



## DNA Barcoding and First report on the confirmation of mud crab *Scylla olivacea* (Brachyura: Portunidae) availability in East coast of India

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### Abstract:

An attempt has been made in the present study in which partial sequencing of COI gene was carried out for the *Scylla olivacea* of genus *Scylla*, collected from Pichavaram mangroves of southeast India. The phylogenetic tree created using Minimum Evolution (ME), Maximum Likelihood (ML) and Neighbor - Joining (NJ) approaches proved that all the three methods produced same topology. The overall average genetic distance is 0.093. The mitochondrial sequence (Cytochrome oxidase subunit I) obtained from the specimen was the first sequence deposition at GenBank from Indian waters. The present study on partial COI sequencing (Barcoding) resolved the taxonomic ambiguity among the members of the genus *Scylla* to a greater extent.

## 1. INTRODUCTION

The mud crabs belongs to genus *Scylla* is one of the largest portunids and is widely distributed throughout the costal Indo-Pacific region. These mud crabs are esteemed palatable seafood item which stands as significant commodity in the international seafood market. They represent a valuable component of small scale coastal fisheries in tropical and subtropical Asia. Unlike the other portunids, *Scylla* has the ability to withstand fluctuations in salinity and low oxygen levels as well as being able to survive out of water, thus simplifying the transportation needed, particularly during exportation (BOPB, 1991). The species identification of mud crab has been controversial for many years. A precise and sound taxonomic identification of species in genus *Scylla* is a prerequisite for hatchery rising of mud crab seeds for aquaculture and also for stock enhancement of mud crab fishery.

Researchers from different localities have reported that the genus *Scylla* includes several species but all these reports are based on morphological and allozyme electrophoresis. One decade before Keenan et al. (1998) revised the genus *Scylla* and proved the occurrence of four species based on morphometry,

allozyme electrophoresis and mtDNA sequencing of Cytochrome oxidase I (CO I) and 16s RNA genes. Subsequently, many authors reported occurrence of four species by using molecular data. In the Indian context little work has been carried out except those of Joel and Sanjeevaraj (1980) and Kathirvel and Srinivasagam (1992) in which the taxonomic status of the Indian mud crabs were portrayed based on morphology concluded the availability of two distinct species namely *Scylla serrata* and *Scylla tranquebarica*. Based on Keenan et al. (1998) classification we first report the confirmation on the availability of *Scylla olivacea* (Herbst, 1796) from Indian waters.

## 2. MATERIALS AND METHODS

Through a routine survey, we collected the mud crabs from Pichavaram mangroves (11°2'N; 79°47'E), southeast coast of India and confirmed as *Scylla olivacea* (Herbst, 1796) based on identification keys proposed by Keenan et al. (1998) (Fig. 1). For the sequencing study, DNA isolation and amplification was done following the method of John et al. 2010. The fragment of COI was amplified by GeneAmp PCR system 9700. Fish F1

[5'-TCAACCAACCACAAAGACATTGGCAC-3'] and Fish R1 [5'-TAGACTTCTGGGTGGCCAAAGAATCA - 3'] primers were employed for COI amplification (John et al. 2010). Sequencing PCR was carried out using Dye terminator mix v3.1 and quantified in Euro bio-agarose gel. The samples were loaded onto MegaBace sequencer (MB 1000) at Bioserve Biotechnologies, Pvt., Ltd., Hyderabad, India.



Figure 1. *Scylla olivacea* dorsal view

### 3. RESULTS AND DISCUSSION

#### 3.1 Diagnosis

Carpus spines of cheliped inner absent, outer reduced. Frontal lobe spines low, rounded with shallow interspaces. Propodus spines of cheliped reduced. Palm of cheliped usually with a pair of blunt prominences on dorsal margin behind insertion of the dactyl, inner larger than outer; may be spinous in juveniles and young adults. Chelipeds, legs, abdomen are without obvious polygonal patterning for both sexes. Colour varies from red through brown to brownish /black.

#### 3.2 Habitat and Distribution

Mud crab *Scylla olivacea* of the genus *Scylla* de Haan are large size portunid crabs inhabit estuaries and coastal waters throughout the tropical to warm temperate zones in the Pacific and Indian oceans associated with mangrove forests (Keenan et al. 1998). In Pichavaram mangroves, it is available throughout the season and pre-dominant in premonsoon season.

#### 3.3 Sequence features

Ninety five sequences (collected from NCBI) belonging to four species of genus *Scylla* disseminated around the world (Table 1). The GenBank submitted sequence (Accession No. JN688965) of the mitochondrial gene Cytochrome oxidase subunit I (Barcode gene) showed 99% similarity to existing sequence of *Scylla olivacea* in GenBank through blast search.

#### 3.4 Phylogram construction

Ninety five sequences (collected from NCBI) belonging to four species of genus *Scylla* disseminated around the world (Table 1)

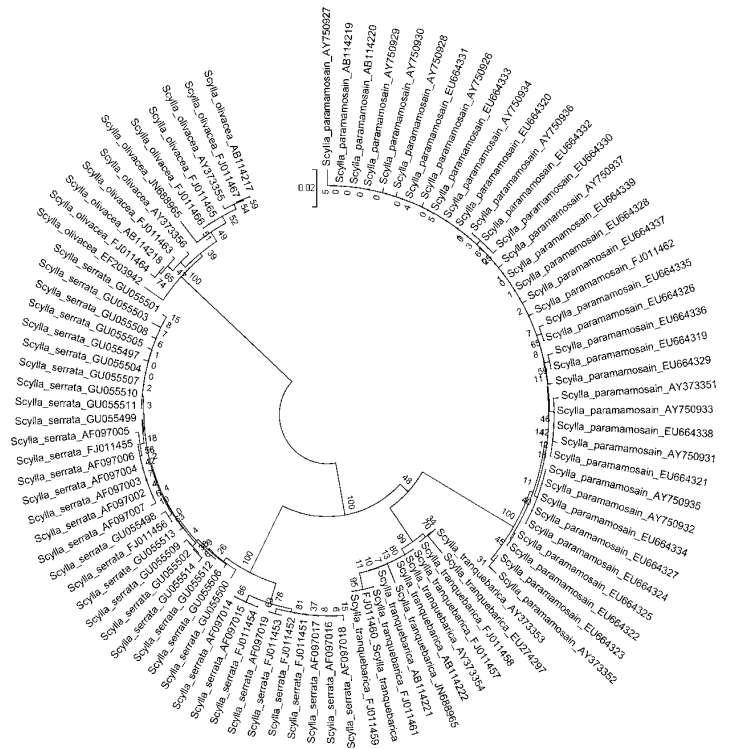


Figure 2. Kimura 2-parameter distance Neighbour-Joining (NJ) tree of 96 sequences from four species belonging to Genus *Scylla*

with the barcode data generated from this work was considered for phylogram construction. Three phylogenetic trees were constructed to verify the distinction of four species and their evolutionary relationship. *Thalamita* spp. was used as an out group which was collected from NCBI and this has been clearly distinguished as an out group in the phylogenetic tree. The constructed phylogram (Fig. 2) proved the congeneric relatedness existing among the four species.

#### 3.5 Genetic distance

The overall genetic distance between the four species of genus *Scylla* calculated using pair-wise distance analysis via Kimura 2 parameter. The overall genetic distance between species of genus *Scylla* in 1-3<sup>rd</sup> and noncoding codon position was 0.093.

Mud crab *Scylla olivacea* (Herbst, 1796) is an Indo-West Pacific species, the type locality was East Indies which was then extended from the east coast of India through to the Indo-Malaysian region. It was first recorded as *Cancer olivaceous* by Herbst in 1796. Identification of *Scylla* species have led to much confusion because of the subtle morphological differences between the species. Several authors used many morphological characters for distinguishing between species (Estampador,

**Table 1.** Sequence of various species of genus *Scylla* from different locations used to construct the phylogram in the present study

Species	Sample location	GenBank Accession No.
<i>Scylla serrata</i>	East-Indies triangle	FJ011456, FJ011455, FJ011454, FJ011453, FJ011452, FJ011451
<i>Scylla serrata</i>	Moreton Bay, eastern Australia	AF097007, AF097006, AF097005, AF097004, AF097003, AF097002, AF097019, AF097018, AF097017, AF097016, AF097015, AF097014
<i>Scylla serrata</i>	Indian Ocean: Western	GU055514, GU055513, GU055512, GU055511, GU055510, GU055509, GU055508, GU055507, GU055506, GU055505, GU055504, GU055503, GU055502, GU055501, GU055500, GU055499, GU055498, GU055497
<i>Scylla tranquebarica</i>	India	JN688965
<i>Scylla tranquebarica</i>	East-Indies triangle	FJ011461, FJ011460, FJ011459, FJ011458, FJ011457
<i>Scylla tranquebarica</i>	Bali, Indonesia	AY373354, AY373353
<i>Scylla tranquebarica</i>	Iloilo, Tigbauan, Philippines	AB114222, AB114221
<i>Scylla tranquebarica</i>	China	EU274297
<i>Scylla olivacea</i>	India	JN688965 (Present study)
<i>Scylla olivacea</i>	East-Indies triangle	FJ011467, FJ011466, FJ011465, FJ011464, FJ011463
<i>Scylla olivacea</i>	Australia: western Australia	AY373356, AY373355
<i>Scylla olivacea</i>	Okinawa, Kabira Bay, Japan	AB114218, AB114217
<i>Scylla olivacea</i>	China	EF203942
<i>Scylla paramamosain</i>	East-Indies triangle	FJ011462
<i>Scylla paramamosain</i>	China	AY750937, AY750936, AY750935, AY750934, AY750933, AY750932, AY750931, AY750930, AY750929, AY750928, AY750927, AY750926, EU664339, EU664338, EU664337, EU664336, EU664335, EU664334, EU664333, EU664332, EU664331, EU664330, EU664329, EU664328, EU664327, EU664326, EU664325, EU664324, EU664323, EU664322, EU664321, EU664320, EU664319
<i>Scylla paramamosain</i>	Australia	AY373352, AY373351
<i>Scylla paramamosain</i>	Okinawa, Nakagusuku Bay, Japan	AB114220, AB114219
<i>Scylla paramamosain</i>	Okinawa, Nakagusuku Bay, Japan	

1949; Serene, 1952; Chayarat & Kaewridh, 1978; Joel and Raj, 1980 and Kathirvel and Srinivasagam, 1992). However,

Keenan's (1998) detailed studies on revision of genus *Scylla* reduce much of the confusion in species identification in genus *Scylla*.

In India, still peoples believed that only two species available namely *Scylla serrata* and *Scylla tranquebarica*. They believed that former is smaller species and the latter is larger species. But, based on Keenan's classification we first report the confirmation on the availability of *Scylla olivacea* (as *Scylla serrata* by Joel and Raj) in Indian waters based on morphology and molecular level.

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