

## Morphometric and phylogenetic analysis of Portunid crabs, *Portunus reticulatus* (Herbst, 1799) and *Portunus pelagicus* (Linnaeus, 1758) from the west coast of India

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Present article consists the studies of two species from the Konkan coastal region of Maharashtra for comparative analysis using different morphometric ratios as well as phylogenetic analysis with *Cytochrome C Oxidase subunit I (COI)* gene. Morphometric ratios were found to be significantly different between *P. reticulatus* and *P. pelagicus*. Phylogenetic analysis differentiated *P. reticulatus* from *P. pelagicus* producing separate clades in the neighbor joining tree. The study was helpful in corroborating the occurrence of *Portunus reticulatus* (Herbst, 1799) from the west coast of India.

[**Key words:** *Portunus reticulatus*, *Portunus pelagicus*, COI, Morphometric ratio]

### Introduction

Decapoda, estimated to contain nearly 15,000 species covering 2,700 genera<sup>1</sup>. *Portunus* is very important genus of the family Portunidae having two commercially important crabs, viz. *Portunus pelagicus* and *P. sanguinolentus* from the west coast of India<sup>2</sup>. Recently a crab from this genus namely *Portunus reticulatus* was reported from Konkan region of the Maharashtra state of India<sup>3</sup>. As per the available information and literature, this crab had never been recorded from the west coast of India and has distribution from the East Indian Ocean; east of the Indian subcontinent, Sri Lanka and the Bay of Bengal<sup>4</sup>. The availability of this crab on the west coast of India would certainly confer an impact on the biodiversity database of this region as well as nation. This would also influence the policy making strategies and conservation aspects of crabs as a biodiversity database has a significant contribution in research on biodiversity and environment assessment activities.

Classical taxonomy is important and requires additional support from molecular taxonomy when identification has to be made for species having less morphological differences and of specimens that do not conform to the referential standards upon which morphological taxonomy is based<sup>5</sup>. But considering

the fewer morphological differences between *P. reticulatus* and *P. pelagicus*, we applied comparative analysis of the morphometric ratios and the DNA sequences of *Cytochrome C Oxidase I (COI)* gene for validating the difference between these two species as this gene have demonstrated its usefulness in discriminating between closely related species across diverse animal phyla<sup>6, 7, 8</sup>. PCR-direct DNA sequencing is a reliable technique for barcoding species and various researchers have prosperously utilized this technique for such a purpose<sup>9, 10, 11, 12, 13, 14, 15, 16</sup>. One of the classic examples of COI barcoding is confirmation of two species of Asian sea bass carried out by Ward *et al.*<sup>17</sup> who examined a 650 base pair region of the *COI* gene of *Lates calcarifer* from Australia and Myanmar suggesting both as different.

Comparative analysis with all the available sequences on NCBI database was not carried out in earlier work due to different objectives of that study. Present study is involving all the sequences under *Portunus* genus that were available in the database. Present study also tried to compare the species on the basis of the external characters. Thus our study was aimed towards carrying comparative analysis of the morphometric ratios and phylogeny with other *Portunus spp.* sequences to corroborate the occurrence of *P. reticulatus* from the west coast of India.

## Materials and Methods

The portunid crabs *Portunus reticulatus* and *Portunus pelagicus* were obtained from the fishermen of Uran Taluka (18.89° N, 72.95° E), located in the Raigad district of Maharashtra State, India (Figure 1). They were caught in shore seine along with other crabs. In total, four specimens of each species were found during the collection. All crabs were carefully stored in absolute alcohol in plastic bottles and transported back to the laboratory for taxonomic identification.

*Portunus reticulatus* and *P. pelagicus* were identified based on phenotypic criteria such as morphology, colour and appendages. Ten morphometric measurements<sup>2, 4</sup> with seven ratios were used for comparison between *P. pelagicus* and *P. reticulatus*.

Sequences for the genus *Portunus* were obtained from NCBI GenBank and used for phylogenetic analysis. Nucleotide composition, evolutionary divergence and a neighbor joining tree between all sequences were derived using MEGA V 5.2<sup>18</sup>. To verify the robustness of the internal nodes of NJ tree, bootstrap analysis has been carried out using 1000 pseudo replications<sup>19</sup>. Evolutionary distances carried out by the Maximum Composite Likelihood method<sup>20</sup> and are in the units of the number of base substitutions per site.

## Results

External morphology of *P. reticulatus* (♀) is shown in Figure 2. Morphological examination of different

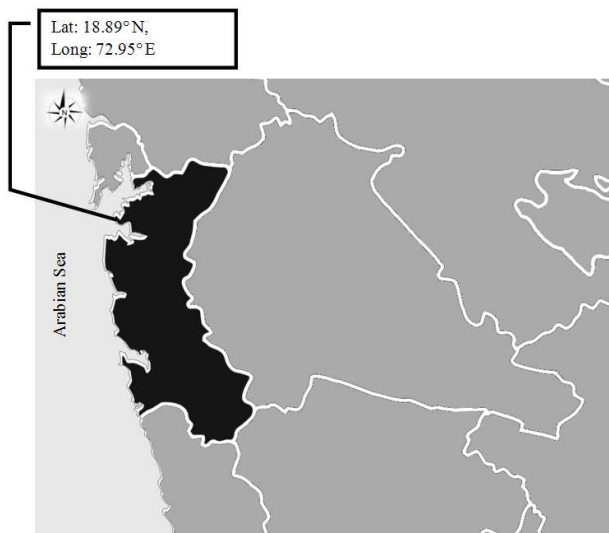


Fig. 1 — Location of *Portunus reticulatus* crab collection in Raigad district (dark shade) of Maharashtra state of India. *Portunus pelagicus* (B) from the west coast of India

crab samples demonstrated two different species viz. *P. pelagicus* and *P. reticulatus*. Aside from previous reported characters we found that *P. reticulatus* has densely crowded fine granules on carapace than *P. pelagicus* (Table 1). The collected samples have both male and female of *P. reticulatus*, but contain only male of *P. pelagicus*. Therefore, our study does not have morphometric data of female *P. pelagicus*. Males were easily differentiated from females due to purple-blue long chelipeds. Variation in ratios derived from the morphometric measurements between portunid crabs are given in Figure 3. These results showed a significant disparity ( $P < 0.05$ ) between ratios except PL and PW. The divergences in the types and ratios derived from the two *Portunus* crabs are observed in the study.

Present study almost covered all the sequences from NCBI GenBank except some, which were pseudogenes (AM410508-AM410510, AM410512 and AM410517) and complete genome sequence (AB093006). We have also omitted sequences, which were devoid of the species names (KC706766, GQ272555). After this we narrowed down the number

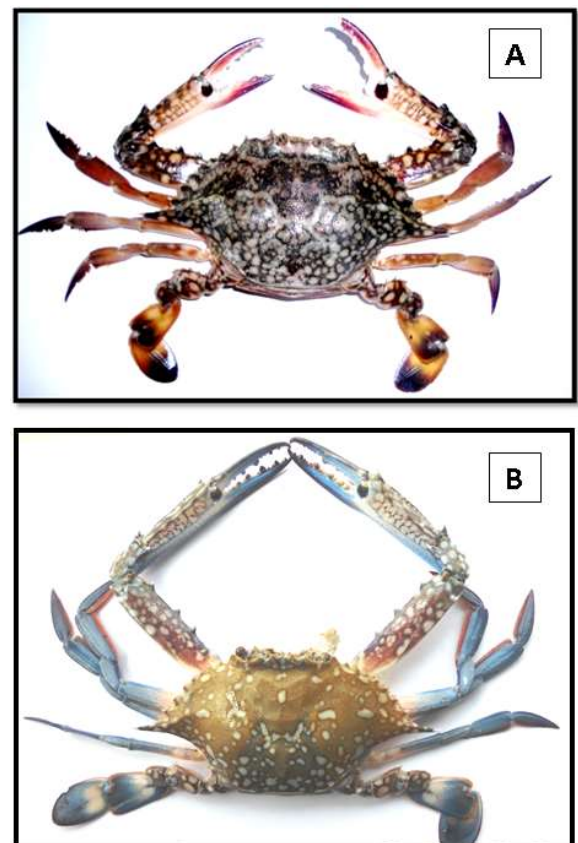


Fig. 2 — Adult *Portunus reticulatus* (A) and *Portunus pelagicus* (B) from the west coast of India

Table 1 — key diagnostic character of *P. pelagicus* and *P. reticulatus* (Lai *et al.* 2010 with addition to observations\*)

Character	<i>P. pelagicus</i>	<i>P. reticulatus</i>
Branchial region of carapace	Prominently swollen	Not swollen
Carapace granulation*	Fine less crowded granules	Fine crowded granules
Cheliped meri	Relatively long and slender	Relatively shorter and stouter
Dactylus of P5	Ovate and relatively elongate	Relatively shorter and more rounded
Carapace colour	Blue-green with spread white spots and purple-blue chelipeds in males. Females with brownish green carapace with red tipped chelipeds	Blue-green with dense white spots with purple-blue chelipeds in males, Brownish-green in females with dense white spots with red tipped chelipeds

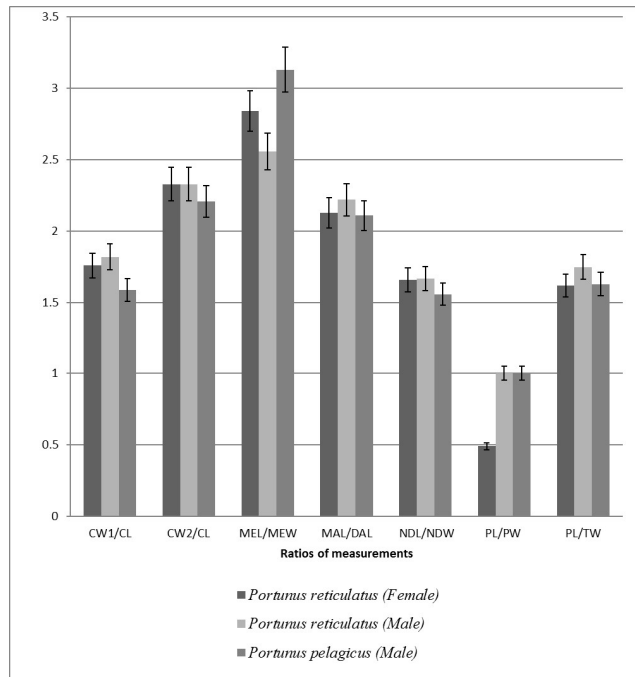


Fig. 3 — The morphometric ratios of *Portunus pelagicus* (♂, N=4) and *Portunus reticulatus* (♂ N=2, ♀=2) from the west coast of India. Measurements: Carapace length (CL), Carapace width (excluding 9<sup>th</sup> anterior-lateral tooth) (CW1), Carapace width (including 9<sup>th</sup> anterior-lateral tooth) (CW2), Major cheliped merus length (MEL), Major cheliped merus width (MEW), Major cheliped manus length (MAL), Major cheliped dactylus length (DAL), Natatory leg dactylus length (NDL), Natatory leg dactylus width (NDW), Penultimate segment length (PL), Penultimate segment width (PW), Telson width (TW) (Lai *et al.* 2010)

of some sequences, which were repetitive and belonged to same author. Accordingly, we selected total 34 sequences out of 162 sequences for the genus *Portunus* reported on NCBI GenBank. Thus, the sequences with mean sequence length of 534 bp per taxon were examined. The number of variable characters was highest (369) for COI gene having 356 numbers of parsimony informative characters. The evolutionary divergence between all the *Portunus* COI sequences is given in Table 2. The divergence was significant between all the *Portunus* species and

ranged from 0.0 to 1.18. We have found significant divergence between two close resembling species i.e. *P. pelagicus* and *P. reticulatus*. This value was  $0.02 \pm 0.01$  between these two species. The within species divergence for *Portunus reticulatus* was zero. The GC content was highest in *P. pelagicus* (36.79) as compared to *P. reticulatus* (36.57). The GC content demonstrated inclining trend from a first codon position (31.51) towards second (38.25) and third codon (40.02) positions in *P. reticulatus*. Comparatively different trend was found in *Portunus pelagicus* at first (29.93), second (40.95) and third (39.49) codon position.

The name of the species with their NCBI GenBank accession numbers are given in neighbor-joining (NJ) tree. The NJ tree supported by high (1000) bootstrap values clustered all *Portunus* species separately from each other except some sequences, which were deposited by Lai *et al.* <sup>4</sup> (Figure 4). Their sequences showed significant divergence than all other *Portunus* sequences from NCBI forming separate clade in the NJ tree. Apart from these sequences, all other sequences showed species specific clades in NJ tree.

## Discussion

Incorrect species identification using classical taxonomy supported with molecular tool leads to wrong molecular database creation. This sort of sequence submissions always misled researchers and create confusion. The concept of DNA barcoding is moving fast on its application for taxonomy and systematic studies. Meyer and Paulay <sup>21</sup> stated the success of DNA barcoding as an effective tool in species recognition, provided based on firm taxonomic base. In this study, DNA barcoding has played an important role with classical taxonomy to correctly comparing species. The difference in the GC content between *P. pelagicus* and *P. reticulatus* differed significantly, which divulged differences in the degree of selective constraint <sup>22</sup>. We have stated



The portunid crabs are valuable for fisheries resources of India as they are edible with good export potential. The exact identification of the species is important for biodiversity as well as food safety. The chances of improper seafood labelling are more if species identification is wrong. This may lead towards the chances of increasing seafood fraud<sup>23</sup>. As the number of crab species and diversity is more, the accurate species identification records are necessary for this group of crustaceans.

The 100% improper labeling of crabs was found in restaurants along the west coast of India which suggested the need of proper identification, nomenclature and record of crustaceans<sup>3</sup>. The occurrence of this species on the west coast of India would not only add the knowledge into the fisheries database but also help in the species diversity management. There are two possibilities of the occurrences of this crab on the west coast of India. The first possibility is the prevalent occurrence of this species on the west coast, but always mistaken with the *P. pelagicus*. Similar information has been found in the study of the first occurrence of green crab *Scylla tranquebarica* from the west coast of India<sup>24</sup> which demonstrated that green crab was prevalent on the west coast, but mistaken with its close relative species mud crab, *Scylla serrata*<sup>25</sup>. But this kind of oversight in species identification creates lots of confusion in the crustacean fishery database and research. Hence the proper taxonomic identification and inventorisation of resources is an important aspect in fisheries resource management. The other possibility is migration of the species from the East Indian Ocean. This migration might be from the Southward side of India. It is necessary to study the phylogenetic relationship of *P. reticulatus* crabs from East and the west coast of India with markers like 16S and 12S etc. Such study will give accurate information about the migration and relationship of crabs between two coasts of India. This will likewise affirm the above mentioned facts on the source of the occurrence of this crab.

### Conclusion

The findings of the present study based on the morphological as well as molecular studies confirmed the occurrence of *P. reticulatus* from west coast of India.

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