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DNA barcoding, phylogenetic relationships and speciation of Genus: *Plectropomus* in Andaman coast

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ABSTRACT:

DNA barcode technique has been recently promoted as a method for both assigning specimens to known species and for discovering new and cryptic species. The present study carried out for the barcode sequences for identification of the *Plectropomus leopardus*. The results of shorter sequence 113bp of *Plectropomus leopardus* is effective to identify a specimen and confirm up to a species level. This sequence also exhibited deep interspecific divergences that allowed for efficient discrimination among the Grouper species. This mini barcode sequence technique might be used efficiently to distinguish *Plectropomus leopardus* with minimal expenditure and efforts. These results also support that formalin fixation samples can also be used for DNA molecular taxonomy.

Keywords:
DNA barcode, Serranidae, Phylogenetic and Andaman Sea.

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INTRODUCTION

Pisces phylum is a highly diverse group among the vertebrate and exhibit a deep phenotypic changes during development; its identification and confirmation needs a modern tool. Among all the methods DNA barcode is an efficient method for species-level identification using an array of species specific molecular tags derived from 59 region of the mitochondrial cytochrome c oxidase I (mt COI) gene (ward et al., 2005). Twenty-eight species have been reported under *Epinephelus*, from the east and west coasts of India and the islands of Lakshadweep in the Arabian Sea and Andaman and Nicobar in the Bay of Bengal (James et al., 1996). Generally groupers are identified by their colour patterns or a suite of morphologic characters like body configuration, size, number of body parts etc. Though generally the colour patterns in medium-sized fishes are distinctive enough to identify different species, one need to be aware of intraspecific variations in colour patterns of juveniles, which may be completely different from the adults of the same species (Heemstra and Randall, 1993). Hence identification based on morphology in groupers has to be supported by other techniques including DNA barcoding. Consequently the phylogenetic based study related to serrianidae fishes are very less reported in Andaman and Nicobar Islands.

MATERIALS AND METHODS

Sample collection and preservation

Family Serrianidae (Grouper) were collected from local fish landing centre at Port Blair, Andaman. Collected samples were identified morphometrically with FAO sheets (Heemstra and Randall, 1993) and stored in formalin. The piece of muscle collected above the lateral line was stored at -20°C for DNA extraction.

DNA extraction and PCR reaction

Total DNA extracted from 0.25g of tissue by the standard proteinase-K/ phenol-chloroform-isoamyl alcohol-ethanol method (Sambrook et al., 1989). An approximately 650bp section of the mitochondrial (mt) DNA genome from the COI gene was amplified using published universal primer of two set (Ward et al., 2005).

The polymerase chain reaction (PCR) components per 50ml reaction were as follows : PCR buffer (10X), MgCl2 (25mM), dNTP (10mm), Forward Primer (30ng/ml), Reverse Primer (30ng/ mL) 1ml, Taq polymerase (3U), ultra pure water, DNA (100ng/ml).

The PCR cycling parameters were as follows, in 95°C for 5min the *Taq* Polymerase were initially denatured followed by 40 cycles of denaturation for 30sec at 95°C, annealing at 58°C for 60 sec and extension at 72°C for 60 sec, After the completion of 40 cycles a final extension step of 7mints at 72°C was performed. The PCR products were tested in 1% Agarose gel, visualized and photographed using Gel Doc System.

Nucleotide sequencing was performed using the Sanger method (Sanger et al., 1977) modified by Chen and Seebug (Chen, et al., 1985). Sequencing was performed using BigDye Terminator Cycle Sequencing kit, following manufacturer's instructions (Applied Biosystems, Foster City, CA, USA). The sequencing was done both in the forward and reverse directions.

Sequence Analysis

The DNA sequences of phenotypically identified fishes were assembled using the SeqMan II version 5.03 (DNASTAR) and aligned using Clustal W pair wise and multiple alignment was completed for phylogenetic identification. Molecular evolutionary analyses were conducted using *MEGA* version 4 (Tamura et al., 2007).

RESULT

DNA sequences were submitted to GenBank (PubMed) and their accession number is JF414594 (**Table.1**). Out of 651 – 655bp basic taxonomic sequences length, the present study exhibit the sequences of 113bp for *Plectropomus leopardus.* All assemblages of conspecific individuals had boot strap support of very vast species divergences compared with other same species DNA sequences collected from NCBI genbank and algorithms used in MEGA 4. The overall average within species K2P distances is 0.21618, with very less in EU595233 *Plectropomus leopardus,* for DQ 101270 *Plectropomus leopardus.* While GU 674047 perciformes species with 52% were slightly showing higher distances. The congeneric distances was 34% which was higher than conspecific distances of species compared with Andaman *Plectropomus loepardus* distance and its range is 10 - 11%.

DISCUSSION AND CONCLUSIONS

The sequence results of DNA barcode for *Plectropomus leopardus* revealed the potential ability of mini-barcodes to discriminate among species. While mini-barcodes produced divergence values comparable to full-length barcodes in both

Table 1 DNA Sequences details of *Plectropomus leopardus* in GenBank files version

Definition Locus	*Plectropomu leopardus* COI gene CDS
Accession Number	JF414594
Submitted Date	VRT 08-APR-2011
Classification	Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi; Actinopterygii; Neopterygii; Teleostei; Euteleostei; Neoteleostei; Acanthomorpha; Acanthopterygii; Percomorpha; Perciformes; Percoidei; Serranidae; Epinephelinae; *Plectropomus*.
Alignment of Partial Sequence of COI Gene 113bp. *Plectropomus leopardus.*	>1 tcattaacat gaaacccct gctatctctc aataccagac ccccctattc gtatgagcag tcctaattac tgcagttctt cttctcctat cactacctgt tctagctgct gga.
Protein_ID, Translate Details	="AEB31228" ="INMKPPAISQYQTPLFVWAVLITAVLLLLSLPVLAAG"

data sets, they were somewhat less effective in discriminating among the species in large assemblages (e.g. 204 species of Australian fishes). However, most applications of mini barcodes will not involve cases that seek to place a specimen among all known species, but rather within a small assemblage. In the species of *Plectropomus leopardus,* mini-barcodes were positioned specifically to discriminate this species pair, and their relatively short lengths took into account the old age of the specimens. In addition, the fact that the forward primers in the two mini-barcodes were designed specifically for this species complex might have positively influenced the chance of amplifying potentially degraded DNA. Interestingly, a similar sized fragment from the other end of the barcode region would have achieved identification. Full-length barcode sequences can be easily and cheaply obtained from recently collected tissue or from those preserved for DNA extraction (Hajibabaei *et al*. 2005).

The resultant records provide a 'gold standard' with high confidence for species discrimination within large species pools (Hebert *et al*. 2003a, 2003b; Smith *et al*. 2005, 2006; Ward *et al* . 2005; Hajibabaei *et al*. 2006). This study confirms that short barcode sequences (113bp) are also valuable for the identification of specimens from selected narrow taxonomic arrays, such as comparing a newly collected specimen. Similarly, it may well be possible to employ mini-barcodes for the identification of formalin-fixed samples, which often contain highly fragmented DNA (Schander & Kenneth 2003). Mini-barcodes may also provide the option to employ alternative sequencing methods, such as pyrosequencing (Fakhrai-Rad *et al*. 2002), that yield only short sequences, but offer lower costs and higher throughput than standard approaches.

The study has successfully proved the utility of COI divergences in identifying all the *Plectropomus leopardus* fishes in Andaman Sea with minimum base pair (113). The present analysis was not meant to be exhaustive, but to highlight the most important feature of mt COI sequences in distinguishing closely related species and intra-specific distances which prove beyond doubt. Further studies involving other family and groups of marine fishes of the area and also by increasing the sample size in future studies will clarify the issues.

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Fig. 1. Neighbor-joining trees based on the mtDNA COI gene nucleotide sequences of *Plectropomus* species analyzed. Numbers at nodes are bootstrap values based on 1000 replicates. The scale bar represents an interval of Tamura-Nei genetic distance for *Plectropomus leopardus.*