International Journal of Zoological Research

ISSN 1811-9778

Academic Journals Inc.

www.academicjournals.com

OPEN ACCESS International Journal of Zoological Research

ISSN 1811-9778
DOI: 10.3923/ijzr.2017.1.5

Research Article
Molecular Characterization of Brachyuran Crab *Dromia dehaani* (Rathbun, 1923) Along the Pazhayar Coast (Southeast Coast of India)

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Abstract

Background: The crab *Dromia dehaani* was predominant in the Pazhayar coast. However, to date there has been no study on the aspects of molecular characterization of crab *Dromia dehaani*. Morphological identification is one of the conventional method and also challenging when the expertise is limited. This is one of the study which describing the molecular aspects of the crab *Dromia dehaani*. **Materials and Methods:** DNA was isolated from *D. dehaani* and the target DNA was amplified using the forward and reverse primers. The PCR products were purified prior DNA sequencing. **Results:** In this present study, 18S rRNA partial sequences were assessed to predict the cladistic status of the study crab *D. dehaani*. Phylogenetic tree was constructed by using 18S rRNA partial sequences. Sequenced conservative portion of the 18S rRNA gene was used to observe the phylogenetic relationship and evolution. **Conclusion:** The phylogenetic relationships were predicted with the subfamilies such as Cyclodorripoidae, Ranninoidae and Eubrachyurans. The molecular phylogeny tree shows that *D. dehaani* belongs to the Dromiidae family, Infraorder-Podotremata and its sister relationship to Cyclodorripoidae, Rannindae and Eubrachyurans. These findings prove that the *D. dehaani* is the ancestor of the above mentioned organisms.

Key words: Decapod, Dromiidae, cladistic, crustaceans, 18S rRNA

Received: July 20, 2016 Accepted: October 21, 2016 Published: December 15, 2016

Citation: S. Ravichandran, D. Karthick Rajan and W. Sylvester Fredrick, 2017. Molecular characterization of brachyuran crab *Dromia dehaani* (Rathbun, 1923) along the Pazhayar Coast (Southeast Coast of India). Int. J. Zool. Res., 13: 1-5.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Crustaceans play a very significant role in the ecology of the marine environment. Brachyura are one of the most diverse groups of crustaceans with approximately 7,000 described species in 98 families occurring in marine, fresh water and terrestrial habitats[1]. Resemble to this crab the podotreme crabs also carry the sponges, debris or live coral. Podotreme crabs taxonomy has been studied extensively over the past several decades although, identification can be still tricky and difficult. The podotreme crabs best recognized by the owing of gonopores on the coxae of the pereopods have variously been regarded as mono, para or polyphyletic or even as non-brachyuran[2]. The probable large scale convergence and extreme morphological diversity of adult features in the Brachyuran has confounded consensus on phylogenetic relationships. Molecular study was used to test hypotheses about the phylogeny of crabs. Combining the morphological characters and Brachyuran were assessed for their phylogenetic position by using 18S rRNA discussed the origin of the Podotremata crabs. The relationships among organisms or genes are studied by comparing homologous of DNA or protein sequences. The molecular phylogenetics of decapod crustaceans has been based on sequence data from a limited number of genes. These have included rapidly evolving mitochondrial genes, which are most appropriate for studies of closely related species and slowly evolving nuclear ribosomal RNA genes, which have been most useful for resolution of deep branches within the Decapoda[3]. Ribosomal RNA gene has been used as one of the most common markers for analyzing the molecular phylogeny[4,5] although its use has occasionally known to be hindered by discrepancies due to the presence of duplicate copies nucleic acid sequences from small-subunit ribosomal RNAs 18S rRNA. The morphological and molecular characters (18S rRNA partial sequences) were assessed to predict the cladistic status of the crab *D. dehaani* done with 18S rRNA partial sequences and phylogenetic tree was constructed in this study.

MATERIALS AND METHODS

The spongy crabs were collected from Pazhayar landing center (11°21'N; 79°50'E) South East Coast of India. The collected crabs were taken for the following measurements of body length carapace length. Length is measured by a ruler vernier calliper to the nearest 0.1 mm and weighed by using the digital weighing balance. Then the samples were stored in 80-95% ethanol for extracting the DNA.

DNA extraction: The total DNA was extracted from the muscle tissue of the spongy crab *D. dehaani* by using the following protocol of earlier studies by Ahyong and O'Meally[2]. Concentration of the DNA was obtained as 21.6 μg mL^{-1}.

PCR and sequencing of PCR products: The PCR amplification, DNA sequencing was carried out by the following methods of Williams and Ozawa[6]. Polymerase chain reaction was carried out on a biometra thermal cycler by using 5X qarta, primers from sigma. The primers used for amplification of DNA are forward primer 18S F 5′-CTGGTTGATYCTGCCAGT-3′ reverse primer used is 18S R 5′-CTTCGAACCTCTGACTTTCG-3′. The DNA ladder of fermentas (#SM1553) was used as molecular weight marker and 10 μL of the same was loaded along with PCR products to compare the amplicon size. The PCR products were loaded in 1% agarose gel to check the presence of DNA under UV transilluminator. The PCR products were purified prior DNA sequencing. The DNA sequencing was performed at MWG Biotech, Bangalore, India.

BLAST analysis: The sequences of PCR products were aligned and the sequences were analyzed by using Basic Local Alignment Search Tool (BLAST). Nucleotide-nucleotide BLAST was carried with facility of National Centre for Biotechnology Information (NCBI) (http://www.ncbi.nlm.nih.gov/BLAST).

RESULTS

GenBank accession number: The total length of the nucleotides obtained is 433 bp and the GenBank accession number was obtained as KC130910. The maximum parsimony phylogenetic tree showed the phylogenetic relationship of the Podotremata and Brachuyuran. The bootstrap consensus tree inferred from 1000 replicates is taken to represent the evolutionary history of the taxa analyzed. Branches corresponding to partitions reproduced in less than 50% bootstrap replicates are collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. In the tree, these studied crab was closely clustered with *D. dehaani* with 55% bootstrap level confidence. Also this cluster was showed sister relationship with Anomuran group. Moreover, Cyclodorripidae, Ranninoidae and Eubrachyurans show monophyletic group. The phylogenetic tree was shown in Fig. 1.

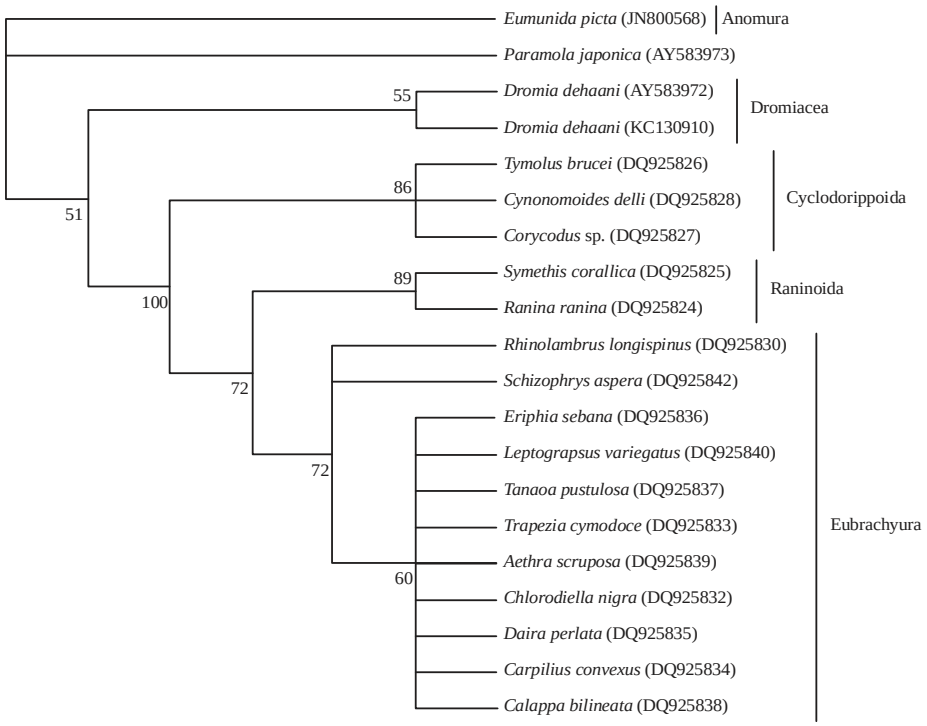

Fig. 1: Maximum parsimony phylogenetic tree of *D. dehaani* with Podotremata and Eubrachyurans

DISCUSSION

The Dromiidae is a family of primitive Brachyuran crabs[7]. The collection of *D. dehhani* was found abundantly in the Pazhayar landing center of the South East Coast of India. The occurrence of Dromiidae family in New Cadelonia and Philippines were reported by Mclay[8]. Likewise Aravindakshan *et al.*[9] reported the abundant occurrence of *D. dehaani* in Bombay the West coast of India. The morphological reports of the present study support the details about the carapace, antennae, gonopod from the study was done in Calcutta, India[10]. So, the morphological investigation of the study crab confirms that the crab to be *D. dehaani.*

Identification of species is necessary, molecular methods highly supports in identification of the animals at species level. The present study describes the molecular characterization of the crab *D. dehaani* from the tissue sample by 18S rRNA partial sequences and 433 nucleotides were obtained.

This 18S rRNA research supports the works done in arthropods. The monophyly of the arthropods and the chelicerates and provides a partial test of arthropod and chelicerate phylogenies that is independent of morphological and developmental characters was conducted by partial 18S rRNA sequences[11]. In *Tachypleus gigas* more than 1800 nucleotide sequences were successfully isolated representing the conservative portions of the 18S rRNA molecule results shown that the homologous sequences of *T. gigas* are phylogenetically more related to *Carcinoscorpius rotundicauda* with the bootstrap value of 99%[12].

Steiner and Dreyer[13] tested the morphological data against an independent data set obtained near-complete 18S rDNA sequences of 12 scaphopod species from five families and aligned them to the existing ones and to a selected set of gastropods, bivalves and cephalopods, using the available polyplacophorans as out group. *Pachycheles chubutensis* are species with a distribution restricted to rocky shores in South-eastern America, that share a troubled taxonomic history. Morphological characters overlap and identification can be imprecise, when both species coexist in the same environment[14]. The relationships among the Brachyuran families are poorly understood due to the high morphological complexity of the group[1]. A molecular phylogeny of the group may help to resolve the long-standing taxonomic question about the validity of the genera *Allopetrolisthes* and *Liopetrolisthes*[15]. Partial sequences of the 18S nuclear and 16S mitochondrial ribosomal genes were obtained for 14 species of *Thalassinidean* shrimp and a

further 6 species in related decapods infraorders[16]. Pycnogonida, group of arthropods are exclusively marine organisms are sequenced the near complete 18S rRNA gene from a large range of Pycnogonida spanning all ten living families recognized. The phylogenetic position of the Phtisicidae and other caprellid amphipods, using 18S rRNA gene sequence data results strongly indicate that the Phtisicidae and other caprellid families form a monophyletic clade[17]. Hence, the partial sequence of 18S rRNA supports the molecular identification in the species level identification.

The analysis of nuclear rDNA which encodes rRNA genes is commonly used in assessing phylogenetic relationships among taxa[18]. The 18S rRNAs have proved useful for phylogenetic analysis in eukaryotes. Because of their ubiquity and evolutionary conservation, these molecules are useful for inferring distant phylogenetic relationships, providing a means of assessing relationships between organisms which lack any informative homologous morphological or developmental traits[19,20]. The 18S rDNA topologies are highly congruent with rbcL based topologies at a diverse array of taxonomic levels[21].

The status of Podotremata is one of the most controversial issues in Brachyuran systematics[22]. In this present study, *D. dehaani* which belongs to Podotremata were studied by the cladistic analysis. The maximum parsimony molecular phylogeny tree was constructed using Podotreme and Eubrachyuran families. The molecular phylogeny tree confirms that *D. dehaani* belongs to Dromidae family and the Infra order belongs to Podotremata with the relationship of 100%. The results obtained supports the study of Ahyong *et al.*[22] where the *D. dehaani* of the gene accession number AY583972 involved in the cladistic analysis also belongs to Infraorder Podotremata have proven under both maximum parsimony and bayesian inference. Podotremata was found to be significantly paraphyletic by comprising three major clades Dromiaceae, Raninoida and Cyclodorippoida.

Similar controversial studies in crabs were investigated by other COI and 16S rRNA, the systematic of the *Calappa lophos* and the species group is clarified using morphological and molecular data[23]. Two species *C. quadrmaculata* and *C. guerini* previously synonymised with *C. lophos* are shown to be valid species using morphological and molecular data. The usefulness of maximum parsimony elucidating the phylogenetic relationships of some of the *Calappa* species based on cytochrome oxidase I is examined. The investigated mud crabs of the family Panopeidae for morphological and molecular analysis was done based on DNA sequences of the mitochondrial large subunit rRNA (16S; 529 bp) and cytochrome oxidase I (COI; 640 bp) genes the results suggest that the species genera *Panopeus* and *Eurypanopeus* are not monophyletic and that their taxonomy does not accurately reflect evolutionary partitions[24].

In two cases *P. herbstii* complex and *E. depressus* and allies, the molecular findings strongly support sister-species relationships that differ from previous morphology-based assumptions. Metaplex belongs to the Grapsidae family and often reported to Ocypodidae as it resembles *Ocypodid* genus characteristic behaviour. Molecular analysis of 16S mitochondrial ribosomal RNA gene of 19 grapsids, 10 ocypodids and 3 camptandriids, including four species of *Metaplax* and four species of *Macrophthalmus* were involved. The resultant phylogenetic tree revealed that both families Grapsidae and Ocypodidae are polyphyletic[25].

CONCLUSION

Thus the crab *D. dehaani* was morphologically examined and confirmed by molecular identification with partial 18S rRNA sequences. The molecular phylogeny tree shows that *D. dehaani* belongs to the Dromiidae family, Infraorder-Podotremata and its sister relationship to Cyclodorripoidae, Rannindae and Eubrachyurans. These findings prove that the *D. dehaani* is the ancestor of the above mentioned organisms.

ACKNOWLEDGMENT

Researchers are thankful to Department of Science and Technology (SB/SO/ AS/063/2013), Government of India, New Delhi for providing financial assistance to carry out this Research work.

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