Molecular Confirmation and Evolutionary Divergence Between Two Species of the Genus *Petrolisthes* Porcellanid Crabs from the Coastal Waters of Pakistan

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INTRODUCTION

Porcelain crabs are the decapod crustaceans and representative of the family Porcellanidae. These crabs have compressed bodies as an adaptation for living in rock holes and underneath the stones. The Genus *Petrolisthes* of Porcelain crabs is common and diverse constituent of the intertidal zone and near shore shallow benthic habitat and has the capability to tolerate higher and lower temperatures throughout low tide (Naz *et al*., 2019). They have elusive body and rapidly break down their limbs when attacked and feeling threat.

Mustaquim (1972), Ahmed and Mustaquim (1974), Kazmi and Siddiqui (2006) has carried out valuable work on the distribution and diversity of Porcellanids from Northern Arabian Sea. Beleem *et al*. (2016) pronounced the five species of porcelain crabs from Gujarat. In present study, the molecular identification of the two species of genus Petrolisthes: *Petrolisthes ornatus* and *P. boscii* have been done based on the mt-DNA Cytochrome oxidase I gene this DNA barcode gene utilized for species identification.

OBJECTIVES

The molecular verification (based on mt DNA COI gene) of the *Petrolisthes ornatus* and *P. boscii* from the coast of Pakistan and estimation of the Genetic divergence and the evolutionary relationship of *Petrolisthes ornatus* and *P. boscii* were the aims of current study.

MATERIALS AND METHODS

Crab samples were collected carefully by hand picking from Manora Channel and Buleji rocky ledge Karachi Pakistan in December 2019. The samples were immediately stored at -20°C for morphological identification through existing taxonomic Keys and later for molecular analyses. The genomic DNA (gDNA) extraction was used for molecular identification of muscles tissues; the process has carried out by Qiagen's DNeasy blood and tissue kit. A 588-base pair (bp) of the target DNA segments were procured by the (PCR) polymerase chain reaction using two universal primers of the COI gene; LCO1490 and HC02198 (Folmer *et al*. 1994). A recent procured set of DNA sequence data were submitted to the Gen Bank later examined for confirmation of species-based on blast (99 % homology). According to Tamura-Nei model (Tamura and Nei 1993) the maximum likelihood method was used to estimate the genetic evolutionary relationship in which all the DNA sequences were primarily aligned using Clustal W through MEGA 7 (Kumar *et al*. 2016).

RESULTS

In the present study the molecular identification of *P. ornatus* and *P. boscii* based on the coding gene of mt DNA Cytochrome Oxidase 1 (COI), Sequence comparison searched by using the Basic Local Alignment Search Tool (BLAST), species confirmed on at least a 99-100% homology. The procured DNA sequences

submitted to GenBank and each isolate get accession number. A total of 5 sequences was used for the phylogenetic analysis and constructed a haplotype network to visualize the relationships between all 5 haplotypes and their frequencies. The average evolutionary divergence over sequence pairs within *P. boscii* was estimated and revealed that the 0.037 divergences was observed. The evaluated sequence diversity between *P. ornatus* and *P. boscii* was observed by the mitochondrial cytochrome oxidase I (COI) gene and the pairwise divergence was 30% in between the two studied species, whereas the evolutionary divergence was 2.48±0.021 within all representative sequences of *P. ornatus* and *P. boscii*.

CONCLUSION

The DNA sequence diversity of the mitochondrial cytochrome oxidase I (COI) gene between *P. ornatus* and *P. boscii* was observed and the network analysis the results revealed that haplotypes differed from each other by a moderate number of mutations.

KEYWORDS

Porcellanid crabs, mt-DNA, Cytochrome oxidase, Evolutionary Divergence, Pakistan.

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