Mitochondrial DNA

http://informahealthcare.com/mdn
ISSN: 1940-1736 (print), 1940-1744 (electronic)

Mitochondrial DNA A DNA Mapp Seq Anal., 27(6): 4638-4642.
2015 Dec 18
 DOI: 10.3109/19401736.2015.1101593

Taylor & Francis
Taylor & Francis Group

SHORT COMMUNICATION

Molecular identification of Bigeyes (Perciformes, Priacanthidae) from Indian waters

Bineesh K. K[1], Gopalakrishnan A[2], Jena J. K[3], Basheer V. S[1], Mohitha C[1], Vineesh N[1], Joselet M[4], and N. G. K. Pillai[2]

[1]National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus, Kochi, Kerala, India, [2]Central Marine Fisheries Research Institute, Kochi, Kerala, India, [3]National Bureau of Fish Genetic Resources, Dilkusha, Lucknow, India, and [4]Nirmalagiri College, Kannur, Kerala, India

Abstract

Thirty-five individuals of six priacanthid fish species were sampled from different localities along the coast of India covering the Arabian Sea and Bay of Bengal. The partial sequence of 16S rRNA and cytochrome oxidase subunit I (COI) genes were analyzed for species identification and phylogenetic relationship among the Indian priacanthids (*Priacanthus hamrur*, *P. prolixus*, *P. blochii*, *P. sagittarius*, *Cookeolus japonicus*, and *Pristigenys refulgens*). The intraspecies genetic distance ranged from 0.000 to 0.002, while distances varied from 0.008 to 0.157 interspecies based on 16S sequences. Using COI data analysis, the intraspecies genetic distance ranged from 0.000 to 0.005, while interspecies distances varied from 0.009 to 0.108. Several sequences labeled *Priacanthus hamrur* in GenBank are shown to be *P. prolixus*. We also observed cryptic speciation in *Heteropriacanthus cruentatus*. Partial sequences of 16S rRNA and COI genes provided phylogenetic information to distinguish thirteen species of priacanthids, indicating the usefulness of molecular markers in species identification.

Keywords

Bigeyes, COI, DNA barcoding, Indian waters, 16S rRNA

History

Received 23 June 2015
Revised 14 September 2015
Accepted 26 September 2015
Published online 9 December 2015

Introduction

The Bigeyes of family Priacanthidae are marine percoid fishes, with four genera and 19 species. They comprise relatively small epibenthic predatory fishes occurring in rocky and coral habitats in Indo-Pacific region with 15 species and four species in the Atlantic (Iwatsuki et al., 2012; Starnes, 1988). The bigeyes are characterized by extremely large eyes, rough scales, bright red coloration, and deep, laterally compressed oval to moderately elongate bodies. They occur as solitary or in small aggregations, but species like *P. hamrur* form large aggregations. Exploratory surveys conducted along the Indian EEZ have revealed higher concentration of priacanthids along the West Coast than the East Coast (James & Pillai, 1989; Sivakami et al., 1998). Bigeyes are concentrated in 40–100 m depth range along the Southwest Coast and 100–200 m depth range along the Northwest coast (Bande et al., 1989; Sivakami et al., 1998).

Priacanthids are generally identified using morphological, meristic, and anatomical characters (Starnes, 1988). But due to morphological similarity and overlapping meristic ranges taxonomic ambiguities exist in several species. Synonym citations in database like FishBase indicate the possibility of taxonomic ambiguity in genera like *Heteropriacanthus* and *Pristigenys*. Most of the species in the family are widely distributed with geographic variations, suggesting the possibility of cryptic species and possible undescribed species.

Accurate identification of exploited fishes is very important in case of morphological similarity, for fisheries management and population studies. Molecular methods in the form of DNA barcodes have been used to differentiate species and identifying cryptic species (Hebert et al., 2003). Mitochondrial DNA genes have been extensively used in fish phylogenetics, since they are highly conserved compared to nuclear DNA, resulting in the accumulation of differences between species (Timm et al., 2008). The 16S rRNA and COI genes have been useful in resolving taxonomic ambiguities, resurrecting species, and identifying market mislabeling in fishes (Iwatsuki, 2013; Keskin & Atar, 2012; Ward et al., 2005).

In this study, the sequences of the COI and 16S rRNA genes were generated for different species of priacanthids along the Indian Coast. In combination with the retrieved sequence data from GenBank, the cryptic species (speciation without an obvious morphological signature) in the genus *Priacanthus* may facilitate further investigations to find out the accurate species diversity and distributions.

Materials and methods

Six species of priacanthids from three genera were collected and the voucher specimens were kept in the National referral museum of the Central Marine Fisheries Research Institute (CMFRI), Kochi and Zoological Survey of India (ZSI), Kozhikode, India. Tissue samples were collected from fragments of white muscle or gill tissues for DNA extraction following the protocol of Miller et al. (1988). The concentration of isolated DNA was checked using the UV spectrophotometer.

The mitochondrial 16S rRNA and COI genes were amplified in 25 µl reaction volume containing 1 × assay buffer (100 mM Tris, 500 mM KCl, 0.1% gelatin, pH 9.0) with 1.5 mM $MgCl_2$ (Genei, Bangalore, India), 5 pmoles of each primer, 200 µM of each dNTP (Genei, Bangalore, India), 1.5 U *Taq* DNA

Correspondence: K.K. Bineesh, National Bureau of Fish Genetic Resources, Central Marine Fisheries Research Institute Campus, P.B. No. 1603, Ernakulam North, PO, Kochi 682 018, Kerala, India. E-mail: kkbineesh@gmail.com

polymerase, and 20 ng of template DNA. The primers used for the amplification of the partial 16S rRNA gene were 16SAR (5′-CGCCTGTTTATCAAAAACAT-3′) and 16SBR (5′-CCGGT CTGAACTCAGATCACGT-3′) (Palumbi et al., 1991) and the partial sequence of COI gene was amplified using primers Fish F1 (5′-TCA ACC AAC CAC AAA GAC ATT GGC AC-3′) and Fish R1 (5′-TAG ACT TCT GGG TGG CCA AAG AAT CA-3′) (Ward et al., 2005). The thermal profile consisted of 4 min initial denaturation at 94°C, 36 repetitions of a three step cycle consisting of denaturation at 94°C for 1 min, annealing 53 °C for 1 min, and extension at 72°C for 1 min including and final extension at 72 °C for 7 min. The PCR products were visualized on 1.5% agarose gels. All samples were sequenced bidirectionally using an ABI3730 capillary sequencer following the instructions of the manufacture.

The raw DNA sequences were edited and aligned using BioEdit sequence alignment editor, version 7.0.5.2 (Hall, 1999). The edited sequences were submitted to GenBank (KF814993–KF815037). The sequence differences between species were calculated by averaging pair-wise comparisons of sequence differences across all individuals. The sequence divergence values within and between species were calculated using Kimura 2 Parameter (K2P) distance model implemented in MEGA V.6.0 (Tamura et al., 2013). The number of polymorphic sites and nucleotide diversity (Pi), nucleotide composition and transition, and transversion between species were determined by DnaSp V 3 (Rozas et al., 2006). Neighbor-joining (NJ) trees of K2P distance were created to provide graphic representation of divergence with 1000 replications.

Results

16S rRNA sequence data analysis

Multiple alignments of partial sequence of the 16S rRNA gene from 28 individuals of 3 genera (*Priacanthus, Cookeolus,* and *Pristigenys*) resulted in a consensus length of 536 sites including base pairs and gaps. The analysis revealed nucleotide frequencies of A = 28.4%, T = 21.9%, G = 24.1%, and C = 25.7%. As expected, average transitional pairs (si = 68.75) were more frequent than transversional pairs (sv = 31.24) with an average ratio of 2.2. The genetic intraspecies distance ranged from 0.000 to 0.002 while interspecies distance varied from 0.008 (between *P.prolixus* and *P. harmur*) to 0.157 (between *C.japonicus* and *P.prolixus*). Neighbor-Joining (NJ) trees of Kimura two parameter (K2P) distances was also suggested to reveal the identical phylogenetic relationships among the species (Figure 1).

Cytochrome oxidase subunit I sequence data analysis

The final alignments of COI gene sequences consisted of 639 bp per taxon. No stop codons were observed in any of the sequences. One to five haplotypes were observed in all the six species.

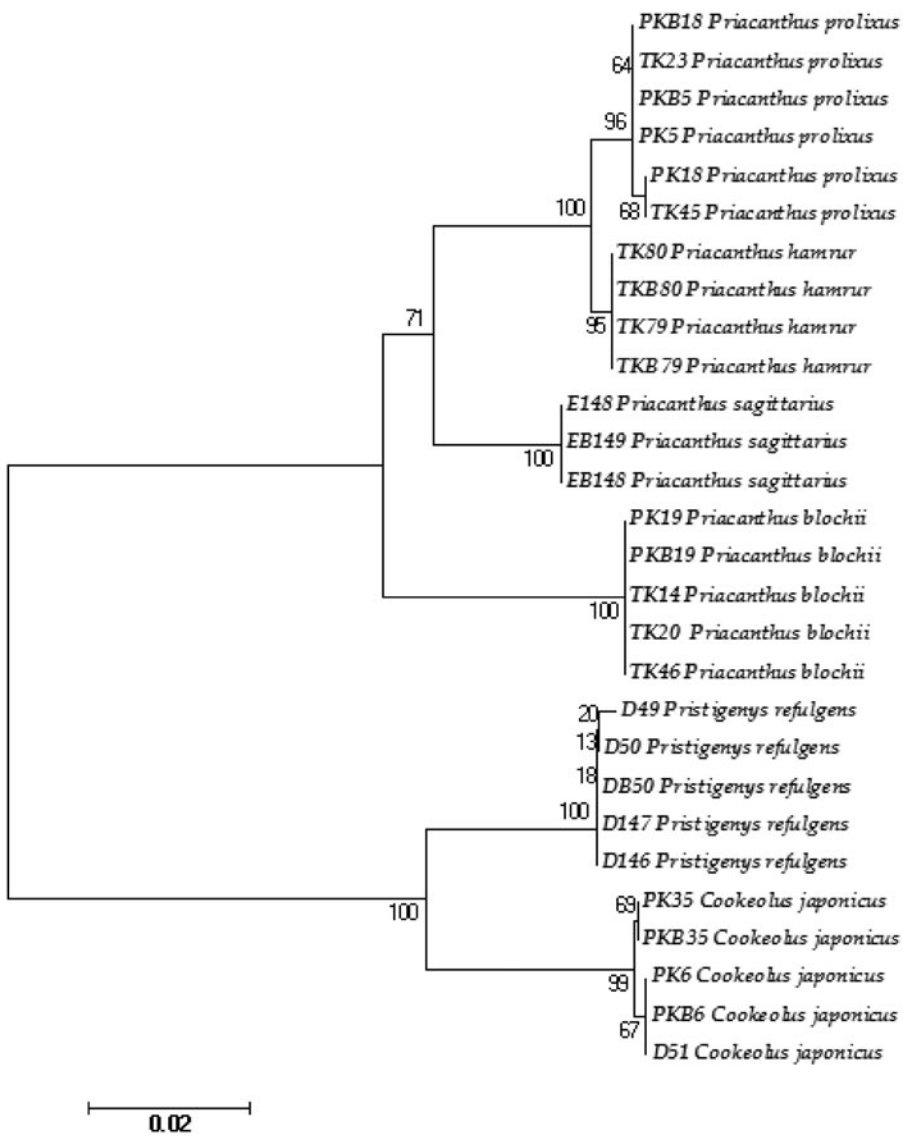

Figure 1. Neighbor-joining tree of 16S rRNA gene sequences derived from Priacanthids using K2P distances.

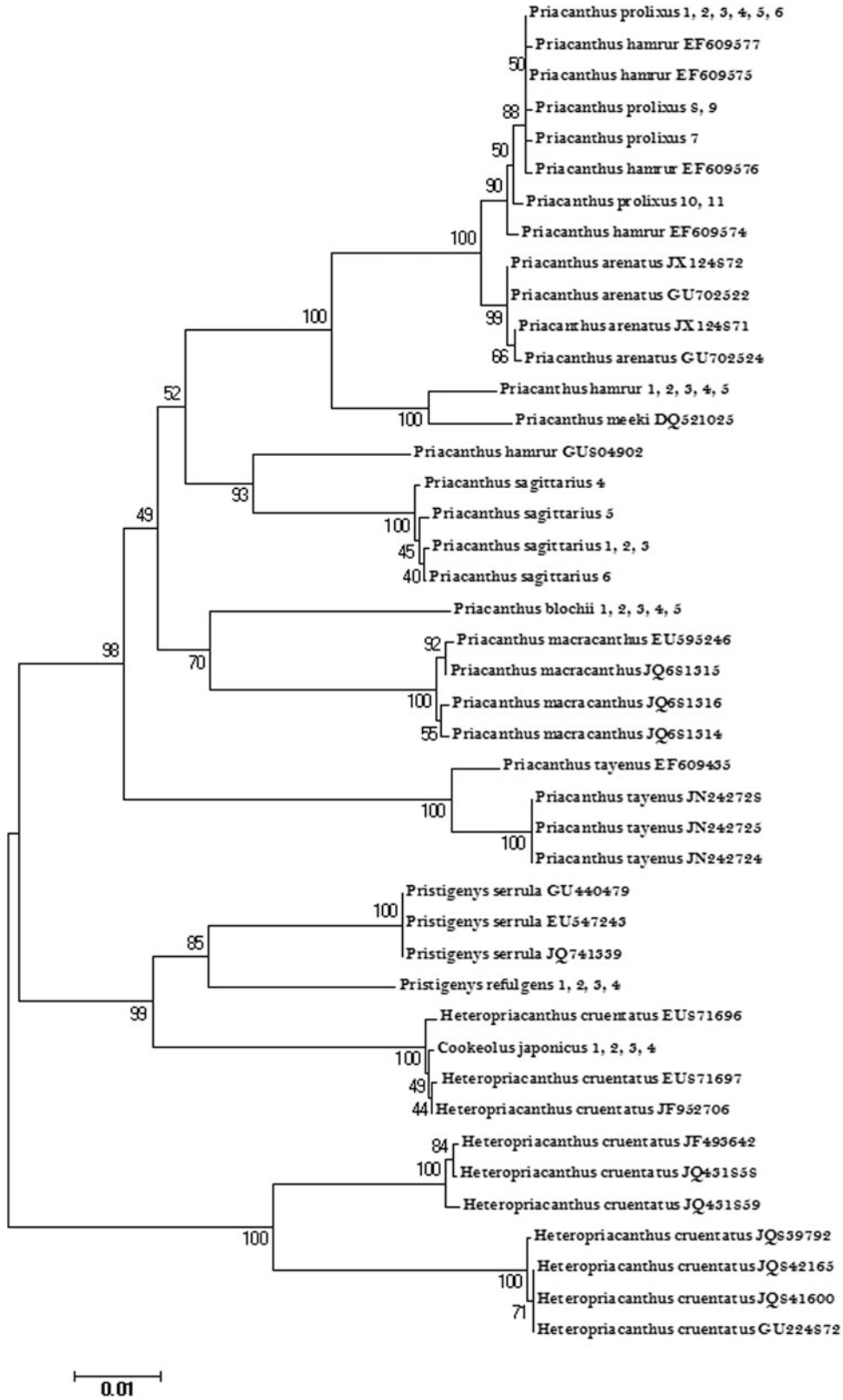

Figure 2. Neighbor-joining tree of COI gene sequences derived from Priacanthids using K2P distances.

Of the 639 sites, 123, 326, 190, 188, and two were missing, conserved, variable, parsimony informative, and singleton, respectively. The analysis showed nucleotide frequencies of A = 23.0%, T = 27.8%, G = 19.0% and C = 30.2%. As expected, average transitional pairs (si = 72.53) were more frequent than transversional pairs (sv = 27.46) with an average ratio of 2.64. Average nucleotide diversity and haplotype (gene) diversity was 0.16144 and 0.994, respectively. The mean diversity in the entire population was found to be 0.007. The genetic intraspecies distance ranged from 0.000 to 0.005, while interspecies distance varied from 0.009 to 0.108. A neighbor joining tree was created to provide a graphic representation of the patterns of divergences (Figure 2). The highest intergeneric distance (0.106) observed was between *P. arenatus* and *Heteropriacanthus cruentatus*.

The lowest intergeneric distance (0.009) was between *P. prolixus* and *Priacanthus arenatus.*

Discussion

Our study provides molecular evidence for species identification of the family Priacanthidae based on two mitochondrial genes. Six species of priacanthids from the Indian waters were found genetically distinct from each other and portioned into three groups without any haplotype sharing. Lakra et al., (2009) reported high nucleotide divergence among the sciaenids species in the Indian waters using 16S rRNA gene sequences and Iwatsuki (2013) shows similar results in *Acanthopagrus latus*, indicating the effectiveness of 16S rRNA gene sequence for accurate identification of species. The high degree of K2P nucleotide divergence with 16S rRNA gene (interspecies 0.008–0.157), indicated its ability to adequately describe interrelationships of priacanthid species. The barcode sequences based on partial sequence information of COI gene has been widely used in species identification and validation of species identity (Lakra et al., 2009; Ward et al., 2005).

Priacanthus prolixus is closely related to *P. arenatus*, *P. harmer,* and *P. meeki* with group sharing characters such as crescentic caudal fin and higher counts of dorsal-fin and anal fins rays. The body color in fresh specimen was similar in all three species except that *P. prolixus* has a reddish-yellow pectoral fin (Motomura et al., 2001). *Priacanthus prolixus* is very similar to *P. hamrur.* The fish called *P. hamrur* in the GenBank and BOLD database from India (eight sequences with NCBI accession numbers: EF609574–EF609577, KJ000235, KF830276, and FJ265857) should be *P. prolixus* based on the intraspecies genetic distance observed (D = 0.3%). The GenBank sequence FJ265856 shows 4.6% average divergence with other *P. prolixus* sequences, possibly due to sequence errors. It may represent another species of *Priacanthus*, but due the lack of voucher specimens, we cannot assign any species name. During the collection period, we observed that *P. prolixus* are moderately abundant in catches along with *P. hamrur* and *P. blochii* along the southwest coast of India. *Priacanthus prolixus* Starnes (1988) was originally described on the basis of 12 samples from the Arabian Sea (Off Somalia) and is endemic to that area (Starnes, 1988). Later, color description was given based on the color photographs of freshly collected materials from Karnataka and Kerala (Motomura et al., 2001). However, our collections of these species from Tuticorin, Chennai and Kolkata shows that *P. prolixus* is not endemic to the Arabian Sea but is widely distributed in the Indian Ocean, including the Bay of Bengal. Our analysis of the COI gene showed *P. arenatus* Cuvier, 1829 sequences from Brazil (NCBI accession nos.: JX124872, GU702522, JX124871, and GU702524) clustering with *P. prolixus* sequences from India with a mean interspecies distance of 0.9%, suggesting *P. arenatus* may be a senior synonym of *P. prolixus*. *Priacanthus arenatus* is very similar to *P. hamrur* and *P. prolixus* of the Indo-Pacific and *P. meeki* of the Hawaiian Pacific region, with differences only in meristic counts and morphometry (Starnes, 1988). However, Caldwell (1962) stated that *P. arenatus* might be synonymous with Indo-Pacific forms. Therefore, there is a need to conduct further taxonomic inquiry into the systematic position of these two species.

Pristigenys refulgens described from Seychelles Islands was considered as junior synonym of *P. niphonia.* However, recently acquired materials have facilitated a reinvestigation resulting in the redescription and designating a neotype for *Pristigenys refulgens* (Iwatsuki et al., 2012). Nair & Geetha (2006) had recorded *Pristigenys niphonia* from Indian waters and this may be a possible misidentification of *P. refulgens,* which is widely distributed in the Indian Ocean and Western Pacific (Iwatsuki et al., 2012). The sequence of *P. niphonia* (JQ681466) from South China Sea shows 3.3% divergence with our sequence of *P. refulgens* from India. The results based on the partial sequences of COI genes supply the molecular evidence to support Iwatsuki et al., (2012) that *P. refulgens* should be a valid species distinct from *P. niphonia.*

COI gene analysis in the current study shows that the family Priacanthidae is split into three major clades (Figure 2), with high bootstrap support (>90%). The first clade includes the genus *Priacanthus,* the second clade includes the *Pristigenys,* and *Cookeolus* with *Heteropriacanthus* in the third clade. However, the *Heteropriacanthus cruentatus* sequences grouped in two very different clusters, one including all the samples from the Society Islands (French Polynesia), and the other samples from Belize. The distance between *P. prolixus* and *P. hamrur* was 9.5% while the distance between the two *Heteropriacanthus cruentatus* clusters was 11.8%, a difference that was statistically significant. These may comprise either some previously synonymized species or may represent a latent species. Three sequences labeled *Heteropriacanthus cruentatus* (NCBI accession nos.: EU871696, EU871697, and JF952706) should be *Cookeolus japonicus* based on their intraspecies genetic distance. Cryptic species have been found in various fishes living in different habitats and more recently found in the lanternfish genus *Benthosema*, that are found on the mesopelagic zone (Zahuranec et al., 2012). Also DNA barcoding studies in the family Carangidae have successfully identified cryptic species diversity within a single known species (Mat Jaafar et al., 2012). Sequence number per species in our study was relatively limited, and it is likely that a more detailed analysis with wide geographical sampling will reveal the true extent of speciation in this family.

Acknowledgements

Authors are grateful to Yukio Iwatsuki (Miyazaki University, Japan) for providing valuable reprints. The authors wish to thank Akhilesh K. V., Rajool Shanis, Manju Sebastine, E. M. Abdussamad (CMFRI, India) and Rajkumar (NBFGR, India) for the support. Authors are grateful to Rahul G. Kumar for suggestions and helpful comments on the earlier version of manuscript. The authors also thank the anonymous reviewers for their helpful comments on the manuscript.

Declaration of interest

The authors report no conflicts of interest. The authors are grateful to the Central Marine Fisheries Research Institute (CMFRI), Kochi, for the support. The authors thank the Center for Marine Living Resources and Ecology and the Ministry of Earth Sciences, India, for providing funding support.

References

Bande VN, Menon NG, Balachandran K. (1989). Studies on the distribution and abundance of Bullseye (Priacanthus spp.) in the EEZ of India. In: Mathew KJ, editor. Proceeding of first workshop on scientific result of FORV Sagar Sampada. Cochin: CMFRI. p. 233–9.

Caldwell DK. (1962). Western Atlantic fishes of the family Priacanthidae. Copeia 417–24.

Hall TA. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symp Ser 41:95–8.

Hebert PDN, Cywinska A, Ball SL, Ward JR. (2003). Biological identifications through DNA barcodes. Proc Biol Sci 270:313–22.

Iwatsuki Y. (2013). Review of the *Acanthopagrus latus* complex (Perciformes: Sparidae) with descriptions of three new species from the Indo-West Pacific Ocean. J Fish Biol 83:64–95.

Iwatsuki Y, Matsuda T, Starnes WC, Nakabo T, Yoshino T. (2012). A 274 valid priacanthid species, *Pristigenys refulgens* (Valenciennes 1862), and a redescription of *P. niphonia* (Cuvier in Cuvier & Valenciennes

1829) in the Indo-West Pacific (Perciformes: Priacanthidae). Zootaxa 3206:41–57.

James PSBR, Pillai VN. (1989). Fishable concentrations of fishes and crustaceans in the off shore and deep-sea areas of the Indian EEZ based on observation made onboard FORV Sagar Sampada. In: Mathew KJ, editor. Proceeding of First Workshop on Scientific Result of FORV Sagar Sampada. Cochin: CMFRI. p. 201–13.

Keskin E, Atar HH. (2012). Molecular identification of fish species from surimi-based products labelled as Alaskan pollock. J Appl Ichthyol 28: 811–14.

Lakra WS, Goswami M, Gopalakrishnan A. (2009). Molecular identification and phylogenetic relationships of seven Indian Sciaenids (Pisces: Perciformes, Sciaenidae) based on 16S rRNA and cytochrome c oxidase subunit I mitochondrial genes. Mol Biol Rep 36:831–9.

Mat Jaafar TN, Taylor MI, Mohd Nor SA, de Bruyn M, Carvalho GR. (2012). DNA Barcoding reveals cryptic diversity within commercially exploited Indo-Malay Carangidae (Teleosteii: Perciformes). PLoS ONE 7:e49623.

Miller SA, Dykes DD, Polesky HF. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acid Res 16:1215.

Motomura H, Burhanuddin AI, Kimura S, Iwatsuki Y. (2001). Fresh colour notes for *Priacanthus prolix*us Starnes, 1988 from the west coast of India (Perciformes: Priacanthidae). Biogeography 3: 77–81.

Nair RJ, Geetha PM. (2006). First record of the Japanese bigeye *Pristigenys niphon*ia (Cuvier & Valenciennes) (Perciforrnes: Priacanthidae) from the Indian seas. J Mar Biol Ass Ind 48:263–6.

Palumbi S, Martin A, Romano S, McMillan WO, Stice L, Grabowski G. (1991). The simple fool's guide to PCR, version 2.0. Honolulu, HI: Department of Zoology and Kewalo Marine Laboratory, University of Hawaii.

Rozas J, Sanchez-DelBarrio JC, Messeguer X, Rozas R. (2006). DNA sequence polymorphism version 4.10.9.

Sivakami S, Vivekanandan E, Nammalwar P, Feroz Khan M, Zacharia PU, MohanRaj G, Grace M, Jayasankar P. (1998). The non-conventional finfish resources of the Indian EEZ. In: Hameed MS, Kurup BM, editors. Cochin: Publication No l. School of Industrial Fisheries, Cochin University of Science and Technology. p. 243–55.

Starnes WC. (1988). Revision, phylogeny and biogeographic comments on the Circumtropical Marine Percoid Fish Family Priacanthidae. Bull Mar Sci 43:117–203.

Tamura Z, Stecher G, Peterson D, Filipski A, Kumar S. (2013). MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–9.

Timm J, Figiel M, Kochzius M. (2008). Contrasting patterns in species boundaries and evolution of anemonefishes (Amphiprioninae, Pomacentridae) in the centre of marine biodiversity. Mol Phylogenet Evol 49:268–76.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN. (2005). DNA barcoding Australia's fish species. Philos Trans R Soc Lond B: Biol Sci 360:1847–57.

Zahuranec BJ, Karuppasamy PK, Valinassab T, Kidwai S, Bernardi J, Bernardi G. (2012). Cryptic speciation in the mesopelagic environment: Molecular phylogenetics of the lanternfish genus *Benthos*ema. Mar Genomics 7:7–10.