International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
(A Peer Reviewed, Referred, Indexed and Open Access Journal)
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 9, Issue 11 -2022

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.11.004

Identification and confirmation of Coral Reef Associated Fish species using Molecular tools, Kilakarai Gulf of Mannar, South East Coast of India

***V. Ammaiappan,* M. Thangaraj, *N. Inayathullah and *P. Vijayanand**
*Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University. Parangipettai, Tamilnadu, India.
Corresponding author E-Mail: *ammaiappanv@gmail.com*

Abstract

Coral reef fish are fish which live amongst or in close relation to coral reefs.Coral reefscontain the most diverse fish assemblages to be found anywhere on earth, with perhaps as many as 6,000–8,000 species dwelling within coral reef ecosystems of the world's oceans. The present study focused on coral reef fishes collected from Kilakarai landings, which were captured from Appa Island- Gulf of Mannar, during January to December 2019. About 25 mechanised trawler boats 20 vallam and 15 catamarans are engaged in fishing activity and they are concentrating their catch mainly on live ornamental fishes trade, lobsters and molluscan fisheries. 102 species belonging to 60 genera representing 25 families were identified. In order to confirm the species identification DNA barcoding was done for these fishes, *Chetodon collare, Chetodon octafasciatus, Chetodon decussatus, Chromis cinerascens, Pempheris malabarica, Coris Formosa, Pomacanthus semicirculatus, Cheilio inermis, Cephalopholis sonnerati, Halichoeres timorensis, Chetodon plebeius, Abudefduf vaigiensis, Cheilinus chlorourus, Abudefduf bengalensis, Pomacanthus moluccensis, Scarus ghobban, Caesio caerulaurea, Sargocentron rubrum, Pseudobalistes flavimarginatus and Myripristis hexagona.*Polymerase chain reactions were performed to amplify the COI gene using universal primers. Polymerase chain reactionproceeded inTechGene^{TM}, thermal cycler, **i**nitial denaturation at 95º C for 3 min. Number of cycles 35, denaturation at 95º C for 30 sec, annealing at 50º C for 30 sec, extension at 72º C for 45sec. Final extension at 72º C for 3 min. Agarose gel electrophoresis 1.5% was used for checking the amplified products and the molecular weight was checked by using molecular weight marker (100 bp ladder).All the sequences showed 99% identity of more than 90% query coverage with previously published COI sequences in the NCBI's nucleotide database.The K2P genetic distance between species shows higher value between *Pomacanthus moluccensis* and *Coris Formosa* 0.304, lower with *Chetodon plebeius* 0.134 *Chetodon collare*. For The K2P genetic distance between genus shows *Pomacanthus* and *Coris* 0.304, lower with *Abudefduf* and *Cephalopholis* 0.191. The K2P genetic distance between families indicate higher Pomacanthidae 0.297 with Pempheridae. The evolutionary history was inferred using the Neighbor-Joining method.The tree shows a significant phylogenetic relationship among the studied 20 fish species.

Keywords: Coral reef fishes, PCR, Phylogenetic tree, taxonomy and DNA barcoding.

Introduction

In this Universe everything is beautiful, all living and non-living flora and fauna is ornamental in nature. When we speak about ornaments it includes all jewels in the coral reef ecosystems. The ways one who see the beauty will differ depend upon their individual views. Ornamental fishes are the most diverse elements in the coral reef ecosystem their beautiful colouration and different body forms attract all the creatures in this world. Some families of ornamental fish are valuable groups for monitoring the health of coral reefs. About 170 varieties of marine origin are presence in Andaman; Nicobar and Lakshadweep Islands (Nair, 2006). From India the export takes place through Calcutta, Mumbai, Chennai, Cochin and Trivandrum airports. Presently ornamental fish market is constituted mainly by freshwater fishes (90%) and marine fishes constitute a minor share of 10% with only domestic market in a limited and controlled way. India possess substantial marine ornamental fish resources distributed over the coral islands of Lakshadweep, Andaman and Nicobar islands, Gulf of Kutch, Maharashtra, Cochin to Vizhinjam in Kerala and Gulf of Mannar, Palk Bay and Kanyakumari in Tamilnadu.

The important groups of marine ornamental fishes are clown fishes, damsel fishes, angels, wrasses, moorish idols, surgeon fishes, tangs, butterfly fishes, trigger fishes, parrot fishes, bat fishes, scorpion fishes and sea horses etc. (Nasser and Rajkumar, 2001). Molecular tools were vital for identifying the species level taxonomy. Barcoding analysis was used in customs goods exported to other countries. The fishery value added products are monitored by food and health departments in various countries. Nowadays lot of research is going on finding the source of raw material in the fishery products so many finding concluded the fishery was in danger of exploiting the endangered animals and mixing it with other species. It leads to conservation issues in recent times.

Kiet-Chuan *et al*., (2017) conducted a comparative research to explain and debate the most recent areas of study in fish taxonomy and species identification procedures. Because ichthyology is a complicated field, failing to identify fishes as unique biotic factors in fish-based investigations might lead to incorrect diagnoses in fisheries. As a result, this review research proposes some useful information and images on fish taxonomy and ichthyology. The present study supported with molecular techniques for identification of coral reef fishes. Specimens were collected from landings at Kilakaraifishing village, Gulf of Mannar.The Gulf of Mannar Biosphere reserve area spread over Rameswaram and Kanyakumari to about 19,000 km^2 in which our study area falls. In addition, the present preliminary observation may stimulate better investigations in future for thorough understanding of coral reef fishes ofthe same areas.

Materials and Methods

In the present study coral reef fishes were collected from Kilakarai landings, which were captured from Appa Island- Gulf of Mannar, during January to December 2019. The Gulf of Mannar is one of the four major coral reef areas of India, which is situated between India and Sri Lanka covering an area of about 10,500sq.km from Tuticorin to Rameswaram. Kilakarai which is located at about 70km from Rameswaram (09° 14’ N, 78° 47’ E) has been chosen for the present study (Fig.1). About 25 mechanised trawler boats 20 vallam and 15 catamarans are engaged in fishing activity and they are concentrating their catch mainly on live ornamental fishes trade, lobsters and molluscan fisheries.

(A) (B)

Fig.1.(A). Map shows the India,Tamilnadu district (B). Shows study area Appa Island, Kilakarai.

Crafts used to capture the reef fishes were vallam and mechanized boats (vathai). Gears employed for exploiting the commercial fishes are by trammel nets, scoop nets skin diving and traps (Koodu in tamil).From the landings the dead fresh fish specimens were collected cleaned, washed and stored in ice boxes with crushed ice then it was carried to the laboratory for study and preservation. Each specimen was identified up to species level using text books, monographs, reprints and online databases (Day, 1878; Smith and Heemstra, 1986; Munro, 2000; Froese and Pauly, 2008-2012). The names used in the text followed Froese and Pauly (2012) and classification that of Nelson (2006). After identification fishes were photographed and caudal fin samples were taken stored in 95% ethanol and then the whole fish was preserved in 10% formaldehyde for further examinations.

Totally 200 samples for 20 species were collected and the tissue samples (caudal fin) were stored in 95% ethanol for further analysis. Three individuals from each species were randomly selected and used for the molecular identification system. DNA was isolated from the samples modified from the standardized salting-out procedure (Sambrook *et al*., 1989). Quantity of the extracted DNA was checked in UV spectrophotometer (SHIMADZHU, JAPAN) by taking the optical density (OD) at 260 nm. It was done according to the following calculation: sample showing 1 OD at 260 nm is equivalent to 50 µg of DNA/mL. The OD was measured for each DNA samples at 260 nm and quantified accordingly.The quality of DNA was checked by measuring the absorbance at 260 and 280 nm (260/280) in ratio. The value between 1.7 - 1.8 indicates good quality DNA without protein/RNA contamination. The quality of DNA was also checked on 0.8% agarose gel electrophoresis.The standard mitochondrial COI gene was selected as a DNA barcode region. Polymerase chain reactions were performed to amplify the COI gene using universal primers (Ward *et al.*, 2005) FishF1_t1 1 (TGTAAAACGACGGCCAGTCGACTAATCATAAAGATATCGGCAC) and FishR2_t1 1 (CAGGAAACAGCTATGACACTTCAGGGTGACCGAAGAATCAGAA)Polymerase chain reaction proceeded in TechGene™, thermal cycler, initial denaturation at 95º C for 3 min.Number of cycles 35, denaturation at 95º C for 30 sec,annealing at 50º C for 30 sec,extension at 72º C for 45sec.Final extension at 72º C for 3 min.Agarose gel electrophoresis 1.5% was used for checking the amplified products and the molecular weight was checked by using molecular weight marker (100 bp ladder).

Sequencing the amplified product

The purified DNA products were sent to Macrogen, Inc. (Seoul, Korea) bidirectional sequencing. The DNA sequence analyzer, 3730xl DNA analyzer with Big-Dye Terminator Cycle type Sequencing Kit version3.1 (Applied Biosystems of Foster City, CA, USA) was used to sequence the samples.

The obtained sequences were edited based on the electropherogram peak clarities. Sequences with noisy peaks were excluded from the analysis. The sequences were further assessed to check the insertion or deletions and stop codons in MEGA 5.0 software. Multiple sequence alignment and pairwise sequence alignment were performed using Clustal W program implemented in MEGA 5.0 in all the sequences. Nucleotide differences were carefully monitored and the differences were observed and edited manually. Sequences were translated into amino acid sequences using vertebrate mitochondrial codon pattern in the MEGA 5.0 for checking the pseudo-gene status. All the sequences were correctly translated into amino acid sequences with their respective starting primes without any internal stop codon.

GenBank submission

All the sequences were submitted to the NCBI's GenBank through BankIt according to NCBI's procedure with required information. The amplified sequences belong to DNA barcode region of COI were confirmed by similarity index built in the NCBI's BLAST program. Based on the higher percentage similarity, query coverage and E-value against the reference species, all the species were identified. Nucleotide composition and genetic distance, among 20 fish species were determined by Kimura-2-Parameter method (Kimura, 1980) using MEGA 5.0 software program (Fig.3).

Results

In the present study coral reef fishes collected from Kilakarai landings comprised of 102 species belonging to 60 genera representing 25 families were identified. These are the family groups recorded in the present study namely, Muraenidae, Plotosidae, Holocentridae, Apogonidae, Pomacentridae, Carangidae, Labridae, Scaridae, Pempheridae, Serranidae, Chaetodontidae, Pomacanthidae, Haemulidae, Lutjanidae, Caesionidae, Scorpaenidae, Ephippidae, Zanclidae, Acanthuridae, Nemipteridae, Balistidae, Ostraciidae, Monocanthidae, Tetradontidae and Diodontidae. Initially these fishes are identified taxonomically and grouped. In order to confirm the species identification DNA barcoding was done for these fishes, *Chetodon collare*, *Chetodon octafasciatus*, *Chetodon decussatus*, *Chromis cinerascens*, *Pempheris malabarica*, *Coris Formosa*, *Pomacanthus semicirculatus*, *Cheilio inermis*, *Cephalopholis sonnerati*, *Halichoeres timorensis*, *Chetodon plebeius*, *Abudefduf vaigiensis*, *Cheilinus chlorourus*, *Abudefduf bengalensis*, *Pomacanthus moluccensis*, *Scarus ghobban*, *Caesio caerulaurea*, *Sargocentron rubrum*, *Pseudobalistes flavimarginatus* and *Myripristis hexagona*.

PCR amplification of DNA barcoding region

Among the 50 DNA samples, 40 were successfully amplified the COI gene using universal primers in 20 species(Fig.2).

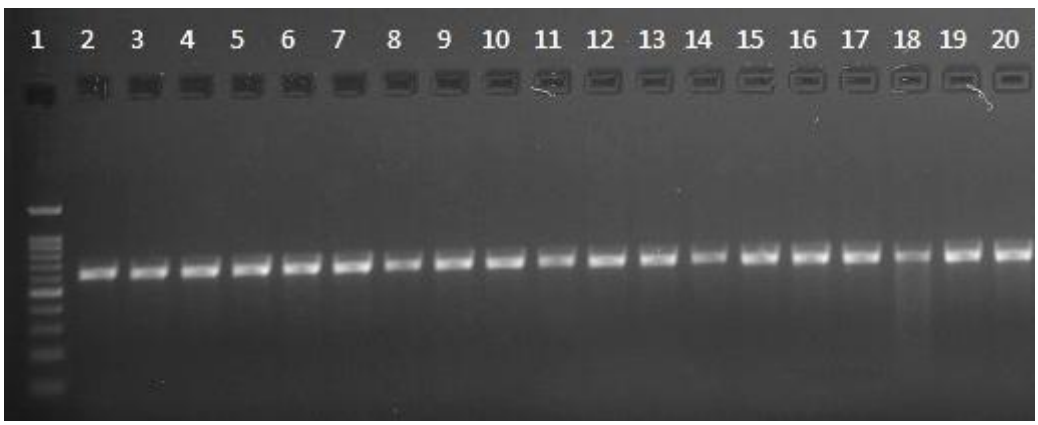

Fig. 2. Agarose gel electrophoresis of amplified COI gene

From the gel analysis, the single dark band were strongly indicated the amplification of COI gene. The size was predicted as approximately 700 bp by comparing with standard molecular marker (100 bp ladder). All the species were given good amplified products.

Sequence characterization

All the sequences showed good length of 594-686 bp and partial at both 5′ and 3′ ends. There were no insertions, deletions or stop codons in any of these sequences.

Species confirmation using COI sequence by BLAST

All the sequences showed 99% identity of more than 90% query coverage with previously published COI sequences in the NCBI's nucleotide database. It is suggested that the present sequences amplified by universal COI primers confirm that sequences belong to the COI gene. NCBI's BankIt protocol was properly followed for generating information regarding our sequences. All the sequences were submitted with unique identification name and accession numbers are given in Table 1.

Table -1. Species included in the phylogenetic analysis with accession number

Sl. no.	Species	Accession number
1.	*Chetodon collare*	ON564509, ON573351
2.	*Chetodon octafasciatus*	ON564510, ON573356
3.	*Chetodon decussatus*	ON564513, ON573363
4.	*Chromis cinerascens*	ON564512, ON573359
5.	*Pempheris malabarica*	ON564570, ON564537, ON573370
6.	*Coris Formosa*	ON565763, ON573364
7.	*Pomacanthus semicirculatus*	ON564519, ON573365
8.	*Cheilio inermis*	ON564520, ON573366
9.	*Cephalopholis sonnerati*	ON564518, ON573367
10.	*Halichoeres timorensis*	ON564535, ON573368
11.	*Chetodon plebeius*	ON566080, ON573369
12.	*Abudefduf vaigiensis*	ON564536, ON573422
13.	*Cheilinus chlorourus*	ON565821, ON573423
14.	*Abudefduf bengalensis*	ON564544, ON573425
15.	*Pomacanthus moluccensis*	ON564607, ON573426
16.	*Scarus ghobban*	ON564556, ON573424
17.	*Caesio caerulaurea*	ON565766, ON573427
18.	*Sargocentron rubrum*	ON564560, ON644428
19.	*Pseudobalistes flavimarginatus*	ON573372, ON644434
20.	*Myripristis hexagona*	ON644446, ON644429

The K2P genetic distance between species shows higher value between *Pomacanthus moluccensis* and *Coris Formosa* 0.304, lower with *Chetodon plebeius* 0.134 *Chetodon collare*. For The K2P genetic distance between genus shows *Pomacanthus* and *Coris* 0.304, lower with *Abudefduf* and *Cephalopholis* 0.191. The K2P genetic distance between families indicate higher Pomacanthidae 0.297 with Pempheridae (Table-2).

Table -2. K2P genetic distance between families

	Chaetodonidae	Pomacentridae	Pembheridae	Labridae	Pomacanthidae	Serranidae	Scaridae	Caesionidae	Holocentridae	Balistidae
Chaetodonidae	*****									
Pomacentridae	0.262	*****								
Pembheridae	0.247	0.246	*****							
Labridae	0.238	0.247	0.260	*****						
Pomacanthidae	0.266	0.275	0.297	0.240	*****					
Serranidae	0.237	0.208	0.259	0.221	0.237	*****				
Scaridae	0.238	0.254	0.243	0.238	0.255	0.245	*****			
Caesionidae	0.217	0.217	0.230	0.236	0.232	0.210	0.208	*****		
Holocentridae	0.222	0.227	0.236	0.232	0.242	0.216	0.227	0.203	*****	
Balistidae	0.252	0.237	0.292	0.253	0.257	0.230	0.232	0.229	0.229	*****

The analysed data for K2P genetic distance within species shows zero values, within genus it shows less than 0.02 and within family it was 0.09 respectively(Fig.3).

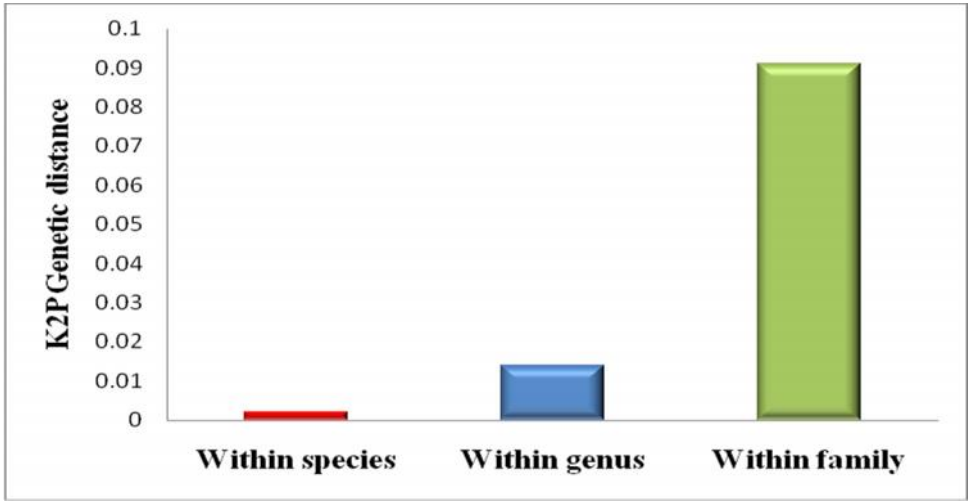

Fig.3. K2P genetic distance within species, within genus and within family

Phylogenetic tree construction

Forty nucleotide sequences of 20 fish species were included in the final analysis. There were a total of 27,076 bp positions were considered in the final dataset. Neighbor – Joining (NJ) statistical method (Saitou and Nei, 1987)Test of Phylogeny - Bootstrap method (Felsenstein, 1985)Bootstrap Replications – 1000, Substitutions Type – Nucleotide Model/Method - Kimura 2-parameter model (Kimura, 1980)Substitutions to Include - d: Transitions + Transversions, Rates among Sites - Uniform ratesPattern among Lineages – Homogeneous. Gaps/Missing Data Treatment - Complete deletion, Codons Included - 1^{st} +2^{nd} +3^{rd} positions.

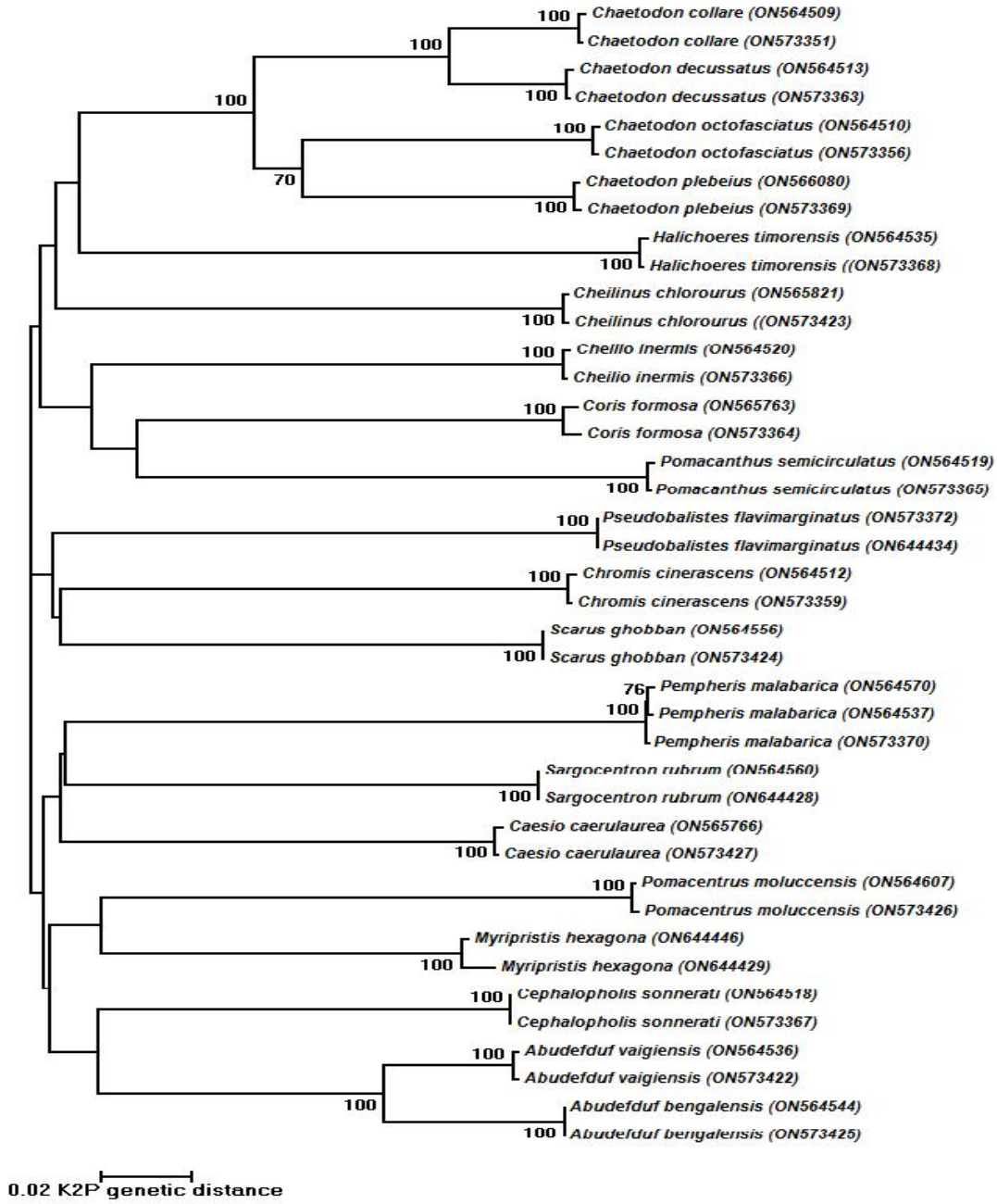

Fig. 4. NJ phylogenetic tree of twenty fish species based on COI sequence data

Phylogenetic status of fish species

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei 1987). The tree was drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Kimura 2-parameter method (Kimura, 1980) and were in the units of the number of base substitutions per site. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. Evolutionary analyses were conducted in MEGA 5.0 (Kumar *et al.,* 2011).The tree shows a significant phylogenetic relationship among the studied 20 fish species (Fig.4).

Discussion

Last few decades molecular studies were extensively carried out in different parts of the world to identify organisms and developed a gene database for reference. This helps the researchers to identify the organisms taxonomically and the controversy in species identification was solved by molecular approaches. Still taxonomy studies play a vital role in the genus as well as species identification of the native occurrences. Earlier the biodiversity and biology of these families have been studied in the Gulf of Mannar province (Venkataramani *et al.,* 2005). In Cuddalore coast 72 species of coral inhabitant finfishes have been recorded. The numerical abundance of coral fishes along the Cuddalore coast strongly suggests the existence of canyons regions formation between Pondicherry and Cuddalore southeast coast of India (Asta Lakshmi and Sundaramanickam, 2011). In Gulf of Mannar, (Manikandarajan *et al*., 2015) reported a total of 113 marine ornamental finfish species with their biodiversity and standing stock biomass. Baiju *et al.,* 2019 reported 232 species of rocky reef associated recorded from the rocky reefs of south Kerala coast of the Indian Subcontinent. Liu *et al.,* (2021) performed DNA barcoding of sharks present in shark products to identify the shark species that are being in traded in the commercial food market of Singapore. Shark is one of the important apex predators and is facing conservation issues in recent times. Sandhya Sukumaran and Gopalakrishnan (2015) reviewed the applications, setbacks and future opportunities of molecular taxonomy. Over the last two decades, the analytical methods have developed rapidly.

Sarhan *et al*., (2021) conducted a research to build reference sequences for *Conus* species from the Egyptian Red Sea coast, as well as to assess the potential of DNA barcodes for specimen identification. The study's findings indicated that COI sequences were compared for maximal similarity with those available in GenBank and the BOLD engine, yielding *Conus* species matches for all analyzed species. Serite *et al*., (2021) created DNA barcodes of dried seahorses and pipefishes intended for TCM in order to examine the limits of DNA barcoding in detecting the provenance of pirated seahorses and pipe fishes. Despite the fact that seahorses and pipe fishes have properly structured genetic groupings, DNA barcoding was only adequate for identifying species but not their nation of origin.

Rodrigues *et al.,* (2021) elucidates the vitality of DNA barcoding for the identification of billfishes (Scombroidei, Teleostei). Barcoding is an efficient technique to trace out illegal trading and product preparation from endangered species. Ahmed *et al.* 2021 conducted a research study that dealt with the molecular identification of small indigenous fish species from Bangladesh with assessment through DNA barcoding of 81 SIS species. The K2P distances was observed to be 15.83% among genera, 19.14% among families and 25.07% among orders with the maximum and minimum genetic divergences were 57.14% and 0.19% respectively. Phylogenetic analysis was also performed through neighbour-joining method. All these sets of analytical experiments emphasized that the genetic diversity of freshwater SIS fishes is vast. With the use of barcoding, 7 new records of small indigenous fish species have been identified which can be further categorized based on further barcoding analysis.

Adibah *et al.* (2020) facilitated the authentication of processed fish products in the seafood industry by evaluation and comparative study of DNA barcoding with Full DNA barcode and Mini DNA barcode. It was identified that the valuable fish species were substituted by non-profit species, indistinguishable fish fillet and sushi roe. Calegari *et al.* (2020) reported the use of DNA barcode authentication targeted on the cytochrome oxidase subunit I (coI) mitochondrial gene to reveal highly fraudulent cod commerce in Porto Alegre, Brazil. COI mitochondrial gene is a validated tool for species identification. Thus this was utilized for the detection of mislabelling and species substitution in the market. 7 of the 10 Cod samples tested during the course of study was identified as fraudulent where the species used in the product was different in terms of quality and price from the one mentioned in the label.

Sachin*et al*., (2018) discussed DNA barcoding, extraction, PCR amplification, and DNA. In DNA barcoding, organisms are distinguished by differences in their individual genetic codes, which are unique to each species, and these unique segments are referred to as Barcodes. DNA barcoding generates barcodes that are kept in databases for future research. This approach allows species to be identified quickly, properly, and in a short amount of time.Reis and Lavery (2020) reviewed marine DNA metabarcoding. DNA metabarcoding facilitates taxonomic identification of various species from a heterogeneous sample mixture. Sensitivity is the prime advantage of this approach making it a key tool in the marine conservation and management and noninvasive environmental sampling.

Xing *et al.* (2020) identified the commercial fishes available in the Taiwan market using Mini DNA barcoding to study and understand the extent of commercial fraud of seafood species. Such species substitution can cause issues to consumers' health. Mini DNA barcoding is an efficient method for fish authentication monitoring and quality control in fish processing units. 55 sequences were obtained from 365 fish samples based on basepair count. The aggregate rate of substitution was 21.97%. The average genetic distances on the grounds of Kimura-2-parameter (K2P) was 0.37% within species, 18.10%、within genera, 22.10% within families and 25.40% within orders The mean interspecific distance and mean intraspecific distance varied by 49-fold. This study demonstrated the mini-DNA barcode was reliable and effective for fish authentication monitoring and could be used by surveillance authorities in the quality control of processed fish products, towards ensuring consumer rights.

Similar methodologies were carried out in the present study. The K2P genetic distance between species shows higher value between *Pomacanthus moluccensis* and *Coris Formosa* 0.304, lower with *Chetodon plebeius* 0.134 *Chetodon collare.* For The K2P genetic distance between genus shows *Pomacanthus* and *Coris* 0.304, lower with *Abudefduf*and *Cephalopholis* 0.191. The K2P genetic distance between families indicates higher Pomacanthidae 0.297 with Pempheridae. The analysed data for K2P genetic distance within species shows zero values, within genus it shows less than 0.02 and within family it was 0.09 respectively. From the findings taxonomy related species identification using molecular techniques have developed extensively around the world. Storing the database and helping the researchers for identifying the species of their country is extra ordinary service provided for them. Systemic taxonomy with molecular level evidence has its own merits. Earlier species where identified for taxonomical classification now a days these techniques are useful to find out the fraudulent happening the consumer products. Because replacement of commercially important fish species with morphologically similar low value fishes is a regular fraudulent activity happening in the market. In the present study all 20 species were confirmed with the help of COI sequence NCBI's by BLAST. And it was submitted in the Genbank for further findings.

Acknowledgments

The authors are thankful to the authorities of Annamalai University, for providing the facilities

and Dr. P. Anantharaman, Dean and M. Kalaiselvam, Director, Centre of Advanced Study in Marine Biology, Faculty of Marine Sciences, Annamalai University for his support and encouragements.

References

Adibah A.B., Syazwan S., Haniza Hanim M.Z., Badrul Munir M.Z., Intan Faraha A.G., and Siti Azizah M.N., 2020. Evaluation of DNA barcoding to facilitate the authentication of processed fish products in the seafood industry. LWT- Food Science and Technology. 129: p.1-7.

Ahmed Md. Sagir., Sujan Kumar Datta., Tonmoy Saha., Zarif Hossain., 2021. Molecular characterization of marine and coastal fishes of Bangladesh through DNA barcodes. Ecology and Evolution. 11:p.3696–3709.

Asta Lakshmi.S., A.Sundaramanickam, 2011. Biodiversity of Reef Ichthyofauna in Cuddalore Coast, Southeast Coast of India. Int. J. Envn. Sci., p.1- 7.

Baiju P.T., Prabhakaran M.P., Benno Pereira F.G and Jayaprakas, V., 2019 Check list of rocky reef associated fishes of south Kerala coast, India. Res. J. Marine Sci., Vol. 7(1): p.1-13.

Calegari B.B., Avila E.F., Reis R.E., Alho C.S., 2020. DNA barcode authentication reveals highly fraudulent cod commerce in Porto Alegre, Brazil. Forensic Science International: Reports 2. p.1-5.

Day, F., 1878. The fishes of India; being a natural history of the fishes known to inhabit the seas and fresh waters of India, Burma, and Ceylon. Text and atlas in 4 Parts. London, xx 778 P.,195 pls.

Felsenstein J., 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39(4): p.783-791.

Froese, R. and Pauly, D., 2008 -2012. Fish Base. world wide web electronic publication. Available from: www.fishbase.org, version (accessed 23 July 2011).

Kiet-Chuan Ng C., Aun-Chuan Ooi P., Wong W.L., and Khoo G., 2017. A Review of Fish Taxonomy Conventions and Species Identification Techniques. J.Surv. Fisheries Sci., 4(1): p.54-93.

Kimura M., 1980. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences Journal of Molecular Evolution vol. 16: p.111–120.

Liu C.J.N., Neo S., Rengifo N.M., French I., Chiang S., Ooi M., Heng J.M., Soon N., Yeo J.Y., Bungum H.Z., Ota K., Koul A.A., Poh Y.H., and Wainwright B.J., 2021. Sharks in hot soup: DNA barcoding of shark species traded in Singapore. Fisheries Research 241:p.1-4.

Manikandarajan, T., K.Ramamoorthy, K.Sundaravarman, D.V.Ramdas, S.Suvitha and G.Sankar, 2015. distribution and diversity of marine ornamental fishes in gulf of mannar region, World Journal of Pharmaceutical Research 4, Issue 6: p.1440-1460.

Munro, I.S.R. 2000. The Marine and Freshwater Fishes of Ceylon. Narendra Publishing House , Delhi, 351pp.

Nair, K.S. 2006. Ornamental fish trade in the Indian context. In: Kurup et al. (eds.), Souvenir, Aquashow 2006. Dept. of Fisheries, Govt. of Kerala, p. 61-70.

Nasser, A. K. V., and Rajkumar U., 2001. Ornamental fish - prospects for culture, Sollvenir, Visakapatnam Regional Centre of CMFRI.p.24-26.

Nelson J.S. (2006). Fishes of the World. 4th (Eds) Hoboken (New Jersey, USA): John Wiley & Sons. 601. ISBN 0-471- 25031-7.

Reis A.L V.D., and Laveery S.D., 2020. Marine DNA Metabarcoding. Encyclopedia of the World's Biomes, Volume 4: p.612-618.

Rodrigues C.E.Jr., Carvalho D.E.V D., Garcia Y., Hashimoto D.T., Foresti F., and Foresti F.P., 2021. DNA Barcode as an effective tool in the identification of billfishes (Scombroidei, Teleostei) from exported specimens. Forensic Science International: Animals and Environments 1: p.1-6.

Sachin S., Mamta A., and Shailesh K.V., 2018. DNA Barcoding: A Significant Molecular Approach for Identification Upto Species Level. Remarking an Analisation Vol-3(3): p.262-269.

Saitou, N. and Nei, M. (1987) The Neighbor-Joining Method: A New Method for Reconstructing Phylogenetic Trees. Molecular Biology and Evolution, 4: p. 406-425.

Sambrook, J., Fritsch, E. R., and Maniatis, T. (1989). Molecular Cloning: A Laboratory Manual (2nd ed.). Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sandhya Sukumaran and Gopalakrishnan, A., 2015. Summer School on Recent Advances in Marine Biodiversity Conservation and Management. Central Marine Fisheries Research Institute. P.157-161

Sarhan M., Wahab M.A., Aly H., and Fouda M.,2021. DNA barcoding of seven cone snail species from Red Sea coast of Egypt. Egyptian J. of Aquatic Research 47: p.93–99.

Serite, C. P., Ntshudisane O. K., Swart E., Simbine L., Jaime G. L.M., and Teske P.R., 2021. Limitations of DNA barcoding in determining the origin of smuggled seahorses and pipefishes. Forensic Science International: Animals and Environments 1:p.1-6.

Smith, M.M. and Heemstra, P.C. , 1986. Smith's Sea Fishes. Springer Verlag Berlin Heidelberg New York, London, Paris, Tokyo, 1047 P

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., and Kumar S., 2011. MEGA5: Molecular Evolutionary Genetics Analysis Using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. *Molecular Biology and Evolution*, Vol. 28 (10): p.2731–2739.

Venkataramani, V.K., P. Jawahar, T. Vaitheeswaran and R. Santhanam, 2005. Marine Ornamental Fishes of Gulf of Mannar. ICAR/NATP/CGP/ publication. 115 pp.

Ward RD, Zemlak TS, Innes BH, Last PR, Hebert PDN (2005) DNA barcoding Australia's fish species. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences, 360, p. 1847– 1857.

Xing B., Zhang Z., Sun R., Wang Y., Lin M., and Wang C., 2020. Mini-DNA barcoding for the identification of commercial fish sold in the markets along the Taiwan Strait. Food control 112: p.1-8.

Access this Article in Online

Website: www.ijarbs.com

Subject: Marine Biology

Quick Response Code

DOI:10.22192/ijarbs.2022.09.11.004

How to cite this article:
V. Ammaiappan,M. Thangaraj, N. Inayathullah and P. Vijayanand. (2022). Identification and confirmation of Coral Reef Associated Fish species using Molecular tools, Kilakarai Gulf of Mannar, South East Coast of India. Int. J. Adv. Res. Biol. Sci. 9(11): 24-34.
DOI: http://dx.doi.org/10.22192/ijarbs.2022.09.11.004