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DNA barcoding of the recorded batfish Halieutaea fitzsimonsi (Gilchrist & Thompson, 1916) from Pazhayar fish landing center, Tamilnadu, India

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ABSTRACT

A batfish halieutaeafitzsimonsi was recorded from pahzyar fish landing centre, Tamilnadu south east coast of India. This species has never been reported earlier from the Indian coast and it has been recognized as new record to the Indian waters. This species was confirmed by co-1 sequencing analysis and confirmed as halieutaeafitzsimonsi (jf493612.1) with the conventional morphometric and meristic characters.

KEYWORDS:Batfish;barcoding; CO-I; novel fish; Pazhayar fish landing centre

INTRODUCTION

The anglerfishes (order-lophiiformes) have been considered as uniquegroup of fishes which have the unusual morphological, ecological adaptations with tremendous diversification and the varied clade of bony fishes. Presently, 18 families of the "Ogcocephalidae" were reported which includes 10 genera and 70 species worldwide[7-9]). These batfishes were considered to be small benthic fish largely seen in tropical and subtropical seas and poorly known group from shallow inshore waters up to 3000m depth. They aremarine bottom-dwellers considering to befed on small invertebrates and fishes. They generally inhabit onflat, relatively open-bottom habitats of rubble,sand, mud, continental shelves and slopes.Even though batfishes are regular in profitable fishing operations, they do not support a fishery due to its low level of edible nature. They appear to give out a fluid that may act as a chemical attractant; however, the chemical properties of these chemical substances are still unexplored.

MATERIALS AND METHODS:

In the present study, a93mm size of specimen was collected by hand picking method from the trashes of Pazhayar fish landing center (Lat 11˚21'32"N; Long 79˚ 49'25"E) since it tends to be novel and unreported species (Figure 1).The specimen was preserved at -20ºcin the National Facility for Marine Natural Product and Drug Discovery Lab, Faculty of Marine Sciences, CAS in Marine Biology, Annamalai Univeristy, Parangipettai, Tamil Nadu. Further, the specimen was archived in formalin and 70% ethanol for measuring their morphometric and meristic characters. Finally, the specimen was subjected to CO-I mitochondrial

sequencing analysis for species identification at RGCB, Trivandrum, Kerala.

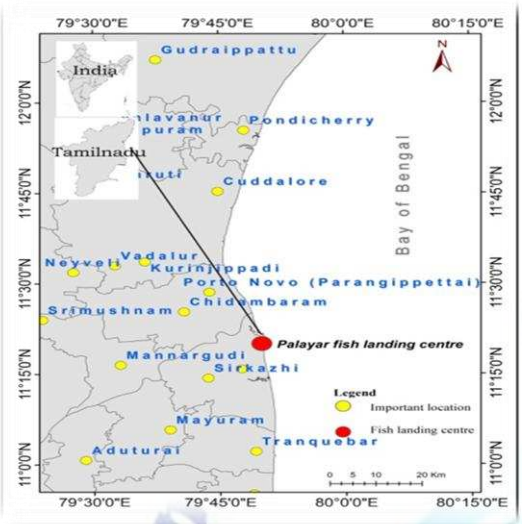

Figure (1) Map showing the location of the collection area the Pazhayar landing center South East Coast Tamil Nadu, India. The arrow represents the collection site (Lat 11˚ 21′32"N Long 79˚ 49′25"E).

DNA Isolation and amplification:

Genomic DNA was isolated from the tissues using NucleoSpin® Tissue Kit (Macherey-Nagel) following manufacturer's instructions. PCR amplification reactions were carried out in a 20 µl reaction volume which constitues 1X Phire PCR buffer (1.5 mM $MgCl_2$), 0.2mM each dNTPs, 1 µl DNA, 0.2 µl PhireHotstart II DNA polymerase enzyme, 0.1 mg/ml BSA and 3% DMSO, 0.5M Betaine, 5pM of forward (TCAACCAACCACAAAGACATTGGCAC)&reverse (TAGACTTCTGGGTGGCCAAAGAATCA) primers[1]. The gels were visualized in a UV trans illuminator (Genei) and the image was captured using Gel documentation system (Bio-Rad).The amplification of DNA was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems).

98°C - 30 sec

98°C - 5 sec
45°C - 10 sec } 10 cycles
72°C - 15 sec

98°C - 5 sec
50°C - 10 sec } 30 cycles
72°C - 15 sec

72°C - 60 sec

4°C - ∞

Sequencing using Big Dye Terminator v3.1 and Data Analysis:

Sequencing reaction was carried out in a PCR thermal cycler (Gene Amp PCR System 9700, Applied Biosystems) using the Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Bio systems, USA) following manufactures protocol. The quality of sequence was checked using Sequence Scanner Software v1 (Applied Biosystems). Sequence alignment and editing of the sequences were done using Geneious Pro v5.1[2]. All aligned sequences were then imported into Basic Local Alignment Search Tool (BLAST) software to ensure the identity of the matched sequences.

RESULTS:

Morphometric measurement and meristic count of *H. fitzimonsi*:

Ogcocephalid Spiny batfishes are morphologically delineated by their strongly depressed body disc, sub triangular or triangular in dorsal view (except box- like in *coelophrys*); tail tapering; enlarged snout, bifurcated dermal spines on ventral surface; tubercles present on entire body, except for gill opening, eyes and illicial cavity; illicial cavity present at tip of the head; a fleshy escal bulb was noticed at the tip of illicium; tubercles on dorsal surface simple or bifurcated. The pectoral fins appeared arm-like structure on the lateral-posterior edge of the disk. The numbers of dorsal fin rays were 2-3 and the pectoral fin rays 10-13 were present. The anal fin rays were 3-4 and caudal fin rays were 8 and the pelvic fins were found on ventral surface disk and spiny batfish was proofed by dissection of digestive system of the purely carnivorous fish. The dorsal and ventral view of the fish *H. fitzimonsi* was given in Figure 2(A - B).In addition, the morphometric parameters and meristic counts were tabulated in Table 1 and 2.

Figure 2 (A). Shows the dorsal view of H. fitzimonsi

Figure 2(B). Shows ventral view of *H. fitzimonsi*

Morphometric parameter	In mm	% SL
Total length	93	100
Standard length	71	76.34
Fork length	87	93.54
Head length	23	24.73
Body depth	12	12.90
Pectoral fin length	25	26.88
Pectoral base	05	05.37
Pelvic fin length	23	24.73
Pelvic base	09	09.67
Dorsal fin length	22	23.65
Dorsal base	06	06.45
Pre-dorsal length	62	66.66
Caudal fin base	07	07.52
Caudal fin length	22	23.65
Caudal peduncle length	06	06.45
Anal base	04	04.30
Pre-anal length	50	53.76
Pre-orbital length	23	24.73
Postorbital head length	46	49.46
Beast length	16	17.20
Eye diameter	06	06.45

Table 1 — Shows the morphometric characters of *H. Fitzimonsi*

Meristic count		Count
Fin rays	Pectoral	13
	Pelvic	05
	Dorsal	05
	Anal	04
Spine	Pectoral	-
	Pelvic	-
	Dorsal	-
	Anal	-

Table 2 — Shows the meristic counts of *H. Fitzimonsi*.

Genomic DNA isolation and PCR amplification:

Genomic DNA was isolated from the fish tissue and its quality check was done by loading in 1 % agarose gel which showed the intact DNA (Fig.3A). CO-1 region of the species was amplified through PCR which shows the molecular weight of 626bp corresponding to that of the DNA ladder. The amplified product (626bp) was further sequenced to identify the genome and it was shown (Fig. 3B).

Figure 3A.Showing the isolated genomic DNA

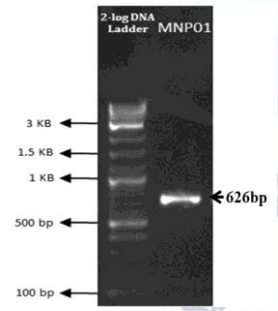

Figure 3B.Showing the amplified DNA along with ladder

DNA SEQUENCING AND ANALYSIS:

The obtained CO-1 gene sequence (626 bp) of the species was preliminary compared with previously obtained sequences of *Halieutaea fitzsimonsi* deposited in GenBank (NCBI) and it indicated that this species is phylogenetically related to the members of the genus *Halieutaea sp*. The phylogenetic tree of CO-1 sequences was constructed by using the three valid representative species of the genus shown in (Fig. 4). The sequence of

Halieutaeafitzsimonsi (JF493612.1) served as the reference of the operational taxonomic unit. The COI sequences related with above species was provided below.

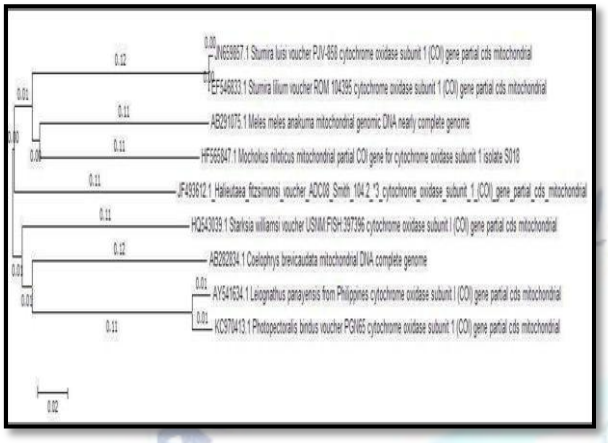

Figure 4.Neighbor joining phylogenetic tree of *H. fitzimonsi*inferred from CO-I gene sequences.

COI sequence (626 bp)

>SR933-MNPO1-CO

CACCCTTTACCTAGTCTTTGGTGCTTGAGCCGGTA
TAGTCGGTACTGCTTTAAGCCTGCTTATTCGTGCC
GAATTAAGCCAACCAGGGGCCCTTTTAGGCGATG
ATCAAATCTACAACGTTATCGTTACTGCCCATGCT
TTTGTTATAATTTTTTTCATAGTTATACCAATCATA
ATTGGAGGCTTCGGAAACTGATTAGTCCCTTTAAT
AATTGGAGCCCCTGACATAGCATTCCCTCGAATA
AATAACATAAGTTTCTGACTGCTCCCTCCCTCTTT
TCTCCTCTTACTTGCATCTTCAGGTGTTGAAGCCG
GTGCCGGTACTGGTTGAACTGTGTACCCTCCCCTA
GCGGGCAACCTGGCTCATGCAGGGGCTTCTGTAG
ACTTAACAATCTTTTCCCTTCATCTTGCAGGGGTG
TCCTCCATTCTAGGAGCTATCAATTTTATTACTAC
TATCTTCAATATAAAACCTCCGTCAACGTCACAA
TACCAAACCCCTTTATTTGTATGGTCAGTTCTCAT
CACCGCAGTACTACTACTCCTAGCCCTTCCTGTAT
TAGCTGCAGGTATCACAATACTACTCACCGACCG
AAACCTAAATACCACGTTTTTCGACCCTGCAGGA
G

DISCUSSION:

The aid of CO-I sequencing as molecular taxonomic tags could facilitate identifying the species. However, in *Halieutaeafitzsimonsi*, CO-I gene sequence has not been reported till date. The present finding is reported as first time in Indian waters; we have successfully amplified 654-bp-long sequence from *H. Fitzimonsi*fish tissue. Theuniversal primer was capable of amplifying the target region an irrespective of any insertions or deletions. The applicability of those primers could act as the global standard for identifying fish species[1]. Moreover, the first Ogcocephalid *Halieutaeastellata* was reported in Taiwan. In 1984, Shen provided color photos of five Ogcocephalid species including two unidentified species and two new records: *Halieutaea fitzimonsi* and *dibranchus japonicas*[6]. Moreover, it seems the similar species was reported from Veeravel landing center and was named as *Halieutaea India* from Chennai, Tamil Nadu coast without any species description[5] and along with species description[3, 4].

Acknowledgment

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