

Identification of *Oplophorus gracilirostris* from South-East and South-West Coast of India using Mitochondrial Genetic Markers



Fisheries

KEYWORDS : deepsea shrimp, genetic marker, phylogenetics, taxonomy

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ABSTRACT

The present study reports the occurrence of *Oplophorus gracilirostris* A. Milne-Edwards, 1881 from south-east and south-west coast of India on the basis of morphological as well as molecular data. It is the first report of insight into the mitochondrial genetic variation of *O. gracilirostris*. For this study, the DNA sequences of three mitochondrial genes, large subunit ribosomal DNA (16S rDNA), cytochrome oxidase I (COI) and cytochrome b (Cyt b) were submitted in the GenBank. Morphological examination of the specimen confirmed the identity of the species as *O. gracilirostris*. The pairwise genetic distance analysis, sequence identity and phylogenetic tree have shown that the specimen has identity with *O. gracilirostris* only on the basis of 16S rDNA sequence of earlier reports. In the case of COI it has shown some identity towards *Oplophorus typus* and *Oplophorus spinosus*, but with a high genetic difference. This is the first submission of Cyt b sequence of *O. gracilirostris*. This study with sequence data of *O. gracilirostris* in GenBank will provide molecular support to the taxonomical identifications carried out on morphological basis.

Introduction

Oplophorids are bathypelagic shrimps with ability to luminescence and exhibit vertical migration (Chan & Yu, 1986). A rare occurrence of the specimens belonging to this family were obtained from few landing centres along the southern coast of India. The common species in *Oplophorus* genus are *Oplophorus gracilirostris* A. Milne Edwards, 1881, *Oplophorus typus* H. Milne Edwards, 1837, *Oplophorus spinosus* Brullé, 1839, and *Oplophorus spinicauda* A. Milne Edwards, 1883 locally known as spiny shrimps. Alcock (1901) reported the first occurrence of *O. gracilirostris* from the Indian coast. *O. gracilirostris* and *O. typus* have the ability to lock their laterally extended scaphocerite by unique structures on the scaphocerite and the basicerite (Chan & Yu, 1986).

Molecular characterization of phylogenetic genes which is also known as DNA barcoding is based on the premise that standardized DNA sequence can distinguish individuals of a species as the genetic difference between species exceeds that within species (Hebert et al. 2003). The nucleotide sequence difference within a gene reflects the evolutionary relationship between two organisms. Mitochondrial genetic markers are extensively used in population and phylogenetic studies of organisms. The large subunit ribosomal DNA (16S rDNA) gene in the mitochondrial genome is the most common genetic marker to elucidate evolutionary relationship as it is one of the slowest evolving genes (Meyer, 1993). Other mitochondrial genes such as cytochrome oxidase I (COI) and cytochrome b are also used as mitochondrial genetic markers. They are used as useful markers for species-specific identification in many fishes (Chauhan & Rajiv, 2010).

The method of identification of species based on genetic markers has revolutionized taxonomic classification process. It is a powerful tool to detect genetic uniqueness of individuals, populations or species (Avisé, 1994; Linda & Paul, 1995). They provide precise knowledge on phylogenetic relationships and resolve taxonomic ambiguities (Backer, 2002; Asensio, 2002; Rasmussen, 2003). It is possible to observe genetic variation in the entire genome using DNA markers. Specific DNA regions on nuclear and mitochondrial genome as novel biological information markers combined with existing morphological characters

can provide reliable taxonomic information required for species identification (Ganopoulos, 2013).

The present study was undertaken to sequence three mitochondrial genes, 16S rDNA, COI and Cyt b of *O. gracilirostris* obtained from south-east and south-west coast of India. Based on these sequences genetic distance, sequence identity and phylogenetic relationship of the specimen with other closely related species were analysed. This study provides preliminary information into the genetic variation of *O. gracilirostris* using DNA sequences of mitochondrial genes, 16S rDNA, COI and Cyt b and it will lay a foundation for identification of specimens on molecular terms in future.

Materials and methods

Materials

Agarose, Ethidium bromide, Tris(hydroxymethyl)aminomethane, Boric acid, EDTA (Ethylenediaminetetraacetic acid), glycerol and Bromophenol blue were purchased from Merck specialities pvt. ltd (India). Primers for 16S rDNA, COI, Cyt b were purchased from Sigma Aldrich (St. Louis, Mo, USA). All other chemicals were locally purchased.

Collection of samples

Six female specimens of the rare deep-sea shrimp *O. gracilirostris* were obtained from the multiday trawlers operating off Sakthikulangara (08° 56.003' N, 076° 32.532' E) on 6 January 2014 and thirteen female specimens from Tuticorin (08° 45.0' N, 078° 18.0' E) on 24 January 2014 along the southern coast of India at 400-800 m depth. Voucher specimens were deposited in sample collections at Crustacean Fisheries Division, Central Marine Fisheries Research Institute, Cochin, India (CMFRI:CFD:OG1&2).

Extraction of total genomic DNA

Total genomic DNA was extracted from pleopod of the individual specimen preserved under 95% ethanol using DNeasy® Blood & Tissue Kit (Qiagen Inc.) according to manufacturer's protocol with some modifications. The cells were lysed by incubating at 56°C for 2 h and all other steps were followed as per the proto-

col. The isolated DNA was stored at -20°C.

PCR amplification of 16S rDNA, COI and Cyt b

Three mitochondrial genes 16S rDNA, COI and Cyt b were amplified, using universal primers 5'-CGCCTGTTTATCAAAAACAT-3' (F), 5'-CCGGTCTGAACTCAGATCAGT-3' (R) (Palumbi et al. 1996), 5'-GGTCAACAAATCATAAAGATATTGG-3' (F), 5'-TAAACTTCAGGGTGACCAAAAATCA-3' (R) (Folmer et al. 1994) and 5'-TGTGGRGCNACYGTWATYACTAA-3' (F), 5'-AANAGGAARTAYCAYTCNGGYTG-3' (R) (Merritt et al., 1998) respectively. Reactions were performed in 25 µl reaction cocktails containing 0.5 µg/µl genomic DNA, 0.05 U/µl Taq DNA polymerase, 1X buffer with MgCl₂, 10 pM/µl of each primer and 200 µM dNTPs. The PCR thermal profile used was 94°C for 5 min for initial denaturation, followed by 35 cycles of 94°C for 1 min, 52°C for 1 min, 72°C for 1.5 min, and a final extension at 72°C for 5 min. The PCR products were electrophoresed on a 1.5% agarose gel containing ethidium bromide, and visualized under UV transilluminator (Lark, India).

Purification of PCR products

Amplified PCR products were purified with XcelGen DNA Gel/PCR Purification Mini Kit (Xcelris Labs Limited, India) according to manufacturer's protocol. The eluted samples were sequenced directly.

Sequencing of 16S rDNA, COI and Cyt b

The PCR purified products were sequenced by dideoxy chain termination method (Sanger et al. 1977) using the Big-Dye Ready-Reaction kit v3.1 (Applied Biosystems) on an ABI Prism 3770 automated sequencer from Scigenome, India. The sequence assemblies and contig editings were performed with DNA Baser Sequence Assembler v4.7 (Heracle BioSoft S.R.L., Romania). The gene sequences obtained were deposited in GenBank and aligned using ClustalX (Thompson et al. 1997) to confirm their identities.

Genetic distance and phylogenetic analysis

The nucleotide sequences of three gene fragments were aligned with sequences from GenBank by means of multiple sequence alignment using ClustalW algorithm. Pairwise genetic distance analysis was performed and phylogenetic trees were inferred for individual loci using the statistical method of neighbour-joining by using MEGA5 software (Saitou & Nei, 1987). All positions containing gaps and missing data were eliminated.

Results and Discussion

This study reports the rare occurrence of nineteen specimens of *O. gracilirostris* from two landing centres in southern coast of India such as Sakthikulangara (6 specimens) and Tuticorin (13 specimens). All specimens were of female individuals and they were in good condition. The specimen of *O. gracilirostris* possesses following distinguishing features in general such as body laterally compressed, rostrum anteriorly slender, upcurved and 1.5 times of the carapace with 10-14 dorsal and 7-8 ventral teeth and the rostral carina extends to the posterior end of the carapace. Orbital tooth is curved inwards between ophthalmopoda and second pair of antennae. Antennal tooth is slightly curved upward and pterygostomial spine is present. The spine of the 3rd abdominal tergum is much lengthier than that of the 4th and 5th. Scaphocerite is peculiar styliform, the outer margin serrated and inner fringed with closely packed hairs. Telson is longer than caudal swimmerets. Postero-lateral angles of the carapace are produced to form a slightly everted tooth (Alcock, 1901; Chan & Yu, 1986). Morphological examination confirmed that the specimens obtained from two landing centres in southern coast of India as *O. gracilirostris* (Fig. 1).



Fig. 1. *Oplophorus gracilirostris* specimen.

The present study was further extended to analyse the specimen using molecular tools. The total genomic DNA from the specimen was isolated and specific mitochondrial genes were PCR amplified. The gel picture of the PCR amplified 16S rDNA, COI and Cyt b showed that they are in the size range of approximately 570 bp, 700 bp and 430 bp respectively (Fig. 2). The PCR amplified gene fragments were sequenced and the partial nucleotide sequences were submitted in GenBank with accession numbers (Table 1). The DNA sequences of each gene fragments from mitochondrial genome of the specimen are available in the GenBank.

Table 1. Nucleotide base composition of gene fragments of the specimen sample.

Gene fragment	Accession No.	Length (bp)	A	T	G	C	GC content (%)
16S rDNA (Isolate 1)	KJ819551	234	76	21	33	84	50.00
COI (Isolate 1)	KJ472213	543	150	133	90	170	47.88
COI (Isolate 2)	KJ472214	513	144	124	82	163	47.75
Cyt b (Isolate 1)	KJ819552	318	98	87	34	99	41.82

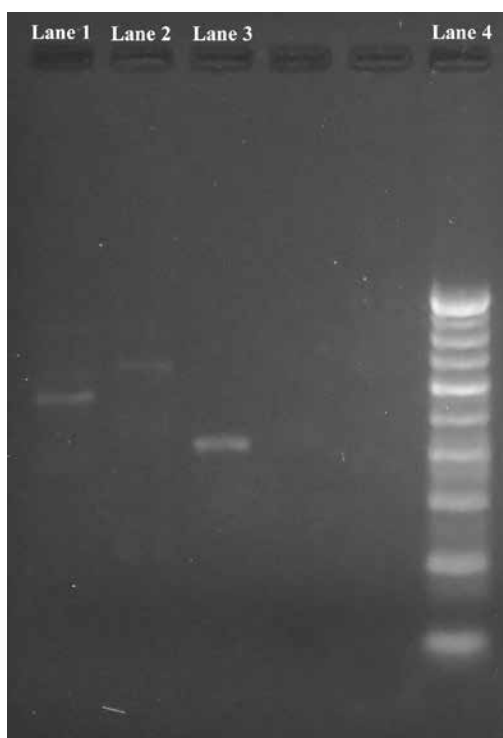


Fig. 2. Agarose gel (1.5%) with Lane 1: 16S rDNA, Lane 2: COI, Lane 3: Cyt b and Lane 4: 100bp ladder.

The nucleotide sequences of 16S rDNA and COI were subjected to pairwise genetic distance and phylogenetic analysis using MEGA5. The pairwise genetic distance and sequence identity of 16S rDNA and COI genes with that of closely related species are given in Table 2 and the single gene phylogenetic tree constructed using the statistical method of neighbour-joining (Fig. 3). There was only one earlier report of DNA sequence submission for *O. gracilirostris* available in the GenBank and that was of 16S rDNA (GQ131906.1). The genetic distance between this sequence and the sequences obtained in this study (KJ819551) was found to be 0.031 and the percentage of sequence identity was 97%. 16S rDNA has shown some sequence similarity towards *O. typus* with 95% sequence identity but in the different node of the phylogenetic tree. At present there are no reports of sequence submissions for COI and Cyt b for *O. gracilirostris* in the GenBank. This is the first report of submission these se-

quences to the GenBank. COI sequence of both Isolate1 and Isolate2 has shown identity towards *O. typus* and *O. spinosus* with a sequence identity of 98% and 89% respectively and a genetic difference of 0.020 and 0.131 respectively with *O. spinosus* being in the different node of the phylogenetic tree. There is no sequence information available for any of the three genes of remaining species of *Oplophorus* genus, *O. spinicauda* and hence sequence comparison with this species is not possible. Therefore, we can say that the landed specimen is *O. gracilirostris* on molecular terms as supporting information for the morphological identification of specimen only on the basis of sequence similarity of 16S rDNA. But other sequences obtained in this study will help in the identification of the specimens in future studies.

Table 2. Pairwise genetic distance and sequence identity of the specimen sample with other closely related species. (-- denotes sequences not available in GenBank)

		<i>O. gracilirostris</i>		<i>O. typus</i>		<i>O. spinosus</i>	
		Genetic Distance	Sequence Identity (%)	Genetic Distance	Sequence Identity (%)	Genetic Distance	Sequence Identity (%)
16S rDNA	Isolate1 (KJ396316)	0.031	97	0.054	95	--	--
COI	Isolate1 (KJ396317)	--	--	0.020	98	0.131	89
	Isolate2 (KJ486493)	--	--	0.020	98	0.131	89

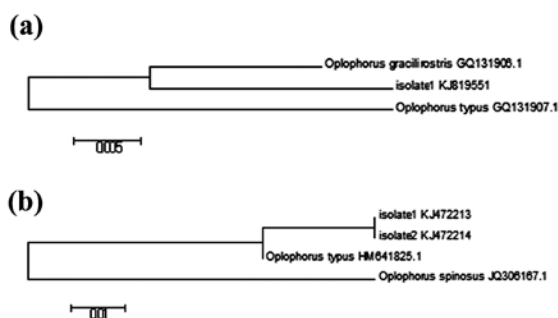


Fig. 3. Neighbour-joining Tree based on the genetic markers (a) 16S rDNA and (b) COI showing the relationship of the specimen sample with other closely related species.

Conclusion

In conclusion, this study reports the occurrence of *O. gracilirostris* from south-east and south-west coast of India on the basis of morphological identification. In addition, this is the first report of the GenBank submission of DNA sequence data for COI and Cyt b of *O. gracilirostris*. This data will help in identification of species on molecular terms and generation of information regarding evolutionary relationship of the species in future.

Acknowledgements

The authors are thankful to the Department of Science and Technology, India for financial assistance (SR/FT/LS-73/2012, SERB). They express their gratitude to Director, CMFRI for the facilities provided and encouragement. They are also thankful to Dr. T.Y. Chan, Ph.D, Professor and Director, National Taiwan Ocean University for the species identification.

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