Progress in Aqua Farming and Marine Biology
ISSN: 2640-6764

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Research Article *Volume 4 Issue 1*

First Record of Red Frog Crab Ranina ranina (Linnaeus, 1758) From Puducherry Coastal Waters with DNA Barcoding as First Report in India

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Received Date: June 04, 2024; **Published Date:** August 06, 2024

Abstract

The present study reported the first record of the rare species of brachyuran spanner crab *Ranina ranina* (Linnaeus, 1758) from Puducherry coastal waters situated on the east coast of India. A single specimen of *Ranina ranina* measuring about140 mm in carapace length was caught by a bottom set gill net operated in Nallavadu landing centre, Puducherry, on 19th November 2019. Crab was morphologically identified at species level through the description of colours, dentations of the carapace, and shapes of cheliped and pereiopods. Molecular identification was carried out by the partial sequencing of the barcode region of the mitochondrial cytochrome oxidase subunit I (COI) gene, which is known to be hypervariable among different crab species. This is the first record of the Red frog crab or spanner crab *Ranina ranina* from Puducherry coastal waters and reported with DNA barcoding for the first time in India. Earlier it was reported from the Gulf of Mannar, South East coast of India and Vizhinjam, Thiruvananthapuram, Kerala located along the south-west coast of India.

Keywords: *Ranina ranina*; Red Frog Crab; Spanner Crab; First Report; DNA Barcoding; Puducherry

Abbreviations

COI: Cytochrome Oxidase Subunit I.

Introduction

Ranina ranina Linnaeus [1], is one of the outsized striking oceanic brachyurans, which comes under order Decapoda, family Raninidae. The red frog crab is the single representative of the genus Ranina under family Raninidae. It is commonly called red crab, frog crab, well known as "spanner crab" in Australia, "krab giraffe" in Seychelles [2-4], "Kona crab" in Hawaii [5-15], and "curacha" in the south western Philippines and it is labelled vernacularly as "Eli Poochi Nandu" in Tamil. It almost looks like a stemware with bright orange shells. It derived the name as spanner crab as their front claws look like a spanner. The species is widespread all over the Indo- Pacific [16], Mauritius, Reunion, East Africa, East Indies to China [15] from the coast of South Africa to Hawaii and the Great Barrier Reef. They are more favour to dwell in bare sandy areas of tidal pools and coastal waters of more than 100m depth, in areas subject to strong currents and adjacent to coral reefs [9,13]. It feeds on small fishes, crabs, shrimps, bivalves, rays, hydroid, copepods, and squids [1]. Spanner crab is infrequent to Indian waters and it has been recorded for the first time in Puducherry coastal waters and for the third time in India. It appeared sporadically in India and it was previously reported in the Gulf of Mannar

[8], Tamil Nadu, and in Vizhinjam [12], Kerala coast. At every turn, it came to light as by catch. Since the previous records demonstrating morphometric based identification, the present study kingpin on the molecular identification of spanner crab and it is done for the first time in India, and the sequence got submitted in the NCBI. We aimed to generate a DNA barcode of the mitochondrial COI marker gene of the crab specimen to authenticate the species identity, which reciprocally enlightens and supports morphology-based identifications [17].

Materials and Methods

A single male spanner crab, *Ranina ranina*, Linnaeus, [1] was collected at Nallavadu landing centre, Puducherry, on 19th November 2019 as a bycatch (Figure 1). It was caught in the bottom set gill net operated at a depth of 30m, 20 km away from the Puducherry coast. The collected specimen was immediately brought in to the laboratory for further identification and observation. Identification up to species level was carried out by a meticulous examination of the morphometric characters using the online database World Register of Marine Species and Wikipedia. The second pereiopod (walking leg) was removed, sliced, and preserved in 95% ethanol and stored at 4°C for DNA barcode study.

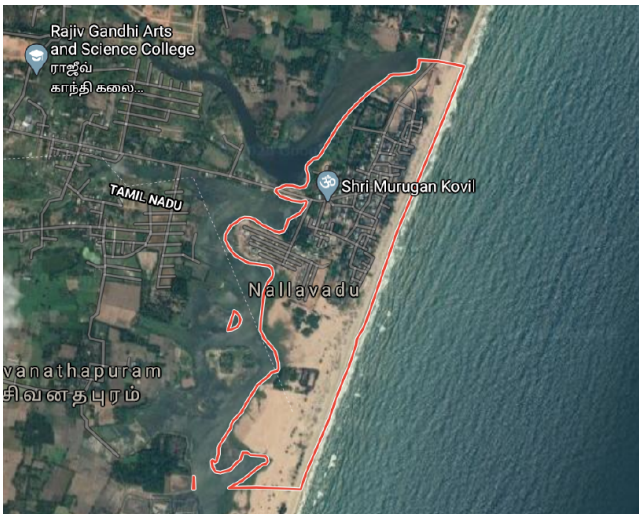

Figure 1: Map shows the Nallavadu Landing Centre, Puducherry.

DNA isolation

DNA was isolated from the stored tissues by the CAGL protocol. Tissue was placed in alcohol and washed four times in tris buffer (pH 8). 25mg of tissue was cut into very small pieces and placed in a 1.5ml Eppendorf tube. 500µl of solution I (20mM EDTA, 50mM tris HCl, 2% SDS) was added to the Eppendorf tube with the tissue sample. The tissue sample was homogenized with sterile homogenizer. 5µl of proteinase K was added to homogenized tissue and mixed thoroughly by a vortex. After the addition of proteinase K, the tissue sample was incubated at 56°C in a water bath for two hours until the tissue was completely lysed. 250µl of solution II (6M saturated NaCl) was added to the sample and mixed thoroughly by a vortex. After vortexing, the sample was chilled on ice for 5minutes. The tissue sample was spun at 8000rpm for 15 minutes. After centrifugation, 500µl of clear supernatant was collected and transferred into a newly labeled 1.5ml of Eppendorf tube. 200µl of 100% ethanol was added to precipitate the DNA and mixed again thoroughly by a vortex. The sample was spun at 1000rpm for 15minutes and supernatant was removed by pipette. 500µl of ice-cold 70% ethanol was added to the vial with a DNA pellet. It was spun at 1100rpm for 5minutes. After centrifugation, the DNA pellet was collected from Eppendorf tube and air-dried. Finally, the pellets were suspended in 20-50µl of Milliq water.

Amplification and Sequencing

The mitochondrial Cytochrome Oxidase Subunit I (COI) gene was amplified at Rajiv Gandhi Centre for Aquaculture (RGCA), a division of MPEDA, Srikali, Tamil Nadu, India. The following COI amplification primer suggested by Herbert, et al. [6] were used,

(Forward) 5'GGTCAACAAATCATAAAGATATTGG3' and Universal COI H

(Reverse) 5'TAAACTTCAGGGTGACCAAAAAATCA3'.

The tubes were loaded into the PCR machine and selected an appropriate program for the region being amplified. PCR products were checked by running on an agarose gel. The amplified products in gel via UV transilluminator with safety shield. The concentration of the amplified PCR product was noted. Amplified PCR products were visualized on 1.5% agarose gels, and the most intense products were chosen for sequencing. The cleaned PCR product was sequenced by sequencing kit-Big Dye® Terminator 3.1 sequence kit (Applied Biosystems, Foster City, California, USA).

Sequence Analysis

The COI partial gene sequence of *Ranina ranina* was unambiguously edited using the Bioedit sequence editor. The edited sequence was aligned by CLUSTAL- W and imported to Bioedit for inspection and toggle translation using MEGA software version 7.

Results and Discussion

Material observed: Paratype, ZSI/MBRC- D1-621, Male, 19 November 2019, Nallavadu, Puducherry, 11°51'32":79°48'56" NW 3543, Nithya Mary Gunalan (Figure 1). Morphometric measurements of *Ranina ranina* is given in Table 1. It measured about 140cm length, 135mm width

carapace, and weighed nearly 585g.

External Traits	Measurements(mm)
Carapace Length	140
Carapace Width	133
Abdomen Length	125
Abdomen width	125
Chelate leg length	
(Right and Left side)	235
Chelate leg spines (Right Side)	
Anterior Portion	10
Posterior Portion	5
Chelate leg spines (Left side)	
Anterior Portion	14
Posterior portion	8
Abdominal segments	6
Carapace spines	21
White spots on the anterior side of carapace	10
Weight	585g
Sex	Male
Colour of the Specimen	Reddish orange

Table 1: Morphometric measurements of *Ranina ranina* from Puducherry coastal waters.

Systematics
Phylum: Arthropoda Von Siebold, 1845
Subphylum: Crustacea Brünnich, 1772
Class: Malacostraca Latreille, 1802
Order: Decapoda Latreille, 1802
Super family: Raninoidea De Haan, 1839
Family: Raninidae De Haan, 1839
Genus: Ranina Lamarck, 1801
Species: *Ranina ranina* (Linnaeus, 1758).

General Description

Shape: *R. ranina* is easily recognizable by their frog-like appearance, has a wide, elongate, oval-shaped carapace. The widest part of carapace is measured in the portion where the pattern of white spots presents at the dorsal side of the carapace. The latero-posterior region is much narrower than its anterior resulting in a shorter posterior boundary giving the carapace an ovate shape (Figures 2a and 2b).

Colour: Unlike any other brachyurans, the uniqueness of *R. ranina* is characterized by bright red-orange colour. The wider part of the carapace at the anterio-dorsal side is reddish-brown, with ten white spots [14].

Figure 2a: Dorsal view.

Figure 2b: Ventral view.

Morphology

The Carapace: An irregular pattern of 12 patchy white spots line across the upper third part of the dorsal carapace with two inferior but prominent parallel white spots centrally located. White spots are more noticeable in large size individuals. The dorsal carapace looks serrated due to the presence of spine-like structures called tubercles. The ventral portion is smooth with setal fields extending thinly along the lateral sides and grows wider towards the posterior aspect.

The orbito-frontal region: The entire frontal and lateral margins of the crab are completely fringed with short setae [7]. The orbito-frontal margin of the crab is characterized by a razor-sharp triangular rostrum circumscribed by symmetrical lateral rostral teeth. In males, the fronto-lateral lobe with a broad proximal base is distinctly longer than the central rostral spine; while the frontal region is shorter with 2 narrower spines directed forward creating a concave anteriorly. This gives the male anterior aspect a squarish

shape with a deep medial groove. The maxillae of *R. ranina* are small and thin. Maxillipeds are modified appendages that serve as feeding accessory organs. The buccal cavity is elongate and completely closed by third maxillipeds [3].

The Pereiopods: The pereiopods are the 5 pairs of walking legs in crabs. The cheliped or the first walking legs are spade-shaped which are laterally flattened with huge size chelae. The dactyl of the cheliped is armed with 7 spines on the outer margin. Anterior to the sharp spines are 5 blunt and very short spines. The dactyl has rounded teeth with a pointed tip that curve downward. The outer and inner margins on the ventral side are lined with very thick short setae. The cheliped is used not only for movement but for food procurement too. The 2nd and 3rd pereiopod commonly referred to as digging legs [11]. Their dactyls are oblate, concave, curved inwardly on the ventral surface with pointed tips specifically act as a digging tool. Fourth Pereiopod has a more extended leg than 2nd and3rd pereiopods. The dactyls take the form of a paddle with a sharp tip which helps to shovel sand forwardly [4]. The fifth pereiopod is highly altered for swimming, although, it also supports the process of digging.

The Abdomen: Abdominal segments are narrow, triangular, and are discernible from the dorsal view. The sexually dimorphic abdomen of *R. ranina* is composed of 6 inflexible segments called pleomeres and the small ovate shaped terminal segment telson which contains the anus. The dorso-lateral region of the abdomen is marked by the presence of setae.

DNA Barcoding

The COI gene was successfully amplified by the primers. After the final alignment about 548 bp were sequenced and submitted to NCBI Genbank and provided the ACC. No. MT 238120. The sequence obtained from the sample was then compared and identified to the species level using sequence databases which showed 99% similarity with *Ranina ranina*.

>mtCOI *Ranina ranina*

CTTATTATTCGAGCAGAGCTTAGCCAACCTGGCACCCTA-ATTGGCAATGACCARATTTATAATGTAGTTGTTACTGCTCAT-GCCTTTGTTATAATTTTCTTTATAGTTATACCCATTATAATTG-GAGGCTTTGGTAACTGACTAGTTCCCCTTATACTTGGAGC-CCCTGATATAGCTTTTCCTCGTATAAACAACATAAGATTTT-GACTTCTTCCTCCTTCCCTAACTCTTCTTCTTATAAGTGG-TATAGTTGAAAGAGGTGTAGGTACCGGCTGAACTGTGTATC-CACCTTTAGCTGCCGCAATTGCCCACGCTGGTGCCTCAGTT-GACCTAGGAATTTTCTCGCTTCATCTAGCAGGTGTCTCCT-CAATTTTAGGTGCCGTAAACTTTATAACCACAGTTATTA-ATATACGCTCTTATGGTATAAGTATAGATCAAATACCCTTATTT-GTATGAGCAGTTTTTATCACTGCTATTTTACTTCTTTTATC-CCTTCCTGTTCTAGCCGGAGCTATTACTATATTGTTAACTGATC-GCAACTTGAATAC

Conclusion

R.ranina is a commercially important, highly priced species in many Asian countries like Japan, Taiwan, Indonesia, Honkong, and topmost crustacean fishery in Australia. *R. ranina* now evolved as a potential species in aquaculture in some parts of the world. But in India, it appeared on and off, mainly caught accidentally in the nets as bycatch and there is almost absence of culturing. Most of the fishermen don't know the real commercial value of spanner crab. Especially in Puducherry, fishermen were just attracted to the colour and unique shape of the red frog crab; still, they are unsure about its edibleness.

Acknowledgement

We thank the Director of Kanchi Mamunivar Government Institute for Postgraduate Studies and Research, Puducherry for permitting us to carry out this work. We also thank our PG and Research Department of Zoology for their intellectual support and encouragement. A special thanks to the fishermen of Puducherry coastal villages who helped us to collect the specimens.

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