Identifying marine fin fishes using DNA barcodes

An estimate of the earth's biodiversity shows that we are one among the 10–15 million species inhabiting the blue planet 'earth'[1]. It took over two centuries for the taxonomists to describe 1.8 million species, but we know this figure might be a gross underestimate of the true biological diversity on earth[2,3]. In addition, human impact, e.g. fragmentation or destruction of habitats, results in a steady decline in diversity due to loss of species and increase in the number of endangered species. Many species have also become extinct without having been described. In view of this trend, there is an urgent need to develop a tool to describe all the earth's species so that the associated societal and economic benefits could be derived in addition to evolving strategies for protecting them and conserving the resources they constitute. Scrutiny of 138 reports and inventories made between 1960 and 2004 has shown that about one-third of specimens collected for assessing the biodiversity is not determined to species level[4]. In order to strengthen the taxonomy and to speed up documentation and understanding of the planet's natural diversity, Hebert *et al.*[5] proposed a concept called DNA barcoding in which a short nucleotide sequence of mitochondrial genome will act as a DNA barcode for species identification of eukaryotes, in particular, animals. This technology has proven to be a rapid tool for precise identification of biological specimens. DNA barcoding works under the principle that inter-species variations are greater than the intra-species variations, allowing one to distinguish the species using nucleotide sequences. Six-fifty nucleotide bases of 5′ cytochrome *c* oxidase subunit 1 gene (CO1) have been accepted as a universal barcode to delineate animal life of this planet. By harnessing the advances in electronics and genetics, barcoding is going to help investigators to quickly recognize known species and to retrieve information about them. This technique will speed up the discovery of many species yet to be named. Thus this technology will provide a vital new tool for appreciating and managing the earth's immense and changing biodiversity.

DNA barcoding of fishes in different parts of the globe gained momentum and it has been well established in Australia[6]. However in Indian waters, only few efforts have been made so far[7–10]. With the view to extending this effort to Indian biodiversity, the present study was undertaken to document and barcode fin fishes of Parangipettai coastal waters (lat. 11°29′N; long. 79°46′E). The barcoded species can be identified quickly, precisely and cheaply using barcode sequences[5]. Fishes were collected live in triplicates per species using cast and gill nets operated in the coastal waters (February 2009 to December 2010). All the fishes were identified up to the species level using the FAO Fish Identification Sheets[11]. The voucher specimens are maintained in the Centre of Advanced Study in Marine Biology, Faculty of Marine Science, Annamalai University, Parangipettai. A cube of lateral tissue was exercised from the specimen, preserved in 95% ethanol and stored at 4°C. Isolation of DNA, PCR amplification, screening of the amplicon through

Table 1. Barcoded fishes of Parangipettai waters and their NCBI accession numbers

Name	Family	Accession number	No. of sequences available earlier*
Mugil cephalus	Mugilidae	EU819545	12
Gerres filamentosus	Gerreidae	EU819546	1
Liza tade	Mugilidae	EU819547	–
Liza parsia	Mugilidae	FJ384677	
Gerres filamentosus	Gerreidae	FJ384678	1
Liza tade	Mugilidae	FJ384679	–
Gerres abbreviatus	Gerreidae	FJ384680	–
Terapon jarbua	Terapontidae	FJ384681	8
Etroplus suratensis	Cichlidae	FJ384682	4
Strophidon sathete	Muraenidae	FJ384683	–
Mystus gulio	Bagridae	FJ384684	1
Johnius dussumieri	Sciaenidae	FJ384685	–
Mugil cephalus	Mugilidae	FJ384686	12
Nematalosa nasus	Clupeidae	FJ384687	–
Eleutheronema tetradactylum	Polynemidae	FJ384688	3
Lates calcarifer	Latidae	FJ384689	37
Valamugil cunnesius	Mugilidae	FJ384690	–
Stolephorus indicus	Engraulidae	FJ384691	–
Lutjanus fulviflamma	Lutjanidae	FJ384692	–
Arothron hispidus	Tetraodonotidae	FJ384693	–
Sardinella longiceps	Clupeidae	FJ384694	5
Polydactylus sextarius	Polynemidae	FJ384695	–
Plotosus lineatus	Plotosidae	FJ384696	19
Cyanoglossus bilineatus	Cyanoglossidae	FJ384697	–
Secutor ruconius	Leiognathidae	FJ384698	–
Stolephorus commersonnii	Engraulidae	FJ384699	–
Himantura uarnak	Dasyatidae	FJ384700	–
Stolephorus indicus	Engraulidae	FJ384701	–
Pampus argenteus	Stromateidae	FJ384702	9
Chirocentrus dorab	Chirocentridae	FJ384703	10
Polydactylus sextarius	Polynemidae	FJ384704	–
Siganus canaliculatus	Siganidae	FJ384705	–
Sillago sihama	Sillaginidae	FJ384706	8
Upeneus vittatus	Mullidae	FJ384707	–
Dussumieria acuta	Clupeidae	FJ384708	4
Himantura imbricata	Dasyatidae	FJ384709	–
Photopectroralis bindus	Leiognathidae	FJ384710	–
Lagocephalus spadiceus	Tetraodontidae	FJ384711	5
Eubleekeria splendens	Leiognathidae	FJ384712	–
Caranx ignobilis	Carangidae	FJ384713	–
Upeneus tragula	Mullidae	FJ384714	–
Platax teira	Ephippidae	FJ384715	–

*Denotes the number of sequences already available at NCBI for the respective fin fishes from other waters.

agarose gel and sequencing were done as described by Prasanna Kumar *et al.*[9]. In brief, CO1 primers, FishF1-5′ TCAACCAACCACAAAGACATTGGC-AC-3′ and FishR1-5′ TAGACTTCT-GGGTGGCCAAAGAATCA-3′ were used for DNA barcode amplification[12]. The PCR was conditioned as follows; 95°C for 2 min, 5 cycles of 94°C for 30 s, 45°C for 40 s, 72°C for 30 s and 35 cycles of 94°C for 30 s, 54°C for 40 s, 72°C for 30 s and final extension was carried out at 72°C for 10 min. Amplicons were sequenced using ABI high throughput sequencer (Bioserve Biotechnologies Pvt Ltd, Hyderabad). The accession numbers of the barcodes at the National Centre for Biotechnological Information (NCBI), USA are given in Table 1.

Use of DNA barcodes for identifying marine fishes has now become an accepted concept[6]. In the present study, 42 barcodes were obtained for 40 species of fin fishes belonging to 32 genera and 23 families. Barcodes for 26 species which were not available in NCBI earlier were barcoded and deposited in this portal (Table 1). The generated sequences will act as a benchmark and reference data for identifying respective species around the world. Recently, Lakra *et al.*[10] barcoded 115 species (belonging to 37 families and 79 genera) of marine fin fishes occurring in the Indian waters. Most of their sampling efforts were restricted to the fish landing centres. Therefore, they could barcode only commercially important species. However, in the present study special efforts were made to collect ornamental and poisonous fishes besides commercially important ones. Therefore, 26 species belonging to 14 families could be barcoded. According to the International Union for Conservation of Nature (IUCN), most of the fishes barcoded have not been evaluated for IUCN status, except *Mugil cephalus* and *Himantura uranak*, which have been evaluated as 'least concerned' and 'vulnerable' species respectively. Among the various species barcoded in the present study, members of Mugilidae are considered to have ambiguity in morphological taxonomy[13,14]. In our previous publication we have shown the occurrence of congeneric species (*Mugil platanus* and *Mugil liza*) in Mugilidae using DNA barcodes[9]. In the present study six DNA barcodes belonging to members of the family Mugilidae have been produced.

Similarity in the sequences of species barcoded presently with those barcoded earlier was analysed through construction of phylogram (Figure 1). Since barcodes of the same species invariably get clustered in same clade, it is clear that across geography barcodes of the same species do not contain many variations. Thus CO1 gene sequences can act as universal DNA markers for identification of fishes. Presence of phylogeographic signals is unclear in the present analysis. Sequences of species such as *M. cephalus*, *Terapon jarbua*, *Etroplus suratensis* and *Dussumieria acuta* collected from Parangipettai waters showed close relationship with those of the respective species collected by Lakra *et al.*[10] from the Indian waters. However, the sequences of species such as *Pampus argenteus*, *Sillago sihama* and *Chirocentrus dorab* barcoded presently showed close relatedness with the respective species barcoded from Australian waters. Barcodes of *Lagocephalus spadiceus* and *Plotosus lineatus* obtained by Lakra *et al.*[10] showed more similarity with those of the respective species sequenced in the Australian waters, rather than with the same species of fishes from Parangipettai waters. Hence the presence of phylogeographic signals is unclear with the species barcoded so far. However, this has to be confirmed through further extensive studies

India is rich in diversity, notwithstanding fish diversity. All the species occurring in the Indian waters have to be barcoded, so that as pointed by the Consortium of Barcode of Life, 'any animal, any plant, any fungus or any organism can be identified on the spot, in an instant and anywhere by anyone'.

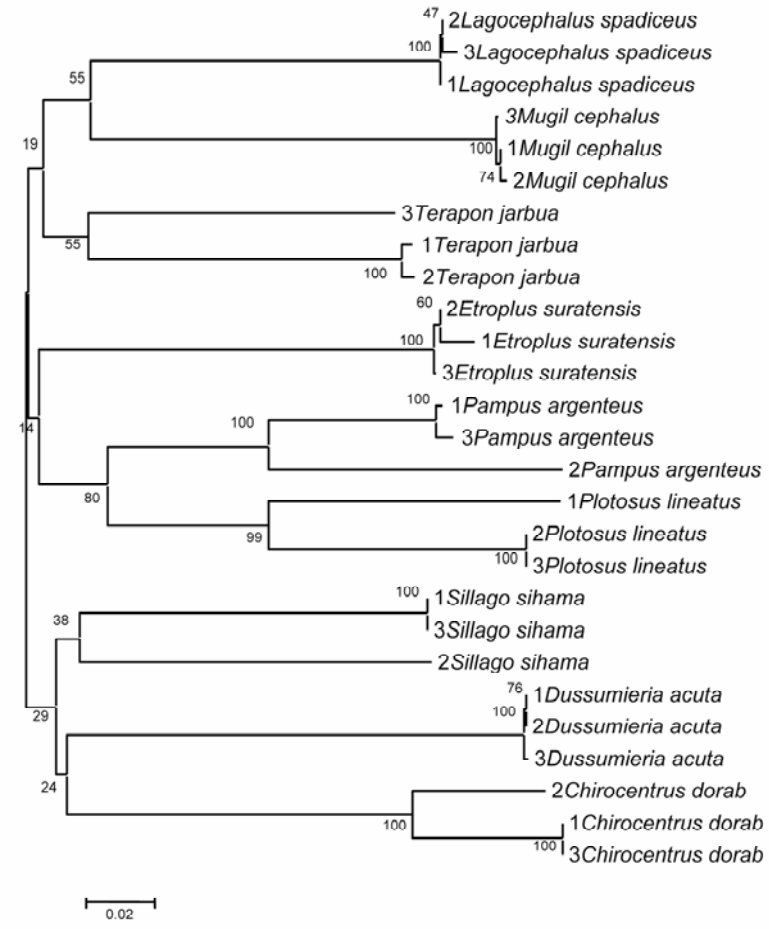

Figure 1. Phylogram showing similarities between DNA barcodes of few species sequenced in earlier studies. The numbers before the species name, viz. 1, 2 and 3 represent the barcode sequences generated in the present study by Lakra *et al.*[10] and Ward *et al.*[6] respectively.

1. Hammond, P., In *Global Biodiversity: Status of the Earth's Living Resources*, 1992, pp. 17–39.

2. Blaxter, M., *Nature*, 2003, **421**, 122–124.
3. Wilson, E. O., *Trends Ecol. Evol*., 2003, **18**, 77–80.
4. Schander, C. and Willassen, E., *Mar. Biol. Res.*, 2005, **1**(1), 79–83.
5. Hebert, P. D. N., Stoeckle, M. Y., Zemlak, T. S. and Francis, C. M., *PLoS Biol*., 2004, **2**(10), e312.
6. Ward, R. D., Zemlark, T. S., Innes, B. H., Last, P. R. and Hebert, P. D. N., *Philos. Trans. R. Soc. London, Ser. B.*, 2005, doi:10.1098/rstb.2005.1716.
7. Ajmal Khan, S., Lyla, P. S., Akbar John, B., Prasanna Kumar, C., Murugan, S. and Jalal, K. C. A., *Biotechnology*, 2010, **9**(3), 373–377.
8. Akbar John, B., Prasanna Kumar, C., Lyla, P. S., Ajmal Khan, S. and Jalal, K. C. A., *Res. J. Biol. Sci.*, 2010, **5**(6), 414–419.
9. Prasanna Kumar, C., Akbar John, B., Ajmal Khan, S., Lyla, P. S., Murugan, S., Rozihan, M. and Jalal, K. C. A., *Trends Appl. Sci. Res.*, 2011, **6**(9), 1028–1036.
10. Lakra, W. S. *et al*., *Mol. Ecol. Resour*., **11**, 60–71.
11. Thomson, J. M., In *FAO Species Identification Sheets for Fishery Purpose. Western Indian Ocean Fishing Area* (eds Fisher, W. and Brianchi, G.), FAO, Rome, 1984, vol. 3.
12. Ivanova, N. V., Zemlak, T. S., Hanner, R. H. and Hebert, P. D. N., *Mol. Ecol. Notes*, 2007, **7**, 544–548.
13. Papasotiropoulos, V., Klossa-Kilia, E., Alahiotis, S. N. and Kilias, G., *Biochem. Genet*., 2007, **45**, 623–636.
14. Gilbert, C. R., *Fla. Sci*., 1993, **56**, 204–210.

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S. AJMAL KHAN
C. PRASANNA KUMAR
P. S. LYLA*
S. MURUGAN

Centre of Advanced Study in Marine Biology,
Faculty of Marine Science,
Annamalai University,
Parangipettai 608 502, India
**For correspondence.*
e-mail: lailanair3@gmail.com