RESEARCH ARTICLE Indian Journal of Animal Research, Volume 56 Issue 8: 933-940 (August 2022)

Character-based Diagnostic Keys, Molecular Identification and Phylogenetic Relationships of Fishes based on Mitochondrial Gene from Pulicat Lake, India: A Tool for Conservation and Fishery Management Purposes

A. Rathipriya[1], A. Kathirvelpandian[1,2], S.A. Shanmugam[1], A. Uma[3], E. Suresh[1], N. Felix[4] 10.18805/IJAR.B-4905

ABSTRACT

Background: Pulicat Lake is one of India's most valuable ecosystems. Correct species identification is essential for the conservation and management of finite resources. Molecular signatures and character-based keys could be used for faster identification. This will be a powerful tool to obtain a significant amount of accurate information quickly and conveniently and could be used for forensic applications and the conservation of fish in Indian waters.

Methods: In addition to morphological identification, the species were identified using various approaches, such as Neighbour-joining (NJ), Maximum Likelihood (ML) trees and the Character-based method. For each species, the LOGic (BLOG 2.0) DNA Barcode analysis method was used to identify different positions of key diagnostic nucleotides in a given set.

Result: DNA barcodes were generated from twenty-nine species of fish representing seven orders, 26 genera and 21 families in this study. The current study's findings show that the 16S rRNA barcodes facilitated successful fish species identification and also provided phylogenetic information to differentiate the fishes. The character-based molecular diagnostic keys for fishes were also developed *in-silico* from the data set comprising 393 mitochondrial 16S rRNA sequences including the sequences generated in the present study and other published resources.

Key words: BLOG 2.0, DNA chip, *In-silico* analysis, Mitochondrial 16S rRNA gene, Pulicat lake.

INTRODUCTION

Pulicat lake in India is the second-largest brackish-water lake running almost parallel to the Bay of Bengal. Govindan and Ravichandran (2016) identified 83 species in Pulicat Lake, categorising them into 14 orders and 50 families. The biodiversity details from this area have been reported by several researchers. So far, no recent consolidated data is available. DNA-based markers, particularly mitochondrial DNA (mtDNA) markers, are commonly used to improve the resolution of systematic connections between species. According to Finizio *et al*. (2006), particular morphological characteristics of fish are sufficient for identifying fish species; however, there is an issue when processed food is involved. Mitochondrial DNA is a useful tool for identifying fish species (Teletchea, 2009) maintaining fish stocks and monitoring seafood (Rubinoff *et al*., 2006). These genes are non-coding and conserved and therefore played an important part in determining phylogenetic relationships. In several fishes, the mitochondrial 16S rRNA gene was employed for molecular phylogenetic analysis (Li *et al*., 2013).

Species categorization with DNA Barcode is a technique for assigning an unknown specimen to a recognised species by evaluating its DNA Barcode sequence and it has been shown to work on a variety of organisms (Schindel and Miller, 2005). A supervised machine learning method termed BLOG (Barcoding with LOGic) is one of the ad-hoc DNA Barcodes classification tools (Weitschek *et al*., 2013). It's a character-based method for classifying specimens into species by using classification criteria that compactly identify species in terms of DNA Barcode positions of essential diagnostic nucleotides. This study will give a DNA barcode database for fish species found in Pulicat lake, which will aid in the

[1]Institute of Fisheries Postgraduate Studies, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, OMR Campus, Vaniyanchavadi-603 103, Chennai, Tamil Nadu, India.
[2]Presently at ICAR-National Bureau of Fish Genetic Resources, PMFGR Centre, Kochi-682 018, Kerala, India.
[3]Dr. M.G.R. Fisheries College and Research Institute, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, Ponneri-601 204, Chennai, Tamil Nadu, India.
[4]Directorate of Incubation and Vocational Training in Aquaculture, Tamil Nadu Dr. J. Jayalalithaa Fisheries University, ECR-Muttukadu-603 112, Chennai, Tamil Nadu, India.

Corresponding Author: A. Kathirvelpandian, ICAR-National Bureau of Fish Genetic Resources, PMFGR Centre, Kochi-682 018, Kerala, India. Email: kathirars@gmail.com

How to cite this article: Rathipriya, A., Kathirvelpandian, A., Shanmugam, S.A., Uma, A., Suresh, E. and Felix, N. (2022). Character-based Diagnostic Keys, Molecular Identification and Phylogenetic Relationships of Fishes based on Mitochondrial Gene from Pulicat Lake, India: A Tool for Conservation and Fishery Management Purposes. Indian Journal of Animal Research. 56(8): 933-940. DOI: 10.18805/IJAR.B-4905.

Submitted: 26-03-2022 **Accepted:** 20-04-2022 **Online:** 23-05-2022

preservation of native diversity and ecological stability for the long-term use of the country's fish genetic resources.

MATERIALS AND METHODS

The study was conducted during March 2020-May 2021 at the Institute of Fisheries Post Graduate Studies, TNJFU, Chennai, Tamil Nadu. The tissue and voucher specimens of twenty-nine species from 21 families were collected from different locations of Pulicat Lake. All of the specimens were morphologically identified using the FAO Fish Identification taxonomy keys. Total DNA was isolated from the muscle tissues that were preserved in 99% ethanol using the salting-out procedure (Miller *et al*., 1988). The PCR amplification of the partial segment of the gene was carried out using primers 16S FP 5′-CGC CTG TTT ATC AAA AAC AT-3′ 16S RP 5′-CCG GTC TGA ACT CAG ATC ACG T-3′ (Palumbi, 1996). Bidirectional sequencing of PCR products was carried out using the Sanger sequencing methodology in ABI 3730 DNA sequencer.

The raw sequences were edited and aligned using BIOEDIT sequence alignment version 7.0.5.2 (Hall, 1999). Firstly, BLAST searches were conducted for each sample to identify highly similar sequences on GenBank. The highly similar sequences were then used to generate a Neighbour-Joining (NJ) and Maximum Likelihood (ML) tree for each of the studied species separately using based on Kimura two-parameter distance model (K2P) as implemented in MEGA version 6.0 (Tamura *et al*., 2013). Sequences of *Litopenaeus vannamei* were selected as the out-group for phylogenetic tree construction. BLOG 2.0 (Weitschek *et al*., 2013) applies a supervised machine learning approach, which involves the usage of test and training data sets. mtDNA 16S rRNA gene sequences of fishes were obtained directly from the Fish Barcode Information system and GenBank Nucleotide Database. For this purpose, the datasets of 393 sequences were divided into a training set (314 sequences, 80% of total) and test set (79 sequences, 20% of total) for the identification of nucleotide positions and classification of species with the help of character-based identification keys. The probe sequences were identified using Gene Runner version 6.2.07.

RESULTS AND DISCUSSION

Nucleotide sequence and genetic divergence analysis

The study represented 29 species of 26 genera, 21 families and 7 orders. The species list and the GenBank accession numbers are provided in Table 1. According to the current study, many researchers utilised the BLASTn similarity approach to check sequences (Rathipriya *et al*., 2019; Lee and Kim, 2020; Das and Choudhury, 2021; Saravanan *et al*., 2021). They studied 16 rRNA gene sequences showed both conserved and variable regions. This could be responsible for the differences in sequence length generated. This may also enable the identification of appropriate taxon-specific primers for phylogenetic applications. The average GC content was (48.1%) similar to the one reported by Ward *et al*. (2005) in teleost (47.10%). The 16S rRNA sequences of fishes were aligned to yield a final size of 523 bp. A total of 260 sites were constant (Fig 1), 263 sites exhibited variable, of which 210 were parsimony informative and 53 were singletons. The estimated transition/transversion bias (R) was 1.297. The sequence analysis revealed average nucleotide frequencies as 28.85% (A), 21.82% (T/U), 25.18% (C) and 24.15% (G). The average overall mean distance was 0.103. The highest distance was observed between *Cynoglossus arel* and *Stolephorus commersonnii* (0.184) and the lowest distance was observed between *Lutjanus fulviflamma* and *L. johnii* (0.020). In this study, K2P% was used to assign an unknown specimen to

Table 1: GenBank accession numbers of 16S rRNA gene for the fishes

Name of the species	GenBank accession no.
Gerres filamentosus	MW534557
Terapon puta	MW578372
Upeneus sulphureus	MW578527
Karalla dussmieri	MW785183
Secutor insidiator	MW785215
Drepane punctata	MW785201
Siganus javus	MW785838
Sillago vincenti	MW785839
Scomberoides tol	MW578517
Alepes kleinii	MW785213
Etroplus suratensis	MW785757
Pomadasys maculatus	MW785204
Lutjanus fulvus	MW785214
Lutjanus johnii	MW888468
Lutjanus fulviflamma	MW959196
Ostorhinchus pleuron	MW785848
Nematalosa nasus	MW534558
Escualosa thoracata	MW785189
Stolephorus commersonnii	MW534695
Encrasicholina heteroloba	MW888466
Opisthopterus tardoore	MW785852
Cynoglossus arel	MW563939
Hippoglossina oblonga	MW945438
Hyporhamphus quoyi	MW578374
Triacanthus biaculeatus	MW578519
Moolgarda perusii	MW578526
Planiliza macrolepis	MW785218
Planiliza subviridis	MW785205
Synodus kaianus	MW785209

Table 2: Summary of 16S rRNA gene sequences genetic divergences (K2P per cent) between various taxonomic levels.

Comparisons between	Distance	
	Minimum	Maximum
Species	2.0%	18.4%
Genera	3.4%	21.4%
Families	5.4%	23.2%
Orders	7.0%	25.6%

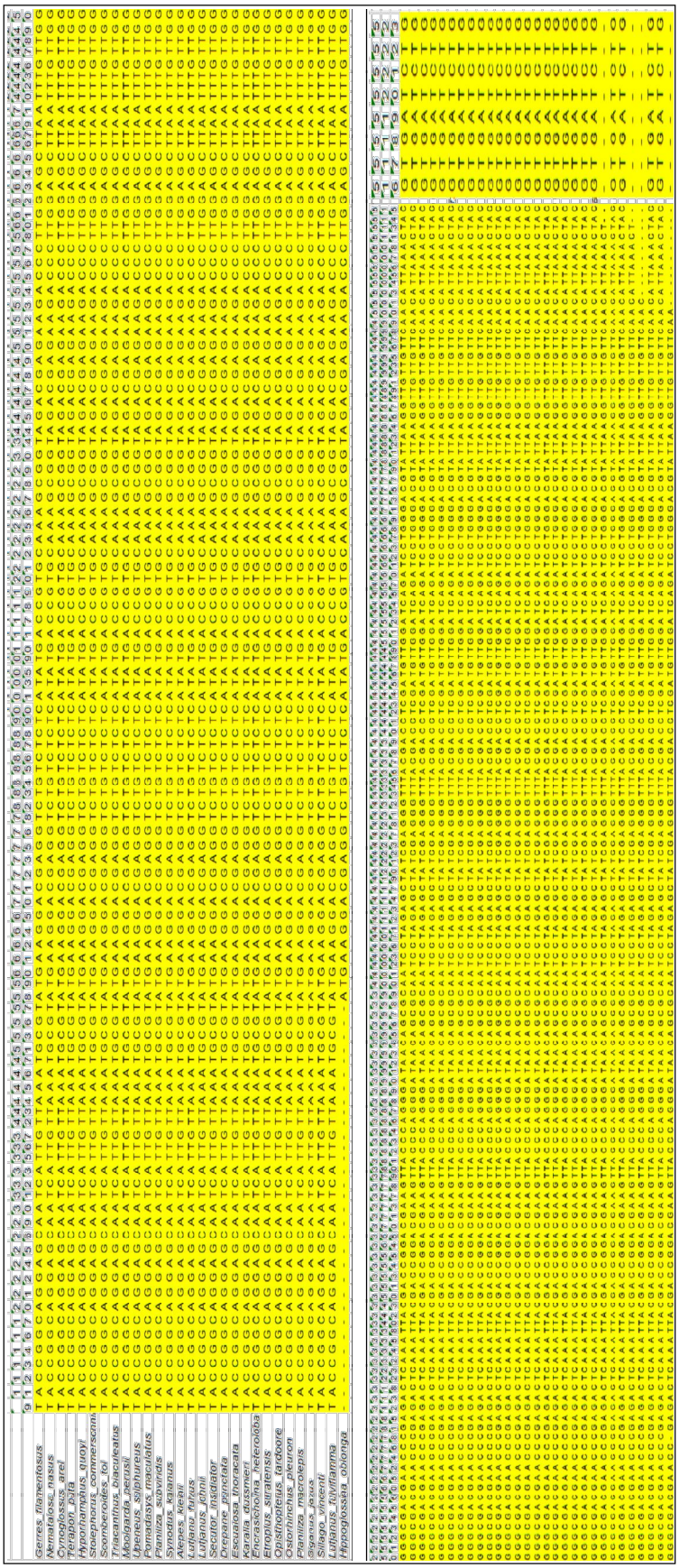

Fig 1: Conserved sites of 16S rRNA sequences.

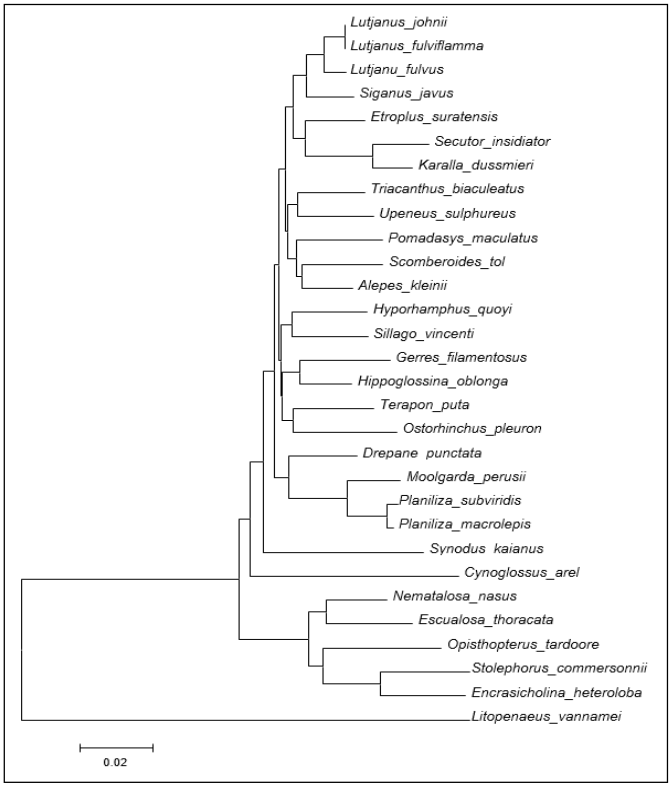

Fig 2: Summary form of Neighbour joining tree of 16S rRNA gene sequences derived from 29 fish species using K2P distances, out-group *Litopenaeus vannamei* sequence.

Fig 3: Summary form of maximum likelihood tree of 16S rRNA gene sequences derived from 29 fish species using K2P distances, out-group *Litopenaeus vannamei* sequence.

Table 3: Species formula for fishes of pulicat lake using BLOG2.0.

Species	Diagnostic nucleotide or species formula
Chanos chanos	pos50=G pos51=T pos55=A pos97=C orpos97=A pos175=T pos195=C
Chirocentrus nudus	pos158=G pos174=A pos175=G
Clarias batrachus	pos158=T pos175=C pos178=G
Diagramma picta	pos84=T pos92=C pos174=C
Diodon hystrix	pos46=T pos130=C pos173=A
Epinephelus lanceolatus	pos11=A pos46=C pos60=C pos97=A
Euthynnus affinis	pos17=A pos69=A pos175=A
Glyptocephalus cynoglossus	pos12=C pos158=C pos175=G
Gymnothorax reticularis	pos68=C pos174=C pos195=T
Kyphosus vaigiensis	pos11=C pos18=T pos60=A pos92=T pos141=T
Eubleekeria splendens	pos12=A pos110=T pos173=G
Mugil cephalus	pos46=T pos68=G pos92=G pos173=C pos195=T or pos60=G pos110=T
Muraenesox cinereus	pos69=A pos97=T pos141=G pos158=C pos178=C
Myoxocephalus octodecemspinosus	pos17=G pos18=A pos92=G
Platycephalus indicus	pos22=A pos24=G pos84=T
Psettodes erumei	pos11=C pos175=C pos178=A
Rachycentron canadum	pos68=T pos175=G pos41=A pos12=C
Scatophagus argus	pos178=A pos84=C pos23=G pos25=T pos51=T pos55=C
Terapon jarbua	pos12=C pos41=C pos51=A pos195=C pos69=G
Valamugil cunnesius	pos12=A pos18=T pos69=G pos175=C
Valamugil seheli	pos23=C pos141=G pos195=C
**Etroplus suratensis*	pos11=T pos68=T pos84=G
**Pomadasys maculatus*	pos18=T pos24=T pos92=G
**Secutor insidiator*	pos22=T pos68=T pos175=G
**Siganus javus*	pos17=A pos174=A pos195=T
**Drepane punctata*	pos193=G pos250=T
**Encrasicholina heteroloba*	pos293=G pos302=A
**Gerres filamentosus*	pos193=T pos219=A pos302=C pos355=C
**Lutjanus fulvus*	pos38=G pos355=C
**Moolgarda perusii*	pos247=A
**Planiliza macrolepis*	pos229=G pos249=A
**Sillago vincenti*	pos193=A pos338=G
**Lutjanus fulviflamma*	pos62=A
**Opisthopterus tardoore*	Pos72=G

*****Indicates the species of the present study.

Table 4: Species-specific probes for the fishes of pulicat lake identified through CBIS (16S rRNA).

Species name	Position	Species-specific probes	Tm	Ta
Chanos chanos	pos195=C	AGACATCCATGCAAGTTGGATCAC	57.9	52.9
	pos175=T	ACAGTTAAGCATCTCCCTTACACCGA	59.9	54.9
Chirocentrus nudus	pos158=G	GTAGAGGTGAAATGCCTACCGAGTCA	60.2	55.2
	Pos174=A	TACCGAGTCAGGTTATAGCTGGTTAC	57.9	52.9
Clarias batrachus	Pos158=T	CCCCCTTCAAGTCAATGAAATTGATCTG	58.7	53.7
	pos175=C	AAAAGTGCCTGAAGGTGGATTTAGTAGT	58.7	53.7
Diagramma picta	pos84=T	GTCTCTTAAATGGGGACCCGTATGAAT	58.6	53.6
	pos92=C	AATGGGGACCCGTATGAATGGCATA	60.3	55.3
Diodon hystrix	pos46=T	GCCCTGTGACTATATGTTTAACGGCC	60.1	55.1
	pos130=C	ATGGCACAACGAGGGCTTAACT	59.2	54.2
	pos173=A	AATGAAATTGATCTCTCCGTGCAGAAG	58.2	53.2
Epinephelus lanceolatus	pos60=C	ATATGTTTAACGGCCGCGGTATTTTG	59.2	54.2
Euthynnus affinis	pos17=A	CTTGCAAAATCAAAGAATAAGAGGTCCAG	57.4	52.4
	pos69=A	CGGCCGCGGTATTTTAACCGT	60.9	55.9

Table 4: Continue.....

Table 4: Continue.....

	pos175=A	AGTGAAATTGATCTCCCCGTGCAGAA	60.8	55.8
Glyptocephalus cynoglossus	pos158=C	GTCAATGAAATTGATCTCCCCGTGCA	60.2	55.2
	pos175=G	TGCAGAAGCGGGGATTAAACCATAA	59.2	54.2
Gymnothorax reticularis	pos68=C	TGCCCTGTGACAGCAGTGTTTAA	59.4	54.4
	pos174=C	TTTACTGTCTCCTCCCCCCAGTCA	60.7	55.7
	pos195=T	TCAATGAAATTGATCTCCCCGTGCAGA	60.8	55.8
Kyphosus vaigiensis	pos11=C	CATCGCCTCTTGCAAAACTGAGG	59.2	54.2
Eubleekeria splendens	pos12=A	CTTGAAAACCAAAAAATAAGAGGTCCCA	56.9	51.9
	pos110=T	GTCCTTTAAATGAGGACCCGTATGAA	57.1	52.1
	pos173=G	CAATGAAATTGATCCCCCCGTGCA	60.4	55.4
Mugil cephalus	pos46=T	ATATACCGCCGTCGTCAGCC	59.2	54.2
	pos92=G	ATGAGAACCAGTATGAATGGCTAGACGAG	60.1	55.1
	pos173=C	CCACAAGCCTCGCCTGTTTAC	58.7	53.7
Muraenesox cinereus	pos178=C	CTGTCTCCCGCACCCAGTC	59.5	54.5
Myoxocephalus octodecemspinosus	pos17=G	TCCCTTACACTGAGAAGTCACCC	57.9	52.9
	pos18=A	CCCTTACACTGAGAAGTCACCCG	59.1	54.1
	pos92=G	ACAAGCCCCTGTAAATACCCCCTAAATA	59.4	54.4
Platycephalus indicus	pos22=A	TAAAGAATTACGGATGATGCACTGAAA	55.8	50.8
	pos84=T	AAGCGGGAAATAGAGCGTCCC	59.4	54.4
Psettodes erumei	pos11=C	CACTTGTCTTTTAAATGGAGACCTGTAT	55.9	50.9
	pos175=C	ACACCCCAAGCTAAAGGAATAAACTGAT	58.7	53.7
	pos178=A	CCCCAAGCTAAAGGAATAAACTGATTG	56.8	51.8
Rachycentron canadum	pos41=A	AACAATACACAATTTATAACCCCTAATAC	56.0	51.0
Scatophagus argus	pos178=A	TTCGGAGCAACAGAGAAAGTACC	57.2	52.2
	pos23=G	ACTGAGAAAGCATCCGTGCAAATC	58.7	53.7
	pos51=T	TGCGGTATCCTGACCGTACGAA	60.2	55.2
Terapon jarbua	pos51=A	GACCGTGCGAAGGTAGCGTAAT	59.8	54.8
	pos69=G	GTGCGAAGGTAGCGTAATCACTTGTC	60.5	55.5
Valamugil cunnesius	pos175=C	AAATAACCCAGTAAAGCCAAAAAAAGCAG	57.9	52.9
Valamugil seheli	pos23=C	CCTGTGACCCCCAGTTTAACG	57.8	52.8
	pos141=G	CCCGGTCAATGAAATTGATCTTCC	56.7	51.7
**Etroplus suratensis*	pos11=T	CTGCCTGTGACCATGAGTTTAACGG	60.4	55.4
	pos68=T	AGCGCAATCACTTGTCTTTTAAATGAAGA	58.6	53.6
	pos84=G	TTTAAATGAAGACCCGTATGAATGGCATA	57.4	52.4
**Pomadasys maculatus*	pos18=T	CCGCCTGCCCTGTGACTTG	60.8	55.8
	pos24=T	GCCCTGTGACTTGTATGTTTAACG	56.9	51.9
	pos92=G	TTTAAATGAGGACCCGTATGAATGGC	57.9	52.9
**Secutor insidiator*	pos22=T	GCATCATGATTTAGCCAGCAATTT	55.5	50.5
	pos68=T	AGCCCCGAAACTAGACGAGCTA	59.6	54.6
	pos175=G	TTTAGTTATAGCTGGTTGCCTAAGAAA	55.0	50.0
**Siganus javus*	pos17=A	CACCGTCGTCAGCTTACCCT	58.9	53.9
	pos174=A	AGGATTTAGCAGTAAGCAGAAAATAGAG	55.9	50.9
	pos195=T	TAAAGGAGGATTTAGCAGTAAGCAGA	56.0	51.0
**Drepane punctata*	pos193=G	TCTGAAGGAGGATTTAGCAGTAAGCAG	58.6	53.6
**Encrasicholina heteroloba*	pos293=G	GTCTTAGGTTGGGGCGACCA	58.8	53.8
	pos302=A	CTAACAACCGATTAACGAACCAAGT	56.2	51.2
**Gerres filamentosus*	pos193=T	TATGGAGCTTTAGACTATTAAGACAGACTAT	55.4	50.4
**Lutjanus fulvus*	pos38=G	GTTTAACGGCCGCGGTATTTTGA	59.2	54.2
	pos355=C	CAAGAGCTCCCGCTCTACTAAACA	58.7	53.7
**Moolgarda perusii*	pos247=A	TTTCAACTACTCCCGCTAAATGAAG	55.8	50.8
**Planiliza macrolepis*	pos229=G	TGGAGCTTTAGACGTCAGAACAGA	58.0	53.0
	pos249=A	CAGACCATGTAAGCTACCTCCATGA	58.2	53.2
**Sillago vincenti*	pos193=A	TGTCTCCTTTTTCAGGTCAATGAAATT	56.1	51.1
	pos338=G	AATGTCTTTGGTTGGGGCGACC	60.3	55.3
**Lutjanus fulviflamma*	pos62=A	GTGCGAAGGTAGCGCAATCACTTGT	62.6	57.6
**Opisthopterus tardoore*	Pos72=G	TTGAAACCCCGAAACCTCCGACG	61.9	56.9

*Indicates the species of the present study.

a known species, to detect novel sequences and to determine whether an unknown specimen is a distinct new species. The average genetic distances between species, genera, families and orders were 18.4%, 21.4%, 23.2% and 25.6%, respectively (Table 2), which was comparable with patterns observed in several fish barcoding studies (Bingpeng *et al*. 2018; Wang *et al*. 2018).

Phylogenetic tree analysis

The species phylogenetic relationships were established, with comparable genera clustered under the same nodes and different genera clustered under separate nodes. High bootstrap values (90-99%) supported the nodes. Basheer *et al*. (2015), Bineesh *et al*. (2015) and Lakra *et al*. (2011) all found similar findings in marine fish species in Indian waters. Several researchers have employed 16S rRNA gene sequences to investigate the phylogeny of various groups, including fish. With a revised classification of the Epinepelini using 12S and 16S rRNA sequences, Craig and Hastings (2007) explained the molecular phylogeny of groupers of the subfamily Epinephelinae (Serranidae). Sparks and Smith (2004) on cichlid fishes, Vinson *et al*. (2004) on Sciaenid fishes, Wiley *et al*., (1998) on lampridiform fishes and Ilves and Taylor (2009) on Osmeridae are some other examples of phylogenetic studies. The summary form of the NJ tree and ML tree is given in Fig 2 and Fig 3.

Character-based species classification of species

In terms of DNA barcode sites, the character-based method aims to identify specimens of essential diagnostic nucleotides for species. In this study, we developed 112 positions of character-based keys using 16S rRNA gene sequences (Table 3). After the species formula was identified, the Primer 3.0 tool was used to check the possible secondary structures to finalise the species-specific probes for each species under study (Table 4). The melting temperature (Tm) of primers in the 55-60°C range and 5°C below the annealing Temperature give the best results, the primers were selected within the range. Paine *et al*. (2007) developed a character-based key for identifying the 17 members of the Scombridae family which is common to the western Atlantic Ocean. Lowenstein *et al*. (2009) described 40 distinguishing locations for blue-fin tuna. Puncher *et al*. (2015) developed a character-based key for identifying Atlantic blue fin tuna larvae (*Thunnas thynnus*). Vargheese *et al*. (2019) utilised BLOG 2.0 to distinguish 82 species of elasmobranchs and found 214 diagnostic nucleotides, which is similar to Rathipriya *et al*. (2021). Mahapatra *et al*. (2020) developed 25 positions of character-based keys for scombrid identification in Indian waters. These diagnostic molecular keys could be translated into a customised DNA chip for precise species identification.

CONCLUSION

Incorrect identification could result in inaccurate estimates of spawning stock biomass and management. Genetic markers are a valid tool for unambiguous identification that is both reliable and rapid and they play a significant role in fisheries management. The probes created could be used to detect substitution in fish and fish products not only for complete specimens but also for products and damaged specimens for fish authenticity.

Conflict of interest: None.

REFERENCES

Basheer, V.S., Mohitha, C., Vineesh, N., Divya, P.R., Gopalakrishnan, A., Jena, J.K. (2015). Molecular phylogenetics of three species of the genus *Rastrelliger* using mitochondrial DNA markers. Mol. Biol. Rep. 42: 873-879.

Bineesh, K.K., Mohitha, C., Vineesh, N., Basheer, V.S., Joselet, M., Pillai, N.K., Jena, J.K., Gopalakrishnan, A. (2015). Molecular identification of three deep-sea fish species of the genus *Chelidoperca* (Perciformes: Serranidae) from Indian waters. Indian J. Fish. 62: 104-108.

Bingpeng, X., Heshan, L., Zhilan, Z., Chunguang, W., Yanguo, W., Jianjun, W. (2018). DNA barcoding for identification of fish species in the Taiwan Strait. PLoS One. 13: e0198109.

Craig, M.T., Hastings, P.A. (2007). A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. Ichthyological Research. 54: 1-17.

Das, P. and Choudhury, R. (2021). Mitochondrial D-loop genetic diversity studies of rhinoceros unicornis in Assam, India. Indian Journal of Animal Research. 55(1): 6-10.

Finizio, D., Guerriero, A., Russo, G., Gian, L., Ciarcia, G. (2006). Identification of gadoid species (Pisces, Gadidae) by sequencing and PCR-RFLP analysis of mitochondrial 12S and 16S rRNA gene fragments. Eur. Food Res. Technol. 225(3): 337-344.

Govindan, S., Ravichandran, R. (2016). Fish Fauna Diversity and Conservation Status of Pulicat Lagoon in Tamil Nadu. Annals of Aquaculture and Research. 3(2): 1018.

Hall, T.A. (1999). BioEdit: A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium Series. 41: 95-98.

Lakra, W.S., Verma, M.S., Goswami, M., Lal, K.K., Mohindra, V., Punia, P., Gopalakrishnan, A., Singh, K.V., Ward, R.D., Hebert, P. (2011). DNA barcoding Indian marine fishes. Mol. Ecol. Resour. 11(1): 60-71.

Lee, D.J. and Kim, Il-C. (2020). The new freshwater crab belongs to the genus Geothelphusa as inferred from mitochondrial and nuclear DNA markers. Indian Journal of Animal Research. 54(4): 424-429.

Li, Y., Ren, Z., Shedlockm, A.M., Wu, J., Sang, L., Tersing, T. (2013). High altitude adaptation of the schizothoracine fishes (Cyprinidae) revealed by the mitochondrial genome analysis. Gene. 517: 169-178.

Lowenstein, J.H., Amato, G., Kolokotronis, S.O. (2009). The real maccoyii: Identifying tuna sushi with DNA barcodes - contrasting characteristics attributes and genetic distances. PLoS ONE 4: e7866.

Mahapatra, S., Rathipriya, A., Priyadarshini, D., Suresh, E., Shanmugam, S.A., Kathivelpandian, A. (2020). Character-based identification system of scombrids from Indian waters for authentication and conservation purposes, Mitochondrial DNA Part B. 5(3): 3239-3242.

Paine, M.A., McDowell, J.R., Graves, J.E. (2007). Specific identification of western Atlantic Ocean scombrids using mitochondrial DNA cytochrome c oxidase subunit I (COI) gene region sequences. Bull Marine Sci. 80: 353-367.

Puncher, G.N., Arrizabalaga, H., Alemany, F., Cariani, A., Oray, I.K., Karakulak, F.S., Basilone, G., Cuttitta, A., Mazzola, S., Tinti, F. (2015). Molecular identification of Atlantic Blue-fin tuna (*Thunnus thynnus*, Scombridae) larvae and development of a DNA character-based identification key for Mediterranean scombrids. PLOS One. 10(7): e0130407.

Rathipriya, A., Karal Marx, K., Jeyashakila, R. (2019). Molecular identification and phylogenetic relationship of flying fishes of Tamil Nadu coast for fishery management purposes. Mitochondrial DNA Part A. 1-11. DOI: 10.1080/24701394.2018.1558220.

Rathipriya, A., Kathirvelpandian, A., Shanmugam, S.A., Suresh, E., Felix, N. (2021). Character-based identification key for commercially important fishes of Pulicat lake: Tool for conservation and management. Mitochondrial DNA Part A. 32:4, 120-125. DOI: 10.1080/24701394.2021.1883009.

Rubinoff, D., Cameron, S., Will, K. (2006). A genomic perspective on the shortcomings of mitochondrial DNA for 'barcoding' identification. J. Hered. 97: 581-594.

Saravanan, K., Kumar, S., Praveenraj, J., Meena, B.L., Kasinath, B.L., Kiruba-Sankar, R. and Roy, S.D. (2021). Molecular characterization of muscle infecting *Myxobolus* sp. causing outbreak in *Labeo rohita*, Rohu: First report from Andaman Islands, Indian Journal of Animal Research. 55(2): 211-216.

Schindel, D., Miller, S. (2005). DNA barcoding a useful tool for taxonomists. Nature. 435: 17-17.

Sparks, J.S., Smith, W.L. (2004). Phylogeny and biogeography of the Malagasy and Australasian rainbow fishes (Teleostei: Atherinoidei): Gondwanan vicariance and evolution in freshwater. Mol. Phylogenet. Evol. 33: 719-734.

Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S. (2013). MEGA6: Molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution. 30: 2725-2729.

Teletchea, F. (2009). Molecular identification methods of fish species: Reassessment and possible applications. Rev. Fish Biol. Fish. 19: 265-293.

Vargheese, S., Chowdhury, L.M., Shijin Ameri, Anjela Mercy, A., Kathirvelpandian, A. (2019). Character based identification system for Elasmobranchs for conservation and forensic applications. Mitochondrial DNA Part A. 30(4): 651-656.

Vinson, C., Grazielle, G., Schneider, H., Sampaio, I. (2004). Sciaenidae fish of the Caete river estuary, Northern Brazil: Mitochondrial DNA suggests explosive radiation for the Western Atlantic assemblage. Genet. Mol. Biol. 27: 174-80.

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D. (2005). DNA barcoding Australia's fish species. Philos Trans R Soc Lond B Biol Sci. 360: 1847-1857.

Wang, L., Wu, Z., Liu, M., Liu, W., Zhao, W., Liu, H., You, F., (2018). DNA Barcoding of Marine Fish Species from Rongcheng Bay, China. PeerJ. 6: e5013.

Weitschek, E., Van Velzen, R., Felici, G., Bertolazzi, P. (2013). BLOG 2.0: A software system for character based species classification with DNA Barcode sequences: What it does, how to use it. Mol. Ecol. Resour. 13(6): 1043-1046.

Wiley, E.O., Johnson, G.D., Dimmick, W.W. (1998). The phylogenetic relationships of lampridiform fishes (Teleostei: Acanthomorpha), based on a total evidence analysis of morphological and molecular data. Mol. Phylo. Evol. 10: 417-425.