal invasive techniques.

Laboratory protocols

Genomic DNA was extracted following the phenol chloroform method (Sambrook & Russell, 2001) with slight modification from all the collected individuals. Amplification of partial sequences of three mitochondrial (COI, 16SrRNA, and 12SrRNA) and one nuclear (18SrRNA) genes was accomplished using various primer sets (Table 2). PCR reactions for gene amplifications were carried out in PTC 200 gradient thermal cycler (M. J. Research Inc., Waltham, MA). All the reactions were performed in 25 µl reactions containing 10 × assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, and pH 9.0) with 15 mM $MgCl_2$ (Genei, Bangalore, India), 5 pmoles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U *Taq* DNA polymerase, 0.5 µl of 25 mM $MgCl_2$, and 25 ng of template DNA to a total volume of 25 ml. The PCR cycling profiles were as follows: 5 min at 95 °C for initial denaturation, 30 cycles of denaturation for 45 s at 94 °C, 30 s annealing at 42–57 °C (depending on primer sequences), 45 s extension at 72 °C, and a final extension for 10 min at 72 °C. The number of cycles was increased to 40 for the Cytochrome oxidase 1 (COI) gene

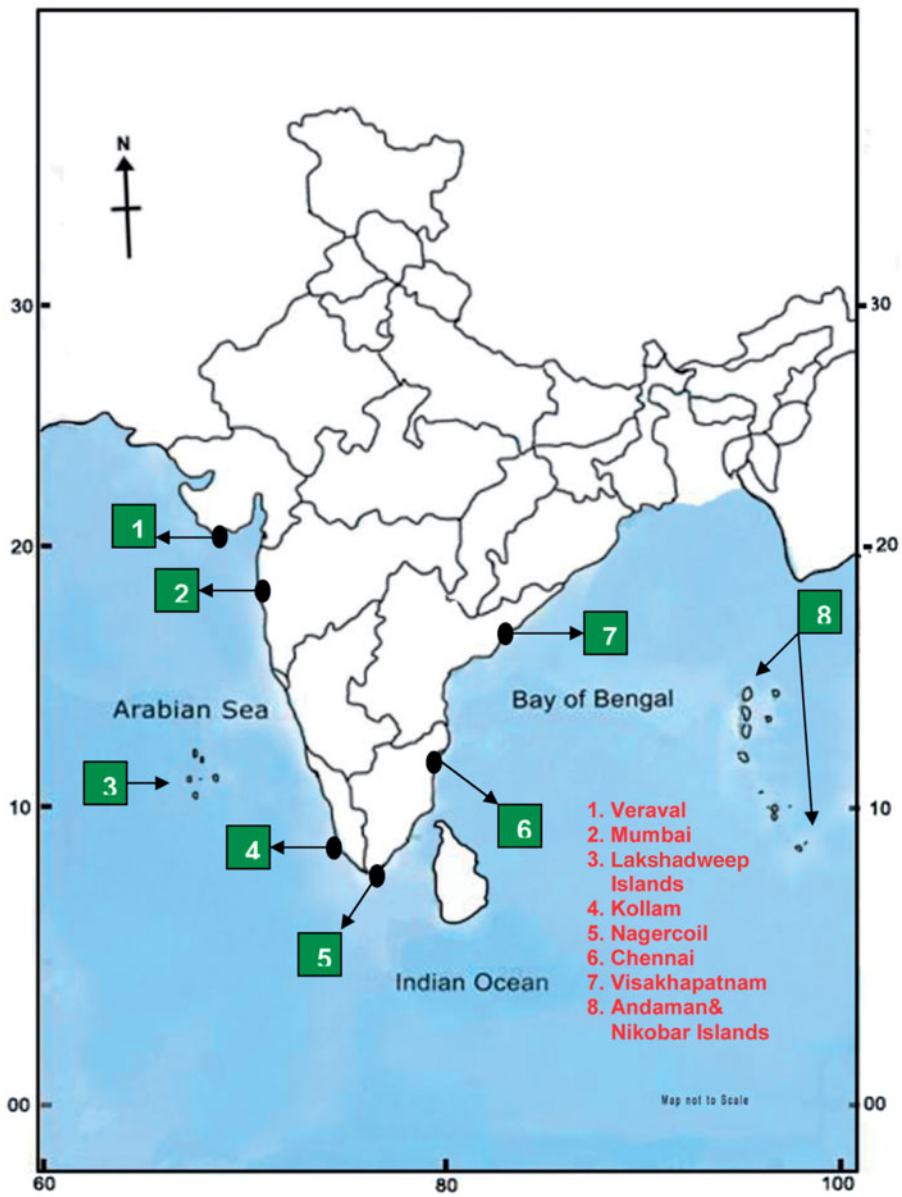

Figure 1. Map of India showing the distribution of the sampling sites of lobster species along Indian coast.

Table 2. Loci and primers used in this study to amplify the mtDNA and nuclear DNA genes.

Locus	Primer name	Sequence	Source	Annealing temp (in °C)	Fragment size (bp)
Mitochondrial genes					
1. Cytochrome oxidase 1 (CO1)	LCO1490	5′-GGT CAA CAA ATC ATA AAG ATA TTG G-3′	Folmer et al. (1994)	42° C – Palinuridae	700
	HCO2198	5′-TAA ACT TCA GGG TGA CCA AAA AAT CA-3′		48 °C – Scyllaridae	
2. 16S rRNA	16S-L2510	5′-CGCCTGTTTATCAAAAACAT-3′	Palumbi et al. (1991)	50	550
	16S- H-3080	5′-CCGGTCTGAACTCAGATCACGT-3′			
3. 12S rRNA	L13337-12S	5′-YCTACTWTGYTACGACTTATCTC-3′	Machida et al. (2002)	57	600
	H13845-12S	5′-GTGCCAGCAGCTGCGGTTA-3′			
Nuclear genes					
1. 18S rRNA	18S 1f	5′-TAC CTG GTT GAT CCT GCC AGT AG-3′	Whiting (2002)	48	900
	18S b2.9	5′-TAT CTG ATC GCC TTC GAA CCT CT-3′			
	18S 5FrRNA	5′-GCG AAA GCA TTT GCC AAG AA-3′	Carranza et al. (1996)	50	900
	18S_9RrRNA	5′-GAT CCT TCC GCA GGT TCA CCT AC-3′			

Table 3. GenBank depository of species from the present study.

Sl No.	Species	No. of individuals sequenced	GenBank accession numbers COI	16S	12S	18S
Palinuridae						
1	*Panulirus homarus homarus*	60	JQ229883-JQ229888	JQ229862; JQ229866-JQ229871	JQ229841–JQ229848	JQ229940
2	*Panulirus ornatus*	5	*HM446347; *GQ223286	JQ229863-JQ229864	JQ229850–JQ229851	JQ229942
3	*Panulirus versicolor*	6	JQ229882	JQ229877	JQ229858–JQ229859	JQ229948
4	*Panulirus polyphagus*	10	*AF339469; *JN418939	JQ229873	JQ229852–JQ229853	JQ229943
5	*Panulirus penicillatus*	5	JQ229881	JQ229874	JQ229854	JQ229944
6	*Panulirus longipes longipes*	5	JQ229879	JQ229872	JQ229849	JQ229941
7	*Linuparus somniosus*	2	JQ229880	JQ229865	JQ229840	JQ229939
8	*Puerulus sewelli*	5	JQ229890	JQ229876	JQ229857	JQ229947
Scyllaridae						
1	*Petrarctus rugosus*	5	JQ229889	JQ229875	JQ229855–JQ229856	JQ229945– JQ229946
2	*Thenus indicus*	3	JQ229890–JQ229891	JQ229878	JQ229860–JQ229861	JQ229949
3	*Thenus unimaculatus*	72	JQ229893–JQ229900	JQ229901–JQ229909	KC951871	JQ229950
No. of sequences used in this study			26	26	22	12

*Sequences retrieved from GenBank.

amplification of scyllarid lobsters. Prior to sequencing, PCR products were purified using GeNei™ Quick PCR purification kit (Genei, Bangalore, India) following the instructions given by the manufacturer. The purified PCR products were used as template for sequencing PCR with same primer pairs by cycle sequencing using ABI PRISM BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA). Sequencing was done at Rajiv Gandhi Centre for Biotechnology, Thiruvananthapuram, India, and SciGenom Labs Pvt. Ltd, Kakkanad, Cochin, India. The chromatograms were visually inspected with the aid of ABI sequence editor 3.3 (Applied Biosystems, Waltham, MA).

Data analysis

The raw DNA sequences were edited manually and the forward and reverse sequences of the genes were combined using cap contig assembly program in BioEdit sequence alignment editor version 7.0.5.2 (M. J. Research Inc., Waltham, MA) (Hall, 1999). The two segments of 18SrRNA sequences were combined into a single 1.8 kb sequence for analysis. The sequences generated varied in length from 655 bp to 702 bp for COI, 528 bp to 567 bp for 16SrRNA, 576 bp to 608 bp for 12SrRNA, and 1683 bp to 1726 bp for the 18SrRNA. Multiple alignments of sequences for each gene were performed using CLUSTAL W algorithm (M. J. Research Inc., Waltham, MA) in BioEdit and the alignments were manually checked and corrected. The number of mtDNA haplotypes for each species and nucleotide sequence characteristics after alignment were analyzed using the program DnaSP version: 5 (DNASTAR, Madison, WI) (Librado & Rozas, 2009). All sequences generated from this study have been deposited in GenBank (Table 3).

For the succeeding phylogenetic analysis, individual sequence set for each gene and combined mtDNA sequences were used. For the concatenated mtDNA data, a representative sequence from each species was chosen randomly when conspecific sequence divergence from the same locality was less than 1%. In order to avoid an outgroup selection effect on phylogenetic reconstruction, both closely and distantly related outgroups were included in the analysis. The nephropid lobster *Homarus americanus* (GenBank accession no. NC_015607.1) and cray fish *Cherax destructor*

(NC_011243.1) were included as outgroups for mtDNA sequence analysis. *H. americanus* (AF235971) and *Cherax quadricarinatus* (AF235966) from GenBank were included as outgroup species for the nuclear 18SrRNA gene analysis. The data analysis of the individual gene and combined mtDNA for all species was carried out with outgroup sequences to create five alignments: COI, 16S, 12S, 18S, and the concatenated COI+16S+12S.

Phylogenetic trees were constructed in MEGA version 5 (MEGA Inc., Englewood, NJ) (Tamura et al., 2011) for individual gene, using distance (Neighbor-Joining/ NJ) and Maximum Parsimony (MP) methods. For the combined mtDNA data set, in addition to the above, the Maximum Likelihood (ML) method was also used for phylogeny reconstruction. The Tamura–Nei model (Tamura & Nei, 1993) with a gamma distribution and a proportion of invariable sites (TN 93+G + I) were selected as the best-fit model of nucleotide substitution in ML analysis. Relative support for tree topology was obtained by bootstrapping using 1000 iterations of the data matrix. Estimates of sequence divergence (based on the Kimura two-parameter model) between species pairs and genera, base composition, and rate of transitions/ transversions were also calculated using the software. All characters were equally weighted and alignment gaps were treated as missing data in phylogeny reconstruction.

Results

The COI dataset included 26 sequences from all the species. The final dataset was of 655 bp long without indels of which 259 were variable and 253 were parsimony informative characters. The A+T base frequency was 58.7% and the average T_S/T_V ratio across pairwise sequence comparisons was 1.24. The value ranges of mean K2P distance observed for the gene were 0.3–0.7% within species, 15–26.8% within genus *Panulirus*, and 16.2% in genus *Thenus*. It was 21.5–26.4% in Palinuridae and 21.4% in Scyllaridae. For the five genera taken together, the value ranged from 21.3% (between *Petractus* and *Thenus*) to 26.9% (*Linuparus* and *Thenus*). The mean evolutionary diversity was 20.8% in entire dataset. Tree topologies from the NJ and MP analysis indicated four major clades: *P. homarus homarus*, *P. versicolor*, *P. ornatus* and *P. polyphagus* formed the clade I, *P. longipes* and *P. penicillatus* formed the second clade, *L. somniosus* and *Petractus sewelli* formed the third clade, and *P. rugosus*, *T. unimaculatus* and *T. indicus* formed the fourth clade. *P. versicolor* and *P. ornatus* were found to be sister taxa in the first clade. *Thenus unimaculatus* and *T. indicus* formed one subclade within the fourth clade. *Linuparus somniosus* and *P. sewelli* were grouped together with the Palinuridae with weak to moderate bootstrap support and formed a basal group to the rest of the Palinurid species. Conspecific individuals from different sampling localities were always clustered together.

The 16SrRNA analysis involved 26 nucleotide sequences. The final dataset consisted of an alignment of 541 bp including indels. There were 230 variable characters of which 172 were parsimony informative. The A + T bases frequency was 66.3%, indicating moderate AT bias. Sequence divergence between conspecific individuals ranged from 0.2 to 0.5%. The average T_S/T_V ratio across pairwise sequence comparisons was 1.09. The interspecific sequence divergence ranged from 4.6 to 26.4% in Palinuridae and 4.9 to 18.1% in Scyllaridae. The average evolutionary divergence over sequence pairs within the above families was 10.9% and 6.7% respectively. The mean evolutionary divergence over sequence pairs was 0.173 for the entire dataset. Intergeneric distance ranged from 19.8 to 22% in Palinuridae and 18% in Scyllaridae. It ranged from 18% (between genus *Petrarctus* and *Thenus*) to 32.1% (*Linuparus* and *Thenus*) among five genera of lobsters. The methods yielded trees with the same overall topology with four major clades. Bootstrap analysis of the 16S rRNA data resulted in 100% support for the monophyly of all eight Palinurid species and the three Scyllarid species. Topologies derived from NJ and MP were congruent except that the latter recovered a topology similar to the NJ with some minor differences in relationships among species within the first major clade.

The 12SrRNA analysis involved 22 nucleotides. The final dataset consisted of an alignment of 592 bp with indels. Among the ingroup taxa, 334 were variable sites out of which 293 were parsimony informative. There was moderate A+T bias of 68.6%. Sequence divergence between conspecific individuals ranged from 0.4 to 0.6% in the ingroup taxa. The inter-specific sequence divergence for 12SrRNA ranged from 5.8% to 38.6% within Palinuridae and 7.9 to 30% within Scyllaridae. The average evolutionary divergence over sequence pairs for the two families was 21.8% and 17.5%. A mean evolutionary divergence value of 30% for the entire dataset and an average T_S/T_V ratio of 1.02 were observed. Intergeneric distance ranged from 26.8 to 36.6% in three genera of Palinuridae and 28.1% in Scyllaridae. It ranged from 26.8% (between genus *Puerulus* and *Linuparus*) to 46.1% (*Linuparus* and *Thenus*) among the five genera of lobsters. The NJ and MP methods yielded trees with the same overall topology similar to the previous analyses.

The analysis of nuclear 18SrRNA gene involved 12 nucleotide sequences without outgroups. The final dataset consisted of 1723 bp with indels. There were a total of 1672 positions in the final dataset. About 183 variable characters were observed out of which 118 were parsimony informative. The overall A+T content was 49.2%. The interspecific sequence divergence ranged from 0.3% to 7.8% within Palinuridae and 0.2 to 1% within Scyllaridae. The average evolutionary divergence over sequence pairs in the two families was 4.8% and 0.4%. The mean evolutionary divergence over sequence pairs was 3.9%. The average T_S/T_V ratio in the dataset was 1.22. The evolutionary history was inferred using the NJ and MP methods which could not resolve the phylogeny.

The concatenated mtDNA data analysis involved 32 nucleotide sequences of ingroup taxa. The combined mitochondrial data set (COI, 16SrRNA, and 12SrRNA) was 1790 bp long including indels and excluding the two outgroup species. In the ingroup taxa, 746 were parsimony informative of 829 variable characters. There were 1674 positions in the final ingroup dataset excluding all indels. The A+T base frequency was 64%. The interspecific sequence divergence ranged from 9 to 34.7% within Palinuridae and 10.2 to 25.3% within Scyllaridae. The overall divergence value ranged from 9.0 to 39.4% (Table 4). The average evolutionary divergence over sequence pairs was 20.9% within Palinuridae and 8.7% within Scyllaridae. It was 25.7% over all sequence pairs. The mean evolutionary divergence over sequence pairs between the families was 37.6% with a mean evolutionary diversity of 27.5% for the entire dataset. The net sequence divergence between them was 22.7% and the average T_S/T_V ratio across all pairwise sequence comparisons was 1.04. The Intergeneric distance ranged from 22.4% (*Linuparus* and *Puerulus*) to 28.3% (*Linuparus* and *Panulirus*) in Palinuridae and 22% (*Thenus* and *Petrarctus*) in Scyllaridae. It ranged from 22.4% (*Petractus* and *Thenus*) to 33% (*Linuparus* and *Thenus*) among the five genera of lobsters (Table 5).

Since the tree topologies are almost identical, only the cladogram generated using the ML analysis based on TN93+G+I model was shown (Figure 2). The tree topologies were supported by >80% bootstrap support at all nodes except for conspecific individuals. Four major clades were recognized congruent with the individual gene trees with mtDNA genes.

Table 4. Average K2P distances of total mtDNA data set (below diagonal) between haplotypes of different species of lobsters.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
	Palinuridae																	
1	*P. homarus homarus-QLN1*																	
2	*P. homarus homarus-CHE1*	0.002																
3	*P. homarus homarus-VZG1*	0.005	0.004															
4	*Panulirus ornatus*	0.093	0.092	0.090														
5	*Panulirus versicolor*	0.121	0.119	0.118	0.127													
6	*Panulirus polyphagus*	0.119	0.119	0.118	0.129	0.140												
7	*P. longipes longipes*	0.226	0.226	0.228	0.256	0.257	0.241											
8	*Panulirus penicillatus*	0.262	0.260	0.260	0.282	0.271	0.283	0.176										
9	*Linuparus somniosus*	0.320	0.321	0.324	0.333	0.311	0.326	0.330	0.347									
10	*Puerulus sewelli*	0.291	0.289	0.288	0.305	0.277	0.285	0.308	0.326	0.250								
	Scyllaridae																	
11	*Thenus unimaculatus-QLN1*	0.365	0.365	0.362	0.363	0.374	0.353	0.365	0.367	0.394	0.369							
12	*T. unimaculatus-QLN2*	0.365	0.365	0.362	0.363	0.372	0.353	0.367	0.367	0.391	0.366	0.003						
13	*T. unimaculatus-CHE1*	0.366	0.366	0.363	0.364	0.375	0.354	0.366	0.370	0.392	0.366	0.003	0.004					
14	*T. unimaculatus-VZG1*	0.369	0.369	0.366	0.365	0.377	0.355	0.367	0.372	0.392	0.370	0.005	0.004	0.003				
15	*T. unimaculatus-VZG2*	0.365	0.365	0.362	0.361	0.373	0.351	0.367	0.370	0.391	0.366	0.004	0.002	0.004	0.003			
16	*T. unimaculatus-VER1*	0.364	0.364	0.360	0.360	0.369	0.351	0.369	0.368	0.392	0.367	0.005	0.005	0.005	0.004	0.003		
17	*Thenus indicus*	0.371	0.371	0.370	0.370	0.368	0.361	0.363	0.364	0.384	0.349	0.104	0.102	0.104	0.107	0.105	0.105	
18	*Petrarctus rugosus*	0.345	0.345	0.346	0.353	0.363	0.355	0.353	0.359	0.368	0.333	0.244	0.243	0.245	0.248	0.244	0.245	0.253

QLN, CHE, VZG, and VRL represent sampling sites Kollam, Chennai, Visakhapatanam, and Veraval, respectively.

Table 5. Average K2P distances of concatenated mtDNA sequences between five genera (below diagonal) of lobsters.

	Panulirus	*Linuparus*	*Puerulus*	*Thenus*
Palinuridae				
Panulirus				
Linuparus	0.283			
Puerulus	0.259	0.224		
Scyllaridae				
Thenus	0.302	0.330	0.315	
Petrarctus	0.294	0.309	0.288	0.222

Discussion

Molecular markers used in the present study were proven to be successful in discriminating between different as well as closely related species of lobsters. Specifically, COI as a barcoding tool helps to identify an organism based on DNA sequence variability and assignment to a certain species previously described and for classification of potentially new species (Da Silva et al., 2011). In view of the species revision of the previously believed monotypic *Thenus* spp., using the COI barcodes, the species of genus *Thenus* distributed and caught widely along the Indian coast was ascertained to be *Thenus unimaculatus* (Burton & Davie, 2007; Jeena et al., 2011). The presence a less abundant species, *Thenus indicus* along the east coast, could also be confirmed. It was also identified that the subspecies of *Panulirus homarus* distributed along the coastline was *P. homarus homarus*. Morphology of all the collected specimens was also examined and it was found that they belong to the microsculpta form described by Berry (1974) which is the nominotypical *P. homarus homarus* (Linnaeus, 1758). No other subspecies could be found in sampling even though Berry (1974) and Holthuis (1991) suspected occurrence of another sub species *P. homarus megasculptus* Pesta, 1915 along the west coast of India. Identification of wild-caught phyllosoma of lobsters was possible based on DNA analysis (Palero et al., 2011; Torres et al., 2014) and the data from this study can have potential application in precise larval identification. However, other genes and phylogenetic methods are also required to evaluate the evolution or phylogenetic information contained in the barcode region of COI (Da Silva et al., 2011). The use of multiple genetic markers is often recommended since different genes may reflect different evolutionary histories (Funk & Omland, 2003). Here, phylogenetic relationships are inferred based on an analysis of a combined mtDNA dataset utilizing these genes.

George & Main (1967) classified species groups in genus *Panulirus* into four based on the condition of second and third maxilliped and geographical differences. Phylogenetic studies based on phyllosoma and puerulus morphologies (Baisre, 1994; McWilliam, 1995), and the genetic studies (Ptacek et al., 2001; Ravago & Juinio-Menez, 2003) also supported this grouping. Ptacek et al. (2001) concluded that two major lineages, rather than four, better represented the previous findings. Of the species of *Panulirus* considered here, *P. longipes longipes* and *P. penicillatus* belonged to groups I and II, respectively, or together in the first major lineage. The second major lineage included *P. polyphagus* of group III and *P. homarus*, *P. versicolor*, and *P. ornatus* of group IV. *Puerulus* and *Linuparus* of Palinuridae are two deep-sea genera (George, 2006; George & Main, 1967). All selected species of Palinuridae in the present study belong to the Stridentes (George & Main, 1967; Parker, 1884). *Puerulus rugosus* and the two *Thenus* species belong to the subfamilies Scyllarinae and Theninae, respectively, of Scyllaridae.

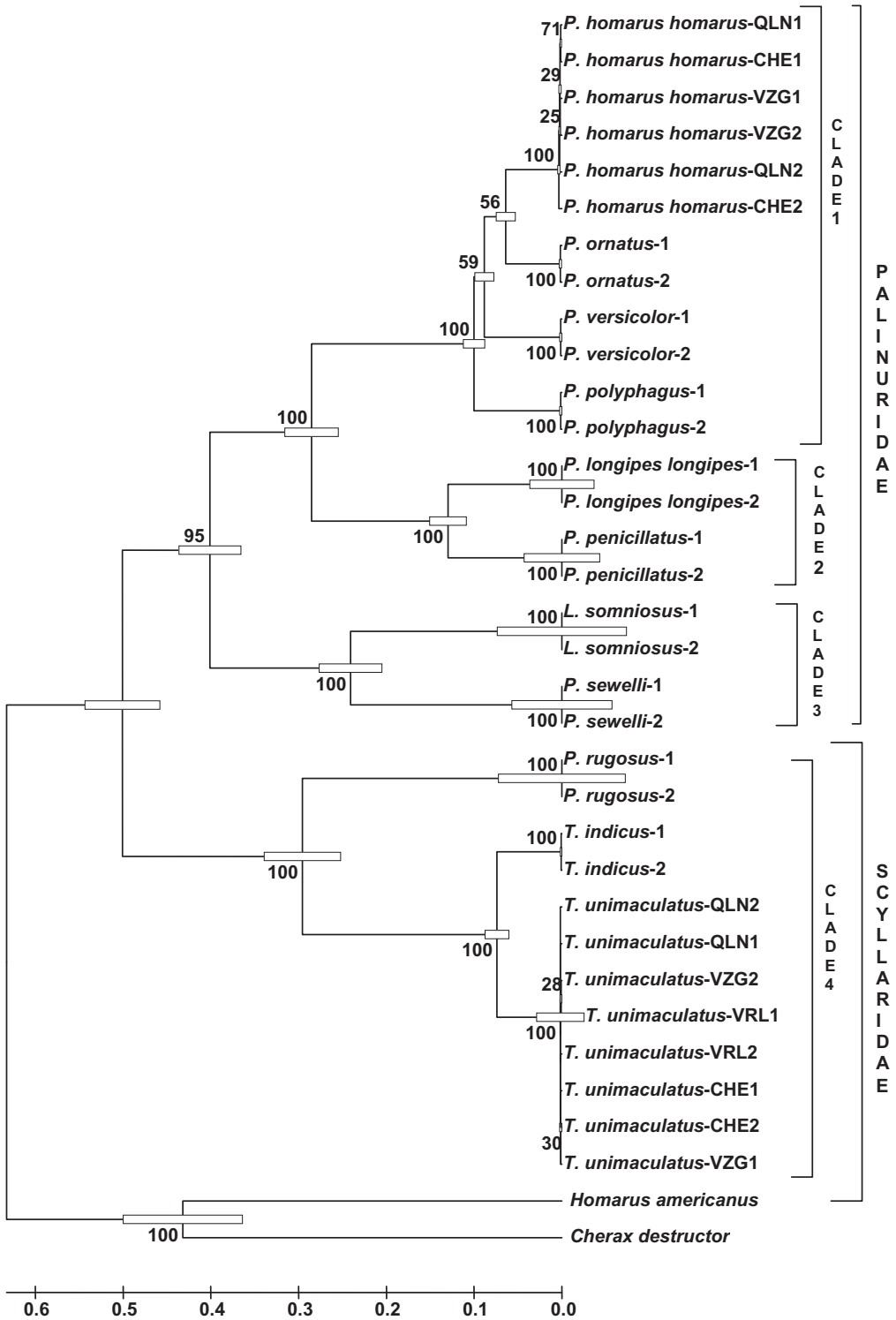

Figure 2. Maximum likelihood tree of the 11 lobster species based on TN93+G+I model in MEGA 5 (MEGA Inc., Englewood, NJ), inferred from haplotype sequence variation of the 1790 bp mtDNA region. The tree with the highest log likelihood (−11,909.7592) is shown. Numbers at nodes indicate the bootstrap values. NC_015607.1 (*H. americanus*) and NC_011243.1 (*C. destructor*) from GenBank are included as outgroup species. VRL, QLN, CHE, and VZG represent samples from Veraval, Kollam, Chennai, and Visakhapatnam, respectively.

Nucleotide composition comparisons and parsimony information from the sequence data

The 12SrRNA region was the most A+T rich while the content was relatively low in the 18SrRNA gene. Decapod mitochondrial genome is observed to be A+T biased with the highest A+T content in the putative control regions (Lin et al., 2012). Regions rich in A+T nucleotides are indicators of reduced selective constraints (Nigro et al., 1991). Nucleotide bias can have drastic effects on phylogenetic reconstructions, especially if the bias is different for individual taxa (Simon et al., 1994). Such bias in phylogenetic analysis can result in the grouping of taxa based on nucleotide composition rather than on shared history (Steel et al., 1993) and can reduce the amount of phylogenetic information in the data because of the increased chance of homoplasious changes. Comparable values of the A+T content had been

observed in genus *Linuparus* (Tsoi et al., 2011) for 12S; in genera like *Jasus* (Ovenden et al., 1997), *Metanephrops* (Chan et al., 2009), etc. for COI as well as 16SrRNA and in decapod reptantia (Ahyong & O'Meally, 2004) for 18S. The A+T content of genes can be correlated to the level of conserved nature of the genes with 18S, the slowest evolving of the four, displaying the least bias.

The number of parsimony informative sites varied among the genes of ingroup taxa from 118 informative sites in the 18SrRNA sequence data set to 293 informative sites in the 12SrRNA. As expected, fewer variable and parsimony informative characters were obtained in the ingroup taxa for the 16S rRNA compared with the protein coding *COI* gene. A similar observation of higher number of variable and parsimony informative sites in the 12S region was reported in grapsoid crabs (Schubart et al., 2006). The slow evolving 18SrRNA sequences were found to have lowest number of parsimony informative sites similar to a number of studies in crustaceans (Ahyong & O'Meally, 2004; Cristescu & Hebert, 2002).

Sequence divergence for various markers

Sequences of individual genes demonstrate that the diversity of DNA markers increases with the rise of taxon rank having the lowest diversity within species (Kartavtsev, 2013). It is less than 1% between conspecific individuals in the analysis. The great sequence divergence in COI is generally considered as of inter-specific or even inter-generic differences in decapod crustaceans. The value ranges of mean K2P distance within species, within genus, and within family for mtDNA COI from the present study are comparable with the reports in malacostracan decapods (Da Silva et al., 2011). As expected, less divergence was found for the *rDNA* genes than for the faster evolving protein coding COI gene. The degree of sequence divergence between groups I and II (11% for 16SrRNA and 20.2% for COI gene) and also between groups III and IV (4.6–7.1% for 16S; 15.3–17.3% for COI) in Palinuridae lies well in the limit reported by Ptacek et al. (2001). The inter-generic value for 16S gene appears to be higher at one end in this study probably because of analysis of data from two families. The value of the same for 12S revealed to be higher than the other genes. Usually the 12SrRNA region is highly conserved (Ballard et al., 1992) and 16S is more variable than 12S (Hwang & Kim, 1999). Variation is extremely low at the 3'half of the molecule but it is also observed that the third domain of 12S is A+T rich and phylogenetically informative (Simon et al., 1994). The sequence data were sufficiently long (592 bp) and could resolve the phylogeny at the species level with high divergence between species. There are reports of sequence divergence for 12SrRNA in the range of 0.65–43.4% in freshwater crabs of Potamonautidae (Daniels et al., 2002). A low divergence rate for nuclear 18SrRNA was observed in concordance with the moderate value among species (5.8–7.2%) in decapod suborder Pleocyemata (Toon et al., 2009).

The magnitude of inter-specific sequence divergence as well as that between the four genes was variable. Within Palinuridae, the level of sequence divergence in pairwise comparisons was higher compared to Scyllaridae. The higher level of sequence divergence among species in the former may also be due to a higher rate of molecular evolution at these mitochondrial genes for species in this family. The mean evolutionary divergences in the ingroup taxa of the present study were 3.9% for 18SrRNA, 30% for 12SrRNA, 20.8% for COI, and 17.3% for 16SrRNA. Munasinghe et al. (2003) detected percentage average divergence at generic level in crayfishes to be 23.82% for COI, 21.92% for 12S, and 17.21% for 16S. The analysis of this study revealed a higher divergence for *12SrRNA* genes compared with the other genes although there are reports of less divergence in *rDNA* genes than *COI* gene in Onychopods (Cristescu & Hebert, 2002).

Phylogenetic relationships

Phylogenetic information content of the individual gene and concatenated data was analyzed which resulted in phylogenetic trees with four major clades within it. Representatives of the groups III and IV (George & Main., 1967) clustered together into one clade; species of groups I and II into second clade, the deep water genera *Linuparus* and *Puerulus* into third clade, and the scyllarid lobsters in another fourth clade within which *T. unimaculatus* and *T. indius* clustered together into one group and *P. rugosus* formed another. The overall phylogeny of selected species of Palinuridae was in concordance with the morphological groupings proposed initially by George & Main (1967). Independent monophyly of Scyllaridae and Stridentes of Palinuridae as evident in the cladograms generated was in agreement with previous molecular-based studies (Palero et al., 2009; Yang et al., 2012) using more species.

In this study, individual gene tree with *18SrRNA* gene failed to resolve the phylogeny while *mtDNA* genes gave congruent results. Hence the nuclear and mtDNA data were not concatenated due to difference in tree topologies. The discordance in nuclear and mitochondrial genealogies and failure of 18S RNA in resolving phylogeny at generic level has been observed in family Pinnotherodae (Palacios-Theil et al., 2009), Palinuridae (Patek & Oakley, 2003), and Scyllaridae (Yang et al., 2012). The reason for this can be the lack of variation in the marker as well as less percentage of parsimony informative characters. Even though polymorphism has been reported in *Panulirus homarus* from Indian coast (Mon et al., 2011) our study could not observe the same for the 18S gene probably because of the slow rate of evolution of nuclear DNA resulting in accumulations of mutations at a low pace.

Even though the origin of two families Palinuridae and Scyllaridae cannot be accurately correlated from the results with the limited number of taxa in the present study, the gene trees from this study indicate that the family Palinuridae is older than Scyllaridae (Figure 2). Based on fossil records, an earlier origin of Palinuridae compared with Scyllaridae has been suggested (Baisre, 1994; Webber & Booth, 2007). The gene trees indicate a recent origin of subfamily Theninae compared with sub-family Scyllarinae but the number of species is not sufficient to derive a conclusion. Webber & Booth (2007) confirm the close affiliation of *Thenus* and the Scyllarinae, and the likelihood of a more recent speciation of *Thenus*.

The results from this study could establish genetic identities of lobster species along the Indian coast. The bar codes generated from this study will aid in the correct identification of lobster larvae which is often difficult by visual examination and in research related to larval transport and distribution. They can help in further investigations regarding the evolution and biogeography of these valuable decapod resources. The 12SrRNA region was found to be the most A+T rich and parsimony informative among the genes used in this study. Support of the independent monophyly of Scyllaridae and Stridentes of Palinuridae was obvious from the genetic data analysis of selected species. Further analysis using more nuclear genes may be necessary to enhance our current knowledge on lobsters of Indian coast.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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