

## MOLECULAR PHYLOGENY OF *SELATIUM BROCKII* AND *NANOSESARMA MINUTUM* GRAPSID CRABS INFERRED FROM COI GENE

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### ABSTRACT

The phylogenetic relationship of the two grapsid crabs *Selatium brockii* and *Nanosesarma minutum* collected from mangrove creeks have been studied corresponding to the mitochondrial genome cytochrome oxidase subunit I (COI) gene. Genomic DNA was isolated from the claw muscle of the two crabs *Selatium brockii* and *Nanosesarma minutum*. The COI gene of the crabs was amplified and sequenced. The phylogenetic tree was constructed from the COI gene sequence. Present findings show that the genetic relationship between these two species to be 74% and both the species show 99% similarity to *Parasesarma erythroductyla* strain MSCAS9 (KX018517), a red handed shore crab found in the mangrove creeks. COI gene could be a useful molecular marker to determine the monophyly of a crustacean family. No earlier reports were available on the molecular phylogeny of currently studied species. Increase in the taxon sampling of COI gene sequences from different groups could be a promising methodological resolution for determining the genetic divergence of the species.

**Keywords:** Grapsid, mangrove, DNA, COI, Phylogeny

### INTRODUCTION

Crustaceans abundant in extreme habitats have wide morphological convergences and renowned for their astounding anatomical, ecological and behavioral diversity, especially those from Sesamididae family found in mangrove ecosystems (Lee, 1998). The Grapsidae and thoracotreme crab families are addressed more in the literature owing to their predominance and have a significant role in the secondary consumers in the intertidal habitats. It includes eight genera with 40 species found in the tropical region; possess a number of morphological and molecular synapomorphies. Under the terminology of DNA taxonomy, two independent tasks have been merged (Desalle et al., 2005). The relationship between taxonomy and molecular divergence has been studied by (Avisé and Johns, 1999). The inter-specific divergence of Cytochrome *b* for vertebrates showed poor equivalence of divergence across the taxa. Cytochrome *b* within the vertebrates showed good relationship between taxonomic classification and molecular divergence, and concluded that mitochondrial DNA promote the convenient taxonomic classification among the species studied (Avisé and Walker, 1999). Recently, a standard sequence threshold of 10 times the mean intra-specific variations was proposed to delimit animal species (Hebert et al., 2004).

In recent years numerous studies have been focused on the taxonomy and molecular phylogeny of grapsid species using molecular approaches. Furthermore, most of the previous studies have been focused on vertebrates, of which taxonomy has been intensively studied and the "taxonomic impediments" are probably fewer than in other phyla (Avisé and Johns, 1999; Avisé and Walker, 1999; Hebert et al., 2004). The classification of the grapsoid genera within the four subfamilies has rarely been studied and questioned (Guinot and Bouchard, 1998). The gecarcinid crabs at the same taxonomic level as the current grapsid subfamilies (Milne Edwards, 1853). In the present study, phylogenetic relationship of two grapsid crabs *Selatium brockii* and *Nanosesarma minutum* is studied from southeast coast of India based on DNA sequence of the mitochondrial COI gene.

### MATERIALS AND METHODS

#### Sample collection

A total no of 5 samples of *Selatium brockii* and *Nanosesarma minutum* were collected from the mangroves of Vellar estuary. These two species were identified using the conventional morphological criteria. The tissue samples from claw muscles were collected, washed three times with saline and preserved in ethanol until DNA extraction.

#### Genomic DNA isolation and COI gene sequencing

Genomic DNA was extracted from the claw muscles of the crabs *Selatium brockii* and *Nanosesarma minutum* by using the following methods of Ahyong and O'Meally, (2004). The COI gene was amplified by Polymerase Chain Reaction with the following forward and reverse primers 5'-GGTCAACAAATCATAAAGATATTGG-3' 5'TTAACCTCAGGGTGACCAAAAATCA-3' as described elsewhere (Williams and Ozawa, 2006). Amplified products were separated and evaluated by electrophoresis through 0.8% agarose gels. The band containing the amplified DNA was excised from the gel. PCR products were cleaned using Wizard PCR purification system (Promega). Further, the PCR products were sequenced by using the genetic analyser (Applied Biosystems 3130). The DNA sequencing was performed at Yaazh xenomics, Chennai, India.

#### BLAST and phylogenetic analysis

The sequenced PCR products were aligned and analyzed by Nucleotide BLAST. The nucleotide sequence of *S. brockii* and *N. minutum* were retrieved from NCBI and aligned by Clustal W using default parameters, manually adjusted and confirmed by computational amino acid sequences using MEGA 5.0 (Tamura et al., 2011). COI gene sequences of *Selatium brockii* and *Nanosesarma minutum* and their similar sequences were obtained from the DNA database and the same was used for phylogenetic analysis. The composition of nucleotide sequences were calculated using MEGA 5.0. J Model test 2.1.3 and Bayesian information criterion (Ip et al., 2015; Schwarz, 1978). Phylogenetic tree was built using the neighbour-joining method and the bootstrap values were calculated.

RESULTS

*Selatium brockii* cytochrome oxidase subunit I gene was partially sequenced with 824 bp and 669 bp for *N. minutum*. The mitochondrial cytochrome oxidase gene sequences of *Selatium brockii* and *Nanosesarma minutum* were submitted in NCBI with an accession number JF809865 and JX979129. The subsequent phylogenetic analysis for two species was carried out using Neighbor joining method using MEGA 5.0. The phylogenetic tree was constructed for *S. brockii* and *N. minutum* along with the species that showed high genetic similarity, which was determined by the BLAST analysis. The genetic relatedness of the species with *S. brockii* and *N. minutum* was also discussed. The phylogenetic tree constructed from the COI gene sequence of *S. brockii* and *N. minutum* showed a close similarity of 99% with *Parasesarma erythroductyla* strain MSCAS9, a red handed shore crab found in the mangrove creeks (Figure 1 & 2). In order to compare the relative performance and information contents of mitochondrial DNA between the *S. brockii* and *N. minutum*, we independently generated the phylogenetic tree for both the species.

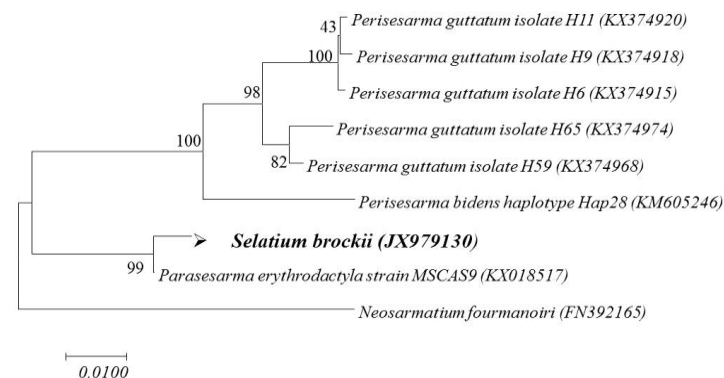


Figure 1 Molecular phylogeny of *Selatium brockii* using mitochondrial COI gene subunit

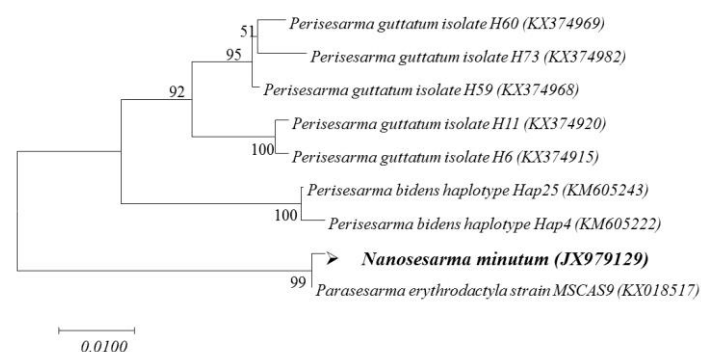


Figure 2 Molecular phylogeny of *Nanosesarma minutum*

The phylogenetic tree of *S. brockii* and *N. minutum* showed 74% of similarity. Since the habitat of the two species are same and, therefore, may have evolved from the ancestor species. In addition, both the species exhibited strong homogeneity (99% sequence similarity) with *P. erythroductyla* strain MSCAS9 (Figure 3). The species studied from grapsidae family showed high bootstrap values, which apparently holds a basal position within the family. Other species showed 92% similarity to *Parasesarma erythroductyla* used in this study. Hence, it acts an ancestor species for *S. brockii* and *N. minutum* which use mangrove creeks as their habitat for their growth and survival. The entire phylogenetic tree constructed for *S. brockii* and *N. minutum* was congruent in their overall topology with some cluster showing high confidence levels consistently. The findings were, therefore, shown together based on the Bayesian inference and neighbour-joining method with all confidence values greater than 60.

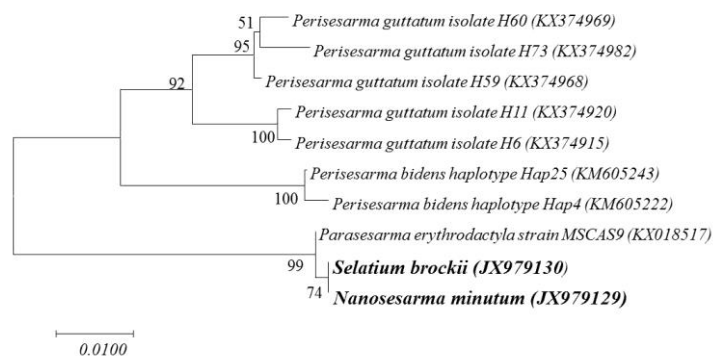


Figure 3 Molecular phylogeny of *Selatium brockii* and *Nanosesarma minutum*

DISCUSSION

In the present study, the phylogenetic relationship of two species under grapsidae family has been studied. The molecular phylogeny of *S. brockii* and *N. minutum* has not been studied and hence an attempt has been undertaken to summarize and discuss those phylogenetic relationship among the other species of grapsidae family. Phylogenetic relationships among the grapsidae crab based on the mitochondrial cytochrome oxidase gene subunit have been proposed in number of earlier studies (Kitaura et al., 2002; Schubart et al., 2002). The grapsidae had been postulated to be monophyletic (Schubart et al., 2006) and the studies showed that grapsidae were paraphyletic and basal to the subfamilies Sesarmidae (Kitaura et al., 2002).

Though lots of attention have been addressed on the grapsidae family due to their predominance, their original classification of some species based on the larval and adult morphology has been questioned. The phylogenetic tree was constructed using the MEGA 5.0 and both the species showed high similarity (99%) to *Parasesarma erythroductyla* strain MSCAS9. *Selatium brockii* and *N. minutum* showed 74% similarity which reveals that they show different morphological and molecular alignments, but both the species have been evolved from *Parasesarma erythroductyla*. The phylogenetic analysis of COI sequences showed high similarities with the decapod species (Li et al., 1993) which was in contrast to the present study. Monophyletic Grapsidae in all of the phylogenetic analysis showed 74-85% and 99% confidence levels, which was found in the present study, thereby validating the morphological results (Schubart et al., 2002; Schubart et al., 2006). Since *S. brockii* and *N. minutum* showed higher relationship with *P. erythroductyla*, it might act as an ancestor for the evolution of these species. The habitat of those species is mud of the mangrove creeks and times back these species might be evaluated with different molecular synapomorphies. The overall topologies for the entire phylogenetic tree were congruent with some clusters showing high confidence levels consistently.

CONCLUSION

Present findings demonstrate that COI gene seems to a useful DNA marker to determine the monophyly of a crustacean family, and increasing the taxon sampling of COI gene sequences from different groups could be a promising methodological resolution for determining the genetic divergence of the species. Here, the phylogenetic relationship of *S. brockii* and *N. minutum* shown together and discussed based on the Bayesian inference and the neighbour-joining method with all confidence values greater than 60. No earlier reports were available on the molecular phylogeny of currently studied species and therefore, the evolutionary relationship has been studied in order to determine their molecular synapomorphies.

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