First Barcode of *Ryphila cancellus* (Crustacea: Decapoda: Leucosiidea) from the Coast of Pakistan

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INTRODUCTION

The Pebble crabs or nut crabs belong to Leucosiid crabs, are the common inhabitant of sub-littoral coastal areas. The crabs of genus *Ryphilia* mainly, *Ryphila cancellus* (Herbst, 1783) are common and have worldwide distribution along the intertidal areas of Indo-pacific region, including Pakistan (Alcock 1896; Tirmizi and Kazmi, 1988; Beleem *et al*., 2016). Due to morphological similarities with other species of the same family, the identification based on the taxonomical assessment is quite challenging due to overlapping. The present study was conducted to investigate the morphological characteristics of Pebble crabs of genus *Ryphila*, in order to clarify the uncertainties in the taxonomic status and amplification of mtDNA to clarify the phylogenetic status. Cytochrome oxidase I (COI) gene will be utilized for phylogenetic analysis, which is a DNA barcoding gene that utilized a short stretch of mitochondrial DNA a highly effective and popular mean to differentiate the biologically diversified species (Folmer *et al*., 1994).

OBJECTIVES

The detailed morphological examination and DNA barcode study has been performed for *Ryphila cancellus* by using Cytochrome oxidase I (COI), a barcoding gene in the present study.

METHODS

In 2016, random sampling of *Ryphila cancellus* was done during the low tide time along the sandy beach of Sandspit. For closer examination, specimens were preserved in 70% alcohol and smaller parts (chela, cheliped merus, 2ndmaxilliped, and G1 of male crab) were dissected and were identified up to species level following Galil (2009). Genomic DNA was extracted from visceral and muscular tissues of animal using the QAIGEN DNA Kit. In order to confirm the amplicon, amplified products were run in 1% agarose gel. Positive amplified products were sent to Macrogen company (Korea) for sequencing. In addition, sequences of *Lyphira perplexa* (KX757764.1), *Ryphila cancellus* (present study), *Philyra pisum* (HM180779.1), *Ebalia cariosa* (KR818220.1), *Iliacanthalio dactylus* (MW412235.1), *Persephonalichten steinii* (JX102082.1), *Persephona crinite* (JX102076.1) were downloaded from GenBank. The model for DNA substitution was selected by AIC criteria Parameter values using Mega 7 software (Kumar *et al*. 2015). Phylogenetic analysis was conducted by applying maximum likelihood (ML) method.

RESULTS

The obtained DNA sequences were deposited in the Genbank after species confirmation through BLAST tool and then aligned CO1 sequences through clustal W including sequences of other crabs retrieved from Genbank. Six CO1 sequences as mentioned above were incorporated for constructing the Maximum likelihood tree. During the current study, a total of 22 specimens were collected, taxonomic characters showed that specimen

in hand belongs to *Ryphila cancellus*. From a single specimen, a 600 bp fragment was amplified and examined in comparison with other species. The overall mean genetic distance obtained between different species of family leucosiidae ranged between 0.18-0.35. Genus *Ryphila* showed highest genetic distance with *Persephona crinite* and *Lyphira perplexa* while the lowest with *Philyra*.

CONCLUSION

This is the first ever barcode study of this Leucosiid species from Pakistan, the ongoing molecular phylogenetic studies constitute a significant contribution to the global barcode reference sequence library for *Ryphila* crabs.

KEYWORDS

Identification, DNA barcoding, Pebble crab, *Ryphilacancellus*, Sublittoral.

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