

Research article

First molecular identification of coral fish, *Cymolutes praetextatus* (Quoy & Gaimard, 1834) in India and first report from Pondicherry coastal waters, South east coast of India

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Abstract: The present study reported the first distributional record of the labrid fish species *Cymolutes praetextatus* with DNA barcoding for the first time in India from Puducherry coastal waters situated on the east coast of India. *Cymolutes praetextatus* was collected at Pillaichavadi landing centre, Puducherry. Fish was morphologically identified at species level through the morphometric characters and description of colours. 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 fish species.

Keywords: *Cymolutes praetextatus*, coral fish, first occurrence, molecular identification, Puducherry

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Introduction

Family Labridae is one of the largest, colourful and remarkably diverse families of reef fishes constitute about more than 60 genera and 500 species worldwide (Parenti Paolo & Randall, J.E, 2011). *Cymolutes praetextatus* falls under the Xyrichtyinae, one of the subfamilies of Labridae, contains five genera which have the habit of burying themselves quickly in sand (Randall and Earle 2004). Many species exhibit incredible changes in colour pattern with age and sexual maturity. The labrid species, *Cymolutes praetextatus* is a ray-finned fish popularly known as knife razor fish or knife wrasse, the wrasses. It is a solitary fish that is commonly found over reef flats and in shallow lagoons, where there are sandy areas which have wreckage and weed (Randall, *et al*, 1990, Lieske, *et al*, 1994). Its diet constitutes mainly of

small benthic invertebrates (Bacchet *et al.*, 2006). They are distributed in Atlantic, Pacific and Indian Ocean (Nelson, 1994; Helfman *et al.*, 2009). *C.praetextatus* was previously reported in the Gulf of Mannar (Mogalekar, 2018) and it is a very common coral fish species in Gulf of Mannar. As Puducherry sustains wide variety of coral fishes *C. praetextatus* displays its first distributional record in Puducherry coastal waters. The present study also pivots on the molecular identification of *Cymolutes praetextatus* and it has been carried out for the first time in India, and the sequence got submitted in the NCBI. *C. praetextatus* and *C. lecluse* share some similar morphological traits where it is being difficult to identify if the specimen is not fresh (Randall *et al*, 2009). So the current study aimed to produce a DNA barcode of the mitochondrial COI marker gene of the fish specimen to

validate the species identity, which equally clarifies and supports morphology-based identifications.

Materials and Methods

Cymolutes praetextatus was collected at Pillaichavadi landing centre, Puducherry, along with the captured commercial fishes especially with *Lutjanus* species. The collected specimen was immediately brought to the laboratory for further identification and observation. Identification up to species level was carried out by a thorough examination of the morphometric characters using the online database FishBase and Wikipedia (Froese and Pauly, 2021, Wikipedia, 2020). The caudal fin was removed, sliced, and preserved in 95% ethanol and stored at 4°C for DNA barcode study.

DNA isolation

As per CAGL protocol DNA was isolated from the stored tissues. 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 labelled 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 c 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., (2005) 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 *Cymolutes praetextatus* 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.

Result and Discussion

Single species of *C. praetextatus* was collected from Pillaichavadi, Puducherry has been identified both taxonomically and in molecular level. The specimen was submitted in Zoological Survey of India, Chennai, India. The gene sample were submitted in NCBI and got the accession number.

Cymolutes praetextatus Quoy & Gaimard, 1834(Fig.1)

Common Name: Knife Razorfish, Knife Razorwrasse, Knifefish, Razon Wrasse

Material observed: Paratype, ZSI/MBRC-F. 2282, Male, 14 December 2019, Pillaichavadi, Puducherry, 12°0'29"; 79°51'30" NW-3543, Nithya Mary Gunalan (Figure 1)

Systematic Account

Phylum: Chordata, Haeckel, 1874

Class: Actinopterygii, Klein, 1885

Order: Perciformes, Bleeker, 1863

Family: Labridae, Cuvier, 1816

Genus: *Cymolutes* Günther, 1861

Species: *Cymolutes praetextatus*, Quoy & Gaimard, 1834



Figure 1. *Cymolutes praetextatus*

General Description

Diagnostic Characters

Cymolutes praetextatus is a pale coloured wrasse which has steep contour to its head. A black spot is present on the dorso-posterior side in the caudal peduncle of the female, an ocellated black spot above the tip of the pectoral fin of the male, and a thin black line in the outer part of the first interspinous membrane of the dorsal fin of both sexes. Dorsal spines (total): 9-10; Dorsal soft rays (total): 12-13; Anal spines: 2-3; Anal soft rays: 11-12 (Myers, 1999). The genus *Cymolutes* is easily separated from the other genera of the sub family Xyrichtyinae by having 72–93 scales in longitudinal series while the other genera have 26 scales; no scales on the head; and 12 branched caudal rays. A pair of slender canine teeth

extended at front of jaws, the lower pair suitably fit to inside and overlapping lips when mouth closed.

Colouration

The flanks are marked with indistinct whitish bars and there is a white stripe along its back which sits above a wide, yellowish or darker coloured stripe, although this may not be present. Series of orange coloured stripes runs throughout the dorsal fin. The males and many of the females of *praetextatus* have a series of orange to pink bars in life on the posterior half of the body. Has a broad yellow lateral stripe. (Myers, R.F., 1999). Margin of dorsal and anal fins deep pink; upper and lower edges of caudal fin pink (Figure 2).

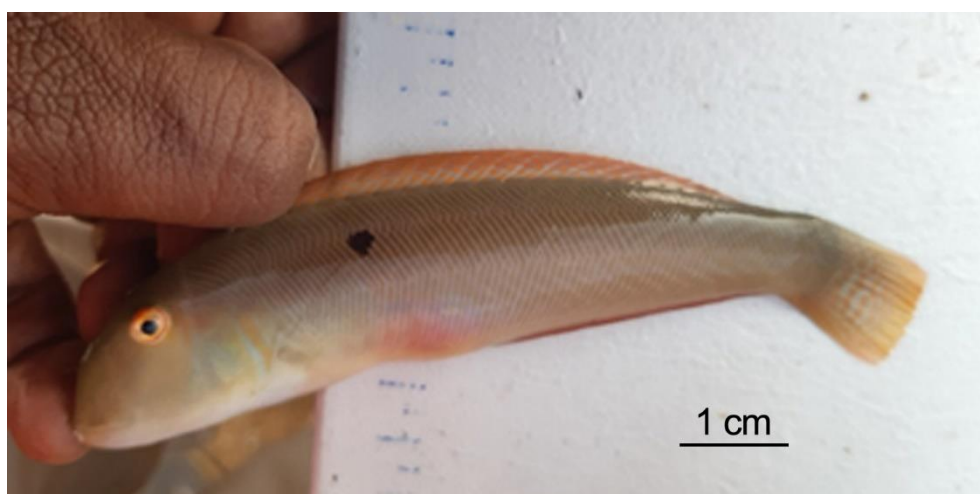


Figure 2. Coloration and pattern of dorsal fin of *Cymolutes praetextatus*

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. MT238118**. The sequence obtained from the sample was then compared and identified to the species level using sequence databases which showed 88% similarity with *Cymolutes praetextatus*.

Remarks

Very rare study has been undertaken about the biodiversity and biology of *C. praetextatus* in India. This species is rarely caught by fisheries. Though labrids are well-known for aquarium trade, this species does not appear often in the aquarium trade. From the fish and fisheries point of view, Puducherry has most fascinating ichthyofaunal diversity, especially the coral habitat fishes. *C. praetextatus* is not very common in Puducherry coastal waters and caught only in countable numbers. It is usually found along with *Iniistius* and *Lutjanus* species. It is considered as *Lutjanus* species by the local fishermen population and vernacularly called as “Kilimooku sangara” which means “Parrot nose snapper” and commercially valuable too.

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Conflicts of Interest

No potential conflict of interest was reported by the authors.

Ethical approval

All applicable national guidelines for the care and use of animals were followed.

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