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Population genetics of *Oithona similis* Claus, 1866 (Crustacea: Cyclopoida) in Arabian Sea: Preliminary evidence of haplotype sharing in two populations

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Present study examined two populations (Vizhinjam and Calicut) of *O. similis* distributed in the Arabian Sea based on the comparative analysis of the 28S rDNA sequence variation and to know the population variation between two large marine ecosystems, Atlantic Ocean and Arabian Sea population. The data's were compared in detail. DNA sequence variation clearly resolved and discriminated the populations, and revealed low levels of intrapopulation variation among Atlantic Ocean and Arabian Sea. The 28S rDNA region was thus shown to provide an accurate and reliable means of identifying the species throughout the sampled domain.

[**Keywords**: Copepod, *Oithona similis*, population genetics, Arabian Sea]

Introduction

Molecular genetic analyses have revealed the prevalence of cryptic speciation in marine invertebrates, resulting in excessive lumping in systematic classifications[1].The advent of molecular genetic technologies in the last decade makes it possible to differentiate morphologically similar species with greater accuracy and arrange them to a phylogenetic tree. Currently, very few nuclear and mitochondrial markers have been successfully employed to resolve phylogenetic relationships in copepods[2,3]. For marine copepods, mitochondrial 16S rRNA gene sequences have been used most extensively to reveal intraspecific or interspecific variations[4,5,6]. Other than 16S rDNA, internal transcribed spacer region[7], cytochrome oxidase I (COI)[8] and nuclear 28S rRNA gene[6] have also been used. The 18S and 28S nuclear ribosomal RNA genes have been used to resolve relationships at the ordinal, familial, or generic levels[8,6,9,10,11]. Phylogenetic analyses of crustaceans have been made based on nucleotide sequence data of the nuclear 18S rDNA[12,13,8].

Cyclopoid family, Oithonidae is often the most abundant group of pelagic copepods in estuarine, coastal and oceanic waters throughout the world[14,15,16]. In this study, the nucleotide sequences of the nuclear 28S rRNA gene of *O. similis* pooled from Southwest Coast of India and examined the molecular diversity among the populations. In an earlier study, both morphology and molecular markers were used to infer

the ordinal status of the Copepod[11]. The relationship among *Oithona* species, including *O. similis*, *O. atlantica* and *O. nana*, have been studied in Pacific and Indian Oceans[2]. These morphological analyses included forty five structural characters and suggested that *O. atlantica* and *O. similis* are more closely related to each other than *O. nana*[2]. In an earlier finding within and among three Oithona species occurring in the South and North Atlantic Oceans, analyzed the 28S rRNA gene sequence and characterized the patterns of variation[3]. In India, only very few works are available on molecular taxonomy/identification of marine copepods using very preliminary molecular tools[17,18,19]. In the study we analyze DNA sequences for a 577 base-pair (bp) region of the 28S rRNA gene and characterize patterns of variation within Arabian Sea and Atlantic Ocean populations of *O. similis*.

Materials and Methods

For the present study, two stations such as Vizhinjam (8º21'56"N; 76º59'39"E), and Calicut (11º13'33"N; 75º46'30"E) (Fig. 1) from Southwest Coast of India were selected. Zooplankton collection was done by horizontal hauling using a Bongo net (mouth diameter 40 cm, mesh size: 60µm) equipped with a calibrated flow meter (General Oceanics, Model-2030) to quantify the volume of water filtered. The net was operated from the deck of a purse seiner for 10 minutes at a speed of 2 knots/hour. Concentrated zooplankton was preserved in 95 % ethanol

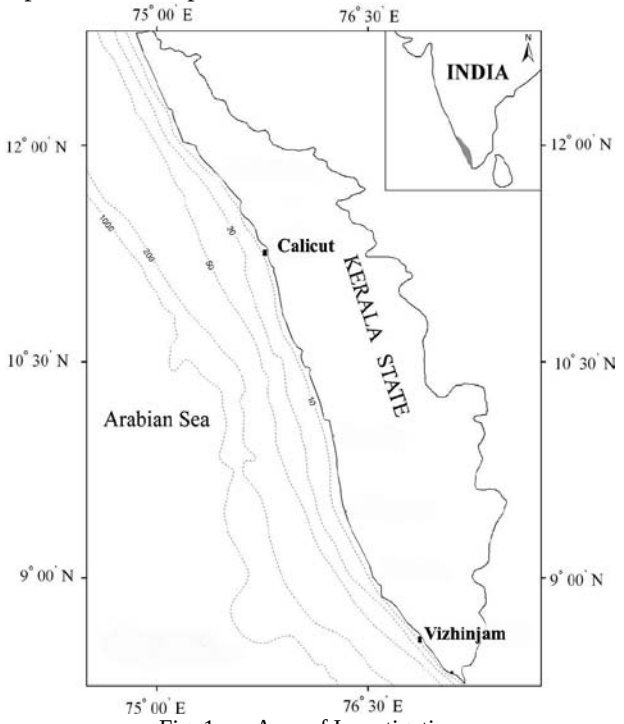

Fig. 1 Area of Investigation

immediately after collection. At each collection, the pre adult and adult *O. similis* were sorted on the same day and kept in 95% ethanol. Species level confirmation of the sorted copepod specimens of *O. similis* was performed according to[20,21,16].

Genomic DNA was extracted from ethanol stored specimens by the standard protocol[22]. DNA samples from each individual was diluted to about 25 ng/µl with deionized distilled water and used for Polymerase chain reaction (PCR) amplification. PCR was used to amplify about 800 bp fragment of the large subunit (28S) ribosomal RNA (rRNA) gene using primers 28SF1: 5′-GCGGAGGAAAAGAAACTAAC-3′and 28SR1: 5′-GCATAGTTTCACCATCTTTCGGG-3′[3]. PCR amplifications were performed in a total volume of 25 µl including 19 µl of double distilled water, 2.5 µl Taq buffer (10 mM Tris-HCl, 10 mM KCl, 15 mM $MgCl_2$, pH 8.0) 1 µl of dNTP mix (0.2 mM each), 1 µl of primer mix (10 pM), 0.75 units of Taq DNA Polymerase (Merck) and 1 µl of the DNA template solution. PCR protocol was: 4 min initial denaturation step at 94^0C; 35cycles of 40s denaturation step at 94^0C, 40s annealing at 50^0C, and 90s extension at 72^0C; and a final extension step of 15 min at 72^0C. Approximately 2 µl of each PCR product was electrophoresed on a 1.5% TBE agarose gel and visualized by UV light with ethidium bromide staining. Both strands of the template DNA were sequenced using the PCR primers in an ABI automated capillary DNA sequencer in a commercial laboratory (Ramachandra Innovis, India)

The 28S rDNA sequences obtained were manually edited, with comparison of aligned sequences for both strands. The DNA sequences of *O. similis* from the two populations were aligned using the default parameters by Clustal W[23], using MEGA Ver. 5.05[24]. The DNA sequences were submitted to the molecular database, GenBank (http://www.nlm.nih.ncbi.org) and were assigned a GenBank Accession Numbers: KC136272-84. Twelve sequences of 28rDNA of *O. similis* of Atlantic Ocean were retrieved from NCBI GenBank (JF419529-40) and were used for comparative analysis.

Molecular analysis was done using a final aligned length of 577 bp of the 28S rRNA gene. Numbers of haplotype sequence and sequence diversities (h) were calculated for each population sampled for the studied populations by DnaSP Ver. 5.10[25]. Haplotype network between Atlantic Ocean and Arabian Sea populations were drawn by Network Ver. 6.1 software[26]. Neighbor-Joining method[27] analysis implemented in MEGA Ver 5.05[24] was used

on the identified haplotype sequences to assess the relationships among the two Oithona populations based on DNA sequence variation; relative support for the tree topology was obtained by bootstrapping[28] using 10,000 iterations. The most appropriate model was found to Jukes-Cantor; the model and estimated parameters were set in MEGA Ver 5.05[24] and the geographic pattern of 28S rDNA variation was assessed. For this analysis, all sequence types found in the populations from Atlantic Ocean and Arabian Sea were considered.

Results

A total of 25 sequences of 28S rDNA for *O. similis* from the two stations in Arabian Sea and Atlantic Ocean were aligned using MEGA Ver. 5.05. DNA sequences of a 577 bp region of the 28S rDNA for 25 individuals revealed the population variation between Atlantic Ocean and Arabian Sea. The distribution and frequency occurrence of *O. similis* haplotypes in Atlantic Ocean and Arabian Sea are given in Fig. 2.

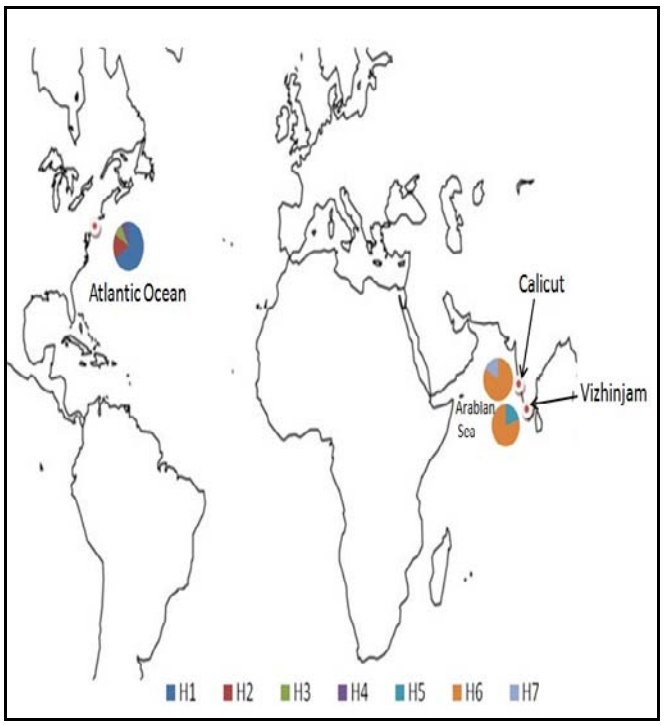

Fig. 2 Frequency distribution of *O. similis* 28S rDNA haplotypes in Atlantic Ocean and Arabian Sea. The seven haplotypes sequence (H1-H7) are represented by different colours

Haplotype network shows Atlantic Ocean population was clustered separately from Arabian Sea populations (Fig. 3).

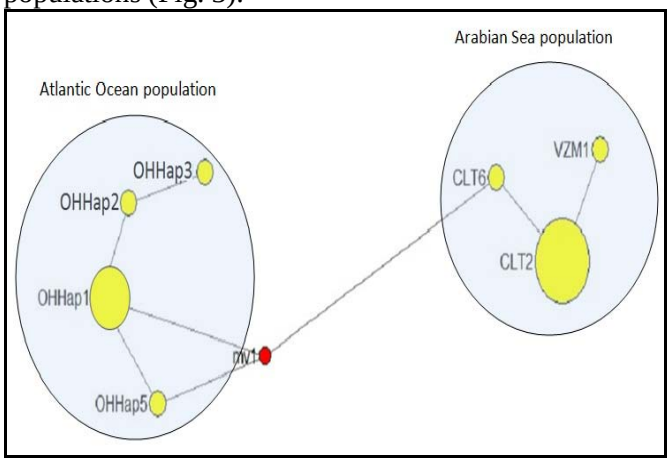

Fig. 3 Haplotype network of Atlantic Ocean and Arabian sea populations of *O. similis* based on 28S rDNA sequence data

Distance among sequence types were analyzed with MEGA using the Jukes – Canter model with alpha parameter of 0.25 to analyze the relationship among and between Atlantic Ocean and Arabian Sea populations based on 28S rDNA sequence is given in Table I.

The mean Jukes-Canter distance between the Atlantic Ocean and the Arabian Sea was 0.137, whereas the distance within Atlantic Ocean population was 0.002 and for Arabian Sea population it was 0.001. The nucleotide diversity (π) among and between Atlantic Ocean and Arabian Sea population based on 28S rDNA sequence is given in Table II. Accordingly, the nucleotide diversity within Atlantic Ocean and Arabian Sea population was 0.001 with a co-efficient of differentiation of 0.980.

The relationship among and between the two populations in Arabian Sea based on 28S rDNA sequence is presented in Table III. Mean Jukes-Canter distance of *O. similis* within Calicut and Vizhinjam was 0.000. The mean Jukes-Canter distance was comparatively more (0.001) between Calicut and Vizhinjam populations. Nucleotide variation in two Arabian Sea populations of *O. similis* is given in Table IV. There was only one segregating site was observed in both Calicut and Vizhinjam populations. Nucleotide diversity in Calicut and Vizhinjam populations was 0.00086.

Table I. Genetic distance among and between Atlantic Ocean and Arabian Sea *Oithona similis* population based on 28S rDNA sequence

Population	**Atlantic Ocean**	**Arabian Sea**
Atlantic Ocean (*n*=12)	0.002	-
Arabian Sea (*n*=13)	**0.137**	0.001

Table II. Nucleotide diversity (π) among and between Atlantic Ocean and Arabian Sea *O. similis* population based on 28S rDNA sequence. Co-efficient of differentiation between the populations is in parenthesis

Population	**Atlantic Ocean**	**Arabian Sea**
Atlantic Ocean (*n*=12)	0.001	-
Arabian Sea (*n*=13)	**0.072** (0.980)	0.001

Table III. Mean Jukes-Canter distance among and between the two populations in Arabian Sea *O. similis* based on 28S rDNA sequence

Population	**Calicut**	**Vizhinjam**
Calicut (*n*=6)	**0.000**	-
Vizhinjam (*n*=7)	0.001	**0.000**

Table IV. Nucleotide variation in two Arabian Sea *O. similis* populations based on 28S rDNA sequence

Population	**N**	**S**	**Ps**	**Θ**	**π**
Calicut	6	1	0.001733	0.00094	0.00086
Vizhinjam	7	1	0.001733	0.00094	0.00086

N = Number of sequence; S = Number of segregating sites, Ps = S/N, Θ = Ps/nb, π = Nucleotide diversity

The Neighbor – Joining phylogenetic tree for Atlantic Ocean and Arabian Sea populations was constructed based on 25 DNA sequence variation (Fig. 4). Neighbor – Joining phylogenetic tree showed *O. similis* in Atlantic Ocean and Arabian Sea were clustered clearly into two separate clade with > 90% bootstrap confidence value. Based on the DNA sequences of a 577 bp region of the 28S rDNA for twenty five *O. similis* individuals, it was concluded that *O. similis* from Arabian Sea was a genetically distinct population. Even for this conserved genetic marker, this species showed significant genetic differentiation among the two locations studied. It seems likely that geographic populations of *O. similis* might be primarily isolated by large scale pattern of ocean circulation, as has been suggested by other genetic analysis of copepod in the Atlantic Ocean basin[3].

The present analysis consisting of inter population pattern of variation of *O. similis* in selected regions of southwest coast of India, Arabian Sea demonstrated the efficacy of the 28S rDNA as an accurate and reliable means of identifying and discriminating the populations.

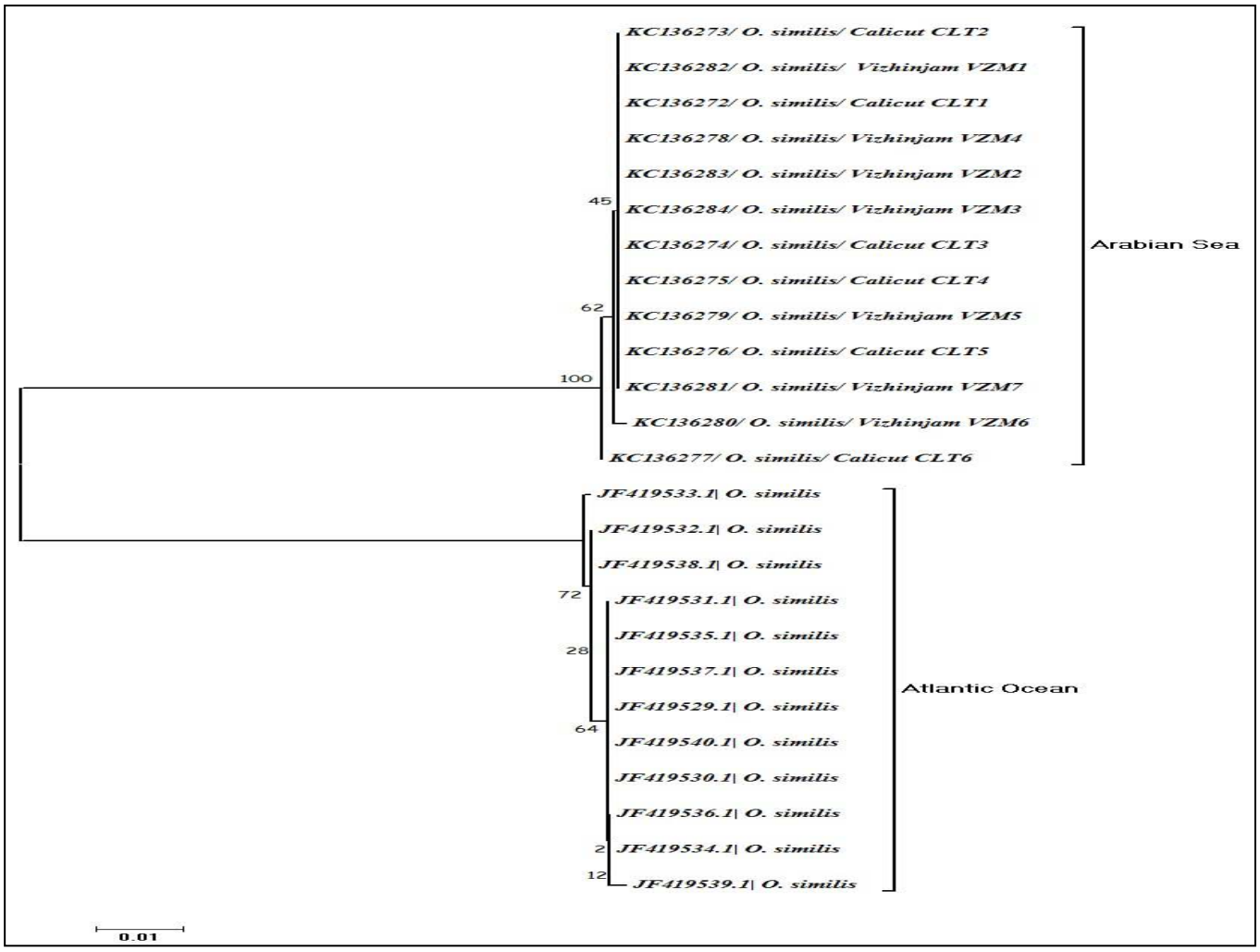

Fig. 4 Neighbor – Joining tree (10000 replications) of *O. similis* in Atlantic Ocean and Arabian Sea based on 28S rDNA gene sequence

Discussion

Accurate and reliable identification of species is a necessary foundation for assessment of biodiversity, especially for important but lesser known populations of the old ocean[3]. Morphological variation may not be give reliable identification key in many of marine organisms because of plasticity. For this instance, molecular markers such as genomic and mitochondrial will give valuable and accurate information about the species. DNA sequence variation of target genes provides invaluable tools for biodiversity analyses[29].

The study examined variation of a portion of 28S rDNA as a marker to identify and discriminate populations of the ecologically important but under studied Cyclopoid copepod *O. similis* found in the Arabian Sea. The species *O. similis* was confirmed by molecular analyses to be a distinct species as previously characterized by morphological taxonomic analyses[30,2]. Such molecular identification of *O. similis*, *O.nana* and *O. atlantica* in Atlantic Ocean has been reported recently[3] and the genetic distances within and between the species of Oithona was agreed somewhat with similar earlier studies[31]. In addition to characterizing differences between species these works provided preliminary analysis of the levels and patterns of 28S rDNA sequence variation within each of the studied species of *Oithona* based on samples collected from a broad latitudinal range of Atlantic Ocean In the present study, among the three haplotypes, one haplotype was shared between the two populations of *O. similis* distributed in the Arabian Sea and is agreed with earlier findings[3]. This finding confirms that 28S rDNA serves as a useful genetic marker for the identification and population delineation of this egg bearing cyclopoid species *O. similis*. Inter population variation of *O. similis* in Arabian Sea was low (0.002) when compared with the earlier report in Atlantic Ocean and it was 0.007[3]. Intraspecific variation measured as the percentage of base variation differed among *O. atlantica* (1.5%), *O. nana* (0.6%) and *O. similis* (0.1%)[3]. According to them, the lower values for *O. nana* and *O. similis* which are both distributed commonly in coastal and shelf waters might be due to in part to their introduction by ballast water. In the present study, *O. similis* exhibited different kind of distribution among populations sampled for this study. The neighbor joining tree showed the Calicut population scattered among the Vizhinjam population. Same kind of observation was documented in Atlantic Ocean where the *O. similis* population was not clearly correlated as per the geographic regions[3].

Based on the 28S rDNA sequence study, *O. similis* is a genetically distinct copepod species throughout the studied distributional range. Even for this conserved genetic marker, this species showed significant genetic differentiation among the two locations studied. It seems likely that geographic populations of *O. similis* might be primarily isolated by large scale pattern of ocean circulation, as has been suggested by other genetic analysis of copepod in the Atlantic Ocean basin[32,33,34].

Present analysis consisting of inter population pattern of variation of *O. similis* in selected regions of Southwest Coast of India, Arabian Sea demonstrated the efficacy of the 28S rDNA as an accurate and reliable means of identifying and discriminating the populations. The 28S rDNA fragment we focused has been suggested as a taxonomic marker due to its variability[35]. Previous studies have used this marker for analyses of copepods[36] and other taxa[37].

Conclusion

DNA sequences of a 577 bp region of the 28S rDNA of *O. similis* revealed the population variation between Atlantic Ocean and Arabian Sea. Based on these results, it was concluded that *O. similis* from the Arabian Sea was a genetically distinct copepod species. Further analyses of inter and intra population variation using more highly variable markers will be needed to address questions of population connectivity, barriers to genetic cohesion of *O. similis* throughout the entire coast line of India. Present analysis consisting of inter population pattern of variation of *O. similis* in selected regions of southwest coast of India, Arabian Sea also suggested the efficacy of the 28S rDNA as an accurate and reliable method to identify and discriminate the populations from different geographical areas. All the 13 sequences were submitted to the NCBI GenBank, USA data base as GenBank Accession no: KC136272 to 84 through BankIt according to NCBI's procedure. This work describes for the first time the molecular characteristics of *Oithona similis* from a tropical coast, typically southwest coast of India and strengthens the route for further related findings from Arabian Sea.

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