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First Record of Yellowbelly Damselfish *Amblyglyphidodon leucogaster* (Bleeker, 1847) from the Lakshadweep, India

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Abstract: The yellowbelly damselfish *Amblyglyphidodon leucogaster* was new record from Lakshadweep, India. Through molecular taxonomy identified as 617 bp region of the mitochondrial cytochrome oxidase subunit I gene and the sequence was deposited in the Gen Bank publication database (Accession number is HM579791).

Key words: Yellowbelly Damselfish % New Record % Sequence % Lakshadweep

INTRODUCTION

DNA Bar-coding (DBC) is a method for taxonomic identification of animals that is entirely based on the 5' portion of the mitochondrial gene, cytochrome oxidase subunit I [1]. DBC is an alternative to traditional taxonomic methods that could become a useful tool for coral reef conservation. The potential value of DBC in marine biology has been commented elsewhere and the Census of Marine Life has included DBC among its recommended methods [2, 3].

Previously, a wide variety of protein and DNA based methods have been used for the genetic identification of fish species [4]. Caterinon [5] reported that the DNA bar-coding initiative offers the opportunity for a standardized system of species identification based on the analysis of small fragments of DNA. Tautz [6, 7] made the case for a DNA-based taxonomic system. DNA sequence analysis has been used to assist species identifications DNA bar-coding is single gene sequence which would be sufficient to differentiate the species and use mitochondrial DNA gene cytochrome oxidase subunit I (cox1) as a universal bio-identification method. The sequence was likened to barcode with species being delineated by a particular sequence of very similar sequences. The target DNA segment of 655 base-pairs near 5' end of the mitochondrial cytochrome oxidase subunit I gene is strongly proposed as a potential for sequence or barcode [8, 9].

In spite of its success, there is still much controversy surrounding this methodological model in terms of species classification, data significance distance, character analysis and taxonomical background of the analyzed groups [10]. However, there are growing numbers of examples for demonstrating the value of DNA bar-coding in different fields of biological sciences including assigning individuals to their corresponding taxa [11-14].

Hence, there is a need for comprehensive and reliable species identification tools or methods combined with early bar-coding success with fishes. DNA bar-coding is an alternative to traditional taxonomic methods that could become a useful tool for coral reef conservation [9,15]. The protocols include standards for the quality of DNA sequence references and the curation of voucher specimens that represent significant improvements over those currently in practice for public sequence databases such as Gen Bank [16]. Hence, in the present study an attempt is made to study a damsel fish species *Amblyglyphidodon leucogaster*, which is a new record from Lakshadweep through molecular taxonomical profile of mitochondrial DNA gene cytochrome oxidase subunit I.

MATERIALS AND METHODS

Sample Collection and DNA Extraction: The damsel fish was collected from the Bangaram Island is located at Lat.

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10° 51' N and Long. 72° 11' E, using perch trap method, small piece of tissue sample was dissected, preserved in 95% ethanol and stored in a deep freezer at (-) 4°C. The total DNA of the tissue sample was extracted by salting out method as proposed by Miller [17].

PCR Amplification: Fragments of the 5' region of mitochondrial DNA primers were suggested by Ward [9].

Fish F1-5'TCAACCAACCACAAAGACATTGGCAC3'
Fish R1-5'TAGACTTCTGGGTGGCCAAAGAATCA3'

PCR thermocycle program suggested by Folmer [18] was adopted. The programme started as initial de-naturation at 94°C for 1 min, five cycles at 94°C for 30 seconds, annealing at 47°C for 40 seconds and extension at 72°C for 1 minute, followed by 35 cycles at 94°C for 30 seconds, 54°C for 40 seconds and 72°C for 1 minute. A final extension at 72°C for 10 minutes was followed by indefinite hold at 4°C. PCR products were electrophoresed on 1 % agarose gel and documented under ultraviolet transillumination [19]. The PCR product was purified by using PCR purification kit (Genei, Bangalore).

RESULTS

Sequencing Analysis: The sequencing result was obtained in chromatogram fail format and read through chromas lite soft. The sequence of cytochrome oxidase subunit I was matched with other sequence data stored in National Centre for Biotechnological Information (NCBI) by Nucleotide BLAST. The sample identified as 617 bp in length and exhibited 92% similarity with the cox1 gene of *Amblyglyphidodon leucogaster* from Gen Bank database. The sequence was deposited in the Gen Bank publication database (Accession number is HM579791).

DISCUSSION

DNA bar-coding is a recent and widely used molecular based identification system that aims to identify biological specimens. Surveys on biodiversity of coral reefs inevitably require taxonomic coverage. DNA bar-coding is a method for taxonomic identification of organisms that is entirely based on the 5' portion of the mitochondrial gene cytochrome oxidase subunit I (COI-5). It can be especially useful for identification of larval forms or incomplete specimens lacking diagnostic morphological characters [1].

According to Ward [20], the simplest way of attempting the identification of an unknown specimen is to paste its COI sequence into the “Barcode of Life Database Systems” (BOLD) identification engine. This was compared with the sequence of all public and private BOLD sequences and all available Gen Bank sequences of the COI barcode region. Gen Bank records are regularly downloaded to BOLD. Although some Gen Bank records include a larger mtDNA region, the COI 59 region alone is used by BOLD for matching. Thus, sequences are available from species that have not been examined through FISH-BOL. The BOLD displays, top 20 matching records with similarity values are preferred. In the present study, the cytochrome oxidase subunit I gene sequence was match with the fish base, which exhibited 92% similarity value of *Amblyglyphidodon leucogaster* from Gen Bank database.

Over the past seven years, a number of campaigns have started to collect and register DNA barcodes from specific families and different regions of life. All of these global or regional campaigns work closely with the “Consortium for the Barcode of Life” (CBOL - http://www.barcodeoflife.org/). “International Barcode of Life Project” (IBOL-http://ibol.org/) and BOLD (http://www.barcodeoflife.org/), an online work bench that aids collection, management, analysis and use of DNA barcodes towards the ultimate goal of a barcode reference library of all life on Earth [21]. The information is then delivered via a data feed to the FISH-BOL website, which uses a taxonomic authority file derived from Fish Base (http://www.fishbase.org), the Catalog of Fishes and the Integrated Taxonomic Information System (ITIS, see http:// www.itis.gov) to monitor progress in barcode species coverage [20, 22].

The problems with the classification of Pomacentridae species are severe [23, 24]. The molecular studies of the damsel fishes have been published previously [25-28]. Bar-coding has the power to provide valuable insight into patterns of genetic divergence affected by species-level or ecological variation [29].

Ward [9] reported about 207 species of marine fish are sequenced or bar-coded as 655 bp region of the mitochondrial cytochrome oxidase subunit I gene (cox1). A 650-bp segment of the 5' region of the mitochondrial cytochrome oxidase subunit I gene is currently used for classification of molecular biodiversity [11, 14, 30, 31]. Several studies have shown the effectiveness of a 650-bp fragment of the cytochrome oxidase I gene for species identification in varied animal lineages [8, 9, 14, 32, 33]. Over all, the preceding findings suggest that the

molecular taxonomical classification for 650-655 bp region of the mitochondrial cytochrome oxidase subunit I gene (cox1) has been identified as fish species. Consequently in the present study, *Amblyglyphidodon leucogaster* species was sequenced and identified as 617 bp region of the mitochondrial cytochrome oxidase subunit I gene, which strongly validated the efficacy of barcodes for identifying the fish species.

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