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DNA Barcoding for the Assessment of the Taxonomy of Fish from Chilika Lagoon, India

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Abstract

DNA barcoding is a technique in which identification of species using DNA barcodes. We generated a verified reference library of cytochrome c oxidase subunit I (COI) sequences for 226 barcodes belonging to 83 fish species from 67 genera, 39 families and 21 orders of fishes with the average divergence within a species is 010%, 13.57% within a genus, and 17.33% within a family with 97-100% identity with comparison to the Genbank database and BOLD of the Chilika lagoon, India. Data analysis done by barcode gap analysis, barcode index number and automatic barcode gap discovery to discriminate the species up to 97.53%, 93.90% and 95.06% respectively. The Barcode Index Number (BIN) discordance analysis reflected 226 specimens belongs to 83 BINs, of which 73.49%were taxonomically concordant and 26.50% were singletons and absence of discordant BIN in our dataset. Finally, the identification success rate of DNA barcoding was evaluated based on the sequencing success rate and species discrimination rate.

Introduction

Chilika lagoon is (190 28'-190 54' N; 850 06'-850 35'E), listed Asia's largest brackish water lagoon, as one of the hotspots of biodiversity and provides shelters to several endangered species listed in the IUCN red list of threatened species. The Lagoon is a unique site of marine, brackish and freshwater ecosystem with estuarine characters. Chilika lagoon is declared as largest coastal wetland (Mohapatra, et al., 2007). Based on its rich biodiversity, unique ecological characters, Chilika Lagoon has been designated as Ramsar site in 1981 with international importance. Unique brackish water ecosystem (Mangla, 1989; Dujovny, 2009) with over hundreds of fish existing and one of the largest tropical lagoons in the world and has designated as Category I of the marine protected areas. Chilika lagoon greatly contributes more than 71% of the total evaluation of the Lagoon ecosystem (Kumar, 2003), so it clearly indicates that the significance of fisheries resources.

Fisheries of Chilika Lagoon has been greatly contributed towards the state economy. The biodiversity of chilika Lagoon is very high due to complicated and long history of its formation which has been estimated to be over 3500-4000 years ago. However, in last two decades huge impact been found on fauna of Chilika Lagoon by natural and anthropogenic threats. Lagoons are found to be most productive aquatic niche and providing several ecosystem services

essential to mankind (Dolbeth, et al., 2016). Densely populated area subjecting lagoons to several anthropogenic pressures (Lopes, et al., 2013). Many fish species belonging to the freshwater, marine and brackish water biota of this Lagoon fall under the category like vulnerable, threatened and endangered (IUCN 2017). This might be attracting attention for the monitoring of ecological changes and need to implement of some advanced technique like molecular based fast and accurate identification for their urgent conservation measures. Species richness and genetic variability are important parameter for long-term maintenance of climax community (Mohanty, et al., 2007). Meanwhile, few new species of fishes have been listed (Mohanty, et al., 2015) needs to update the checklist of existing fish fauna from the Chilika Lagoon. There were few studies based on the taxonomy and enlisting fish species by Bhatta, et al., 2001; Chaudhuri, B L 1916a, 1916b, 1916c, 1917, 1923; Menon, MAS 1961; Hora, S L, 1923; Barman, et al., 2007; Jones, et al., 1954; Mohanty, et al., 2007, 2015; Mohapatra, et al., 2013, 2014; Siddiqi, et al., 1995. Many species are still in an unidentified mode and synonymy while many invalid specific names are also considered valid. In addition to this, there is some 'species complexity' that will form cryptic species concept (Molur, et al., 2011). Furthermore, some specific names are not accepted scientifically because they did not full fill the criterion of national and international codes (Reid, 2010). However, species identification based on morphology (Rosso, et al., 2012), many times lead to misidentification due to high degree of phenotypic plasticity (Khedkar, et al., 2014) in such characters leading injustice of the species number. However, the available list of fish still recognized based on has many generic terms and enigmatic (Freire, et al., 2022). Earlier, the identification of species is based on phenotypic, however, the limited taxonomist experts, made it difficult to covered up biodiversity (Espirito-Santo, et al., 2012). Morphology based identification has some limitation due to phenotypic plasticity, cryptic species, and unrecognized developmental conditions (Hebert, et al., 2003). Furthermore, problems are even more in groups of highly similar species as well as in processed fish, where they lack identification characters.

Therefore, to overcome these ambiguities, it is essential to undertake an assessment of fish diversity by employing advanced molecular technologies, especially, in a biodiversity hotspot such as the Chilika lagoon, where no single molecular studies have been done. The DNA-based barcoding method has been proven to be a valuable molecular technique for species identification, and it is accessible to non-specialists (Hebert, Ratnasingham & Dewaard, 2003; Frezal & Leblois, 2008; Leray & Knowlton, 2015). Several international campaigns are dedicatedly working on the DNA barcoding of fish; FISH-BOL (http://wwwfishbolorg), now well established and to target to DNA barcoding of fishes globally. (Ward, Hanner & Hebert, 2009). DNA barcoding technique has potential to discriminates the whole fish as well as unorganized small parts like larvae, fillets, eggs, fins, and other part of the body that are difficult to identify based on external characters (Trivedi et al., 2016). The mitochondrial COI gene has been popular as a molecular marker extremely effective at discriminating fish species (Ward et al., 2005; Hubert et al., 2008; Valdez-Moreno et al., 2009). The COI gene target region for DNA barcoding (Hebert, Ratnasingham & Dewaard, 2003; Hajibabaei, et al., 2007a; 2007b). COI barcoding distinguished 98% of reported marine fish species, this approach listed and record fish in many geographic regions (Aquilino, et al., 2011; Asgharian, et al., 2011; Cawthorn, Steinman & Witthuhn, 2011; Lakra, et al., 2011; Becker, et al., 2015). However, In India very limited DNA barcoding study on marine fish resources while the diversity of fish in the Chilika Lagoon still untouched.

This is the first cumulative assessment of DNA barcoding of fishes in the Chilika Lagoon, it might be somehow significant contribution towards the global fish DNA barcode library and will be helpful for the management and conservation programmes in this region.

Materials and Methods

Collection of Fish Samples

A total 252 fish were collected from the Chilika Lagoon (19'69" N 85'29" E) in eastern coast of Odisha state, India. All voucher specimens were high quality images while caudal fin was preserved in 95% alcohol. 1 to 7 individual specimens were collected for each fish species. Identification of fish species identified by using standard taxonomic keys (Jayaram, 2009, 2010) and online database like FishBase (http://wwwfishbaseorg/, 2016), Catalogue of Life (http://wwwcatalogueofifeorg/, 2016) and Catalog of Fishes -version of 29 September2016(http://researcharchivecalacademyorg/research/ichthyology/catalog/fshcatmanasp).

DNA Extraction, Amplification, and Sequencing of the COI Gene

Extracted the DNA from the stored caudal fin using a Wizard Genomic DNA Purification Kit (Promega Corporation, Madison, WI, USA), following the manufacturers protocol. Quantification of DNA done by Nanodrop 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA) by targeting the ratio of 260/280 and 230/260. The fragment of the mitochondrial COI gene region is about 650bp was amplified successfully by using the different primers COI 5' Fish F1(TCAACCAACCACAAAGACATTGGCAC3') and COI 5 Fish R1 (TAGACTTCTGGGTGGCCAAAGAATCA 3') (Ward et al., 2009). PCR mixtures included 5 µl of 10 × PCR buffer, 2.5 µl of $MgCl_2$ (50 mM), 1 µl of dNTP (0.05 mM), 1.5 µl of each primer (0.01 mM), 125 U of Taq polymerase, 2.5 µl

of DNA template and Initial denaturation at 94°C for 3 min; 35 cycles of denaturation at 94°C for 30 s, followed by annealing at temperatures of 54.5°C, for 45 s, and initial extension at 72°C for 1 minute, with a final extension of 5 min at 72°C. Furthermore, for the confirmation of amplification was done by used 1.2% agarose gels by stained by EtBr. The PCR products were cleaned up by using Exo-SAP enzymatic treatment, followed by cycle sequencing. The purified PCR products proceed for bidirectional sequencing using an ABI xl 3730 and ABI xl 3130 capillary sequencer (Applied Biosystems, Foster City, CA, USA) using the Big-Dye Terminator Cycle Sequencing Kit (Applied Biosystems).

The DNA sequences were assembled and aligned using Codon code aligner with ClustalW software (Thompson et al., 1994). The resulted DNA sequences were compared with the previously submitted sequences of fishes in BOLD and GenBank databases and we found similarity score over 97-100% for all sequences. Simultaneously both distance and character-based method employed for the better resolution of species. Kimura 2-parameter (K2P) distance model (Kimura, 1980) was used to calculate pairwise genetic distances. Neighbor-joining (NJ) phylogenetic trees of K2P distances giving 1000 bootstrap replications support (Saitou & Nei, 1987) were generated for determining interspecies divergence. MEGA version 5.1 (Tamura et al., 2011) was used to find out K2P distance and the neighbor-joining (NJ) tree. The phylogenetic NJ tree from 226 specimens was constructed based on K2P distances. The Barcode Index Number (BIN) web server analysis platform is on BOLD system by Ratnasingham and Hebert (2013). The BIN is based on an online platform that clusters COI barcode sequences that generates unique operational taxonomic units (OTUs) number for each species sequence set and has proven effective in identification (Kekkonen et al., 2015). Basically, DNA barcoding method is based on the (Hebert et al., 2003) identification of species relies on the algorithm, where an intra-specific divergence is always less than the inter-specific, known to be as 'barcode gap' (Meyer and Paulay, 2005) used to compare existing Barcode Index Numbers (BINs) to all the sequences of the specimens analysed in the present study. To calculate the gap of the given dataset by another method called Automatic Barcode Gap Discovery, where hypothetical species concept is done using the barcode gap, is further divide the DNA sequence dataset (Puillandre et al., 2012). The obtained result regulates the screening efficiency of species diversity to resolve the cryptic species concept and to enlisting the fish species using molecular approach (Lakra et al., 2011; Khedkar et al., 2014). The DNA barcoding analysis was done by using species delimitation method based on the single locus. This method has been useful in resolving the concept of cryptic species and hence, taxonomic uncertainty (Conte-Grand et al., 2017; Araujo et al., 2019). The obtained data like trace files, institutional data, taxonomic information, images, geographical data, and sample id were all submitted to this platform. This information gives the data in the form of number of clusters to determine the BINS, not only the degree of interspecies and intraspecies divergence but genera, families as well, the barcode gap, and to construct a Neighbour-Joining (NJ) tree based on the Kimura 2-parameter (K2P) approach (Kimura, 1980), using 1,000 bootstrap pseudoreplicates. This analysis was run in the BOLD Workbench application (version 3.6). We used this tree to confirm that all species were formed monophyletic clustered. Thus, DNA barcoding technique become effective in identifying organism at species level known to be similar based on morphological observation. Con-familial species are then classified and grouped as independent clades in general phylogenetic analysis.

The BIN discordance report was used to confirmation the species as well as to check for cases of low intraspecific divergence. Intraspecies divergence was calculated in terms of transition (si)/transversion (sv) ratio and genetic distance. Further, the mean ratio of transition verses transversion (si/sv) of all specimens found to be 1.39. This ratio provides information about sequence distance and in phylogeny reconstruction. A high si/sv ratio is indicative of a small genetic distance, and vice versa (Yang & Yoder, 1999). Some families like Cyprinidae, clupidae, cichlidae and siluridae which are dominant in the Chilika Lagoon (shown in Figure 6) using DNA barcodes of the fish collected in this study and the mean si/sv ratios for these families were 3.16, 1.39, 1.81, and 2.57, respectively. Further, the mean genetic distances among species within these dominant families were 17.27%, 21.3%, 25.05%, and 15.61%, respectively. Cyprinidae family shows the highest si/sv ratio (3.16) and the lowest genetic distance among species within families Siluridae (15.61%) and it appears to be a recently diverged group and is youngest among dominant families in the Chilika Lagoon. Mitochondrial DNA has the potential to evolve faster than nuclear DNA while another property of mt-DNA is it has a greater number of variable and informative sites. Rapid substitution rates of mitochondrial DNA also make it useful for analyses at species and genus levels. To verify intraspecific and interspecific genetic distances, we also used another species delineation tool, barcode gap analyses in ABGD (https://bioinfomnhnfr/abi/public /abgd/) ABGD was used with K2P with the transition/transversion ratio (TS/TV) set to 20, 10 recursive steps, X (relative gap width) = 10; the remaining parameters were set to default values (Pmin = 00001, Pmax = 001, Nb bins = 20).

Results

A total of 252 fish specimens were collected from Chilika lagoon and their hierarchical distribution is shown in Table 1. All sequences of >500 bp without any insertions, deletions, stop codons and NUMTs were

Table 1. Taxonomic distribution of species found in the Chilika Lake

Order	Family	Species	Individual
Acanthuriformes	Sciaenidae	*Cynoscion reticulatus*	1
Anabantiformes	Anabantidae	*Anabas testudineus*	3
	Channidae	*Channa kelaartii*	4
		Channa marulius	3
		Channa punctata	3
	Nandidae	*Nandus nandus*	3
Aulopiformes	Synodontidae	*Saurida undosquamis*	3
Beloniformes	Belonidae	*Strongylura strongylura*	3
		Xenentodon cancila	4
	Hemiramphidae	*Rhynchorhamphus malabaricus*	2
Carangiformes	Carangidae	*Alepes kleinii*	3
Characiformes	Serrasalmidae	*Piaractus brachypomus*	2
Cichliformes	Cichlidae	*Etroplus suratensis*	1
		Oreochromis mossambicus	5
		Oreochromis niloticus	6
Clupeiformes	Clupeidae	*Dussumieria acuta*	1
		Escualosa thoracata	1
		Gudusia chapra	3
		Nematalosa nasus	4
		Sardinella fimbriata	1
	Engraulidae	*Stolephorus dubiosus*	1
		Thryssa malabarica	1
		Thryssa setirostris	1
	Pristigasteridae	*Ilisha elongata*	2
Cypriniformes	Cobitidae	*Lepidocephalichthys guntea*	1
		Lepidocephalichthys thermalis	7
	Cyprinidae	*Barbonymus gonionotus*	2
		Cirrhinus mrigala	4
		Cirrhinus reba	3
		Ctenopharyngodon idella	3
		Cyprinus carpio	3
		Gibelion catla	3
		Hypophthalmichthys molitrix	4
		Labeo calbasu	4
		Labeo rohita	8
		Osteobrama vigorsii	3
		Puntius chola	3
		Puntius sophore	3
		Rasbora daniconius	2
		Rasbora rasbora	3
		Salmophasia bacaila	4
		Securicula gora	3
		Systomus sarana	3
Gobiiformes	Gobiidae	*Glossogobius guiris*	2
	Oxudercidae	*Awaous ocellaris*	3
		Trypauchen vagina	1
Moroniformes	Ephippidae	*Ephippus orbis*	1
Mugiliformes	Mugilidae	*Osteomugil perusii*	3
		Planiliza macrolepis	1
		Planiliza tade	4
Osteoglossiformes	Notopteridae	*Notopterus notopterus*	6
Ovalentaria	Ambassidae	*Ambassis ambassis*	2
Perciformes	Gerreidae	*Gerres erythrourus*	2
	Lutjanidae	*Lutjanus fulviflamma*	1
		Lutjanus johnii	1
		Lutjanus rivulatus	2
	Serranidae	*Epinephelus coioides*	1
		Epinephelus latifasciatus	1
	Terapontidae	*Pelates quadrilineatus*	1
Scombriformes	Stromateidae	*Pampus chinensis*	4
Scorpaeniformes	Platycephalidae	*Platycephalus indicus*	1
Siluriformes	Ariidae	*Arius arius*	4
		Plicofollis layardi	1
	Bagridae	*Mystus cavasius*	4
		Mystus gulio	3
		Mystus malabaricus	2
	Clariidae	*Clarias gariepinus*	4
	Heteropneustidae	*Heteropneustes fossilis*	2
	Pangasiidae	*Pangasianodon hypophthalmus*	1
	Plotosidae	*Plotosus nkunga*	3
	Siluridae	*Ompok bimaculatus*	7
		Ompok pabda	3
		Wallago attu	5
Spariformes	Lobotidae	*Datnioides polota*	1
	Nemipteridae	*Nemipterus japonicus*	6
	Sparidae	*Acanthopagrus berda*	2
		Crenidens crenidens	1
		Rhabdosargus sarba	5
Synbranchiformes	Mastacembelidae	*Macrognathus guentheri*	3
		Mastacembelus oatesii	3
Tetraodontiformes	Triacanthidae	*Triacanthus biaculeatus*	2
21 Orders	39 Families	83 *Species*	226 Individuals

submitted to BOLD (https://v4boldsystemsorg/ indexphp/DS-CHILIKA) and NCBI GenBank accession numbers were obtained shown in Table 2. Out of these, sequences were successfully generated for 226 specimens, resulting in 100% amplification rate of COI barcode. The resulted COI amplified sequences of length > 600 bp without any deletion, insertion, or stop codon. The average genetic distances between individuals within species, genera, and families were 0.10%, 13.57%, and 17.33%, respectively found in the current study is shown in the Table 3. Later analysis showed that all specimens exhibited high distance values to their nearest neighbour reflecting's the presence of barcode gap among the 83 putative species.

Similarity score performed using the BOLD identification engine and NCBI nucleotide blast. The nucleotide frequencies were 25.13% (A), 29.79% (T/U), 27.75% (C), and 17.34% (G). A base-composition analysis showed that the average T content was highest, and the average G content was lowest. The AT content (54.92%) was higher than the GC content (45.09%) is shown in Table 4. Like the results for Australian (Ward et al., 2015) Canadian (Hubert et al., 2008), Cu-ban (Lara, et al., 2012) and Taiwan Strait fish species (Bingpeng, et al., 2018).

Table.2. List of the studied fish species from the Chilika lagoon Odisha Indi, their BOLD accession numbers and Genebank Accession Number.

Taxa	Individual	NCBI Accession No.	BIN
Acanthopagrus berda	2	BOLD:ACI0806	OQ569905 and OQ569906
Alepes kleiii	3	BOLD:ADG2635	OQ569908 to OQ569910
Ambassis ambassis	2	BOLD:ACJ3337	OQ880638,OQ880643
Anabas testudineus	2	BOLD:ABA9363	OQ880656 to OQ880657
Arius arius	3	BOLD:AAD9382	OQ880661,OQ880663, OQ880665
Awaous ocellaris	2	BOLD:AEB5275	OR431490 to OR431491
Barbonymus gonionotus	2	BOLD:AAD1940	OQ880676 to OQ880677
Chanda nama	3	BOLD:AAZ1771	OQ539631, OQ539636, OQ539644
Channa kelaartii	4	BOLD:ADP1551	OQ539654 to OQ539657
Channa marulius	3	BOLD:ABA8625	OQ632659, OQ632660, OQ632664
Channa punctata	3	BOLD:AAE8814	OQ632677, OQ632679, OQ632684
Cirrhinus mrigala	4	BOLD:AAF3499	OR431492 to OR431495
Cirrhinus reba	3	BOLD:AAJ3231	OR431496 to OR431498
Clarias gariepinus	4	BOLD:AAB2256	OQ632683 to OQ632686
Crenidens crenidens	1	BOLD:ACL1923	OR431499
Ctenopharyngodon idella	1	BOLD:ACL1923	OQ880698
Ctenopharyngodon idella	2	BOLD:ACL1923	OR431500 to OR431501
Cynoscion reticulatus	1	BOLD:AEC5708	OQ880706
Cyprinus carpio	3	BOLD:AAA7175	OR431502 to OR431504
Datnioides polota	1	BOLD:AAC5920	OR431505
Dussumieria acuta	1	BOLD:ADD5327	OQ880720
Ephippus orbis	1	BOLD:AAD8911	OR431506
Epinephelus coioides	1	BOLD:AAB8391	OQ880724
Epinephelus latifasciatus	1	BOLD:AAC6086	OR431507
Etroplus suratensis	1	BOLD:AAF3969	OQ880730
Gibelion catla	1	BOLD:AAK2267	OQ880739
Glossogobius guiris	2	BOLD:AAC6086	OR431508 to OR431509
Gudusia chapra	3	BOLD:ABA9557	OR431510 to OR431512
Heteropneustes fossilis	2	BOLD:ACR4875	OR431513 to OR431514
Hypophthalmichthys molitrix	2	BOLD:AAF6633	OQ880766 to OQ880767
Ilisha elongata	1	BOLD:ACC0078	OR431515
Labeo calbasu	1	BOLD:AAD7996	OQ622044
Labeo rohita	8	BOLD:ADB9997	OQ536316,OQ536317,OQ536324,OQ536333,OQ536339,OQ536355,OQ536358, OQ536359
Lepidocephalichthys guntea	1	BOLD:ACC0078	OR431516
Lepidocephalichthys thermalis	2	BOLD:ACX6285	OQ880784 to OQ880785
Lutjanus fulviflamma	1	BOLD:ADF5681	OQ880786
Lutjanus johnii	1	BOLD:AAC7492	OQ880788
Lutjanus rivulatus	2	BOLD:AAB7684	OQ880789 to OQ880790
Lutjanus rivulatus	3	BOLD:AAB7684	OQ880796, OQ880798,OQ880799
Mastacembelus oatesii	3	BOLD:AEB8888	OR430236 to OR430238
Mystus cavasius	4	BOLD:ADX0539	OQ554956, OQ554968,OQ554966, OR430239
Mystus gulio	3	BOLD:ACH1421	OR430240 to OR430242
Nandus nandus	3	BOLD:AAZ8464	OR430243 to OR430245
Nematalosa nasus	4	BOLD:ABY2938	OQ880807, OQ880808, OQ880810, OQ880811
Nemipterus japonicus	6	BOLD:AAC1279	OQ880812 to OQ880817
Notopterus notopterus	6	BOLD:AAF2803	OQ536411, OQ536414, OQ536432, OQ536407, OQ536419,OR430246
Ompok bimaculatus	7	BOLD:AAA9421	OR430247 to OR430252,OR430268
Ompok pabda	3	BOLD:AAB0409	OR430253 to OR430255
Oreochromis mossambicus	5	BOLD:AAA8511	OQ726304, OQ726306, OR430264 to OR430266
Oreochromis niloticus	6	BOLD:AAC9904	OQ726314 to OQ726316, OQ726310, OQ726318, OQ726320

Table 2. *Continued*

Osteobrama vigorsii	3	BOLD:ABY3071	OQ555196, OQ555200, OQ555205
Osteomugil perusii	3	BOLD:AAW7354	OQ730270, OQ730272, OQ730273
Pampus chinensis	4	BOLD:AAD2813	OQ730289, OQ730291 to OQ730293
Pangasianodon hypophthalmus	1	BOLD:AAE3237	OQ730294
Parastromateus niger	2	BOLD:AAB3884	OQ730307 to OQ730308
Pelates quadrilineatus	1	BOLD:AAA9700	OQ730310
Piaractus brachypomus	2	BOLD:AAC5682	OQ730314 to OQ730315
Planiliza microlepis	1	BOLD:ACC0087	OR430267
Planiliza tade	4	BOLD:AAE6698	OQ730317 to OQ730320
Platycephalus indicus	1	BOLD:AEC4500	OQ730323
Plicofollis layardi	1	BOLD:AAF3393	OQ730324
Plotosus nkunga	3	BOLD:ACH1329	OQ730325 to OQ730327
Puntius chola	3	BOLD:AAX7390	OQ730332, OQ730335, OQ730336
Puntius sophore	2	BOLD:AAX7390	OQ730339 to OQ730340
Rhabdosargus sarba	1	BOLD:ABX6594	OQ730353
Rhynchorhamphus malabaricus	2	BOLD:ABV4537	OQ730364 to OQ730365
Salmophasia bacaila	3	BOLD:ABA0106	OR430261 to OR430263
Saurida undosquamis	3	BOLD:ACG7154	OQ730380 to OQ730382
Securicula gora	1	BOLD:ACX7514	OQ730387
Stolephorus dubiosus	1	BOLD:ADG4839	OQ730400
Strongylura strongylura	3	BOLD:AAD4770	OQ730402, OQ730404, OQ730406
Systomus sarana	3	BOLD:AAY5233	OQ555181 to OQ555183
Thryssa malabarica	1	BOLD:AAE7811	OR430260
Thryssa setirostris	1	BOLD:AAC1966	OR430259
Triacanthus biaculeatus	2	BOLD:ADI2430	OR430257 to OR430258
Trypauchen vagina	1	BOLD:AAM5072	OR430256
Wallago attu	5	BOLD:AAE1290	OQ555344,OQ555349,OQ555351,OQ555354, OQ555355
Xenentodon cancila	4	BOLD:ABU9035	OQ555359, OQ555360, OQ555362, OQ555364

Table 3. The distribution of sequence divergence at each taxonomic level from 226 analysed specimens.

Label	n	Taxa	Comparisons	Min dist (%)	Mean dist (%)	Max dist(%)	SE dist (%)
Within Species	204	61	289	0	0.10	1.9	0
Within Genus	86	13	182	5.7	13.57	25.79	0.03
Within Family	146	13	1681	6.85	17.33	29.05	0

Min and *max dist.* minimum and maximum distance, *SE dist.* standard error in distance.

Table 4. Summary statistics for nucleotide frequency distribution are provided in the table below

	Min	Mean	Max	SE
G %	14.31	17.34	20.98	0.081
C %	23.38	27.75	32.83	0.1103
A %	19.87	25.13	28.96	0.1222
T %	24.96	29.79	33.33	0.0973
GC %	40.84	45.09	51.76	0.1447
GC % Codon Pos 1	49.46	55.65	59.46	0.1143
GC % Codon Pos 2	41.18	42.67	45.52	0.0463
GC % Codon Pos 3	24.08	36.94	55.28	0.403

Furthermore, the NJ tree showed that all the identified species to align in a monophyletic cluster without found any overlap between species by providing a s bootstrap support of 99% and 1.00 posterior probability is shown in Figure 1.

Further analysis results indicate the presence of gap called as barcode gap found among all the 83 observed species is shown in Table 5. In concordance with Barcoding Gap Analysis, ABGD also generated 83 operational taxonomic units (OTUs) with the initial partition at a prior intraspecific divergence (*P*) (P=0.0022–0.0465) is shown in Figure 2.

Species Delimitation

The assessment of species recognition with previously known sequences and closely related species in BLAST and BOLD databases yielded 97–100% similarity provide species-level resolution. Furthermore, Barcoding Gap Analysis showed that all putative species had a maximum intraspecies distance of less than 1.9%. The mean distance to the nearest neighbour (NN) was 11.14%, is shown in Figure 3.

Subsequent DNA barcodes sequences were analysed with ABGD tool that reflected in a stable

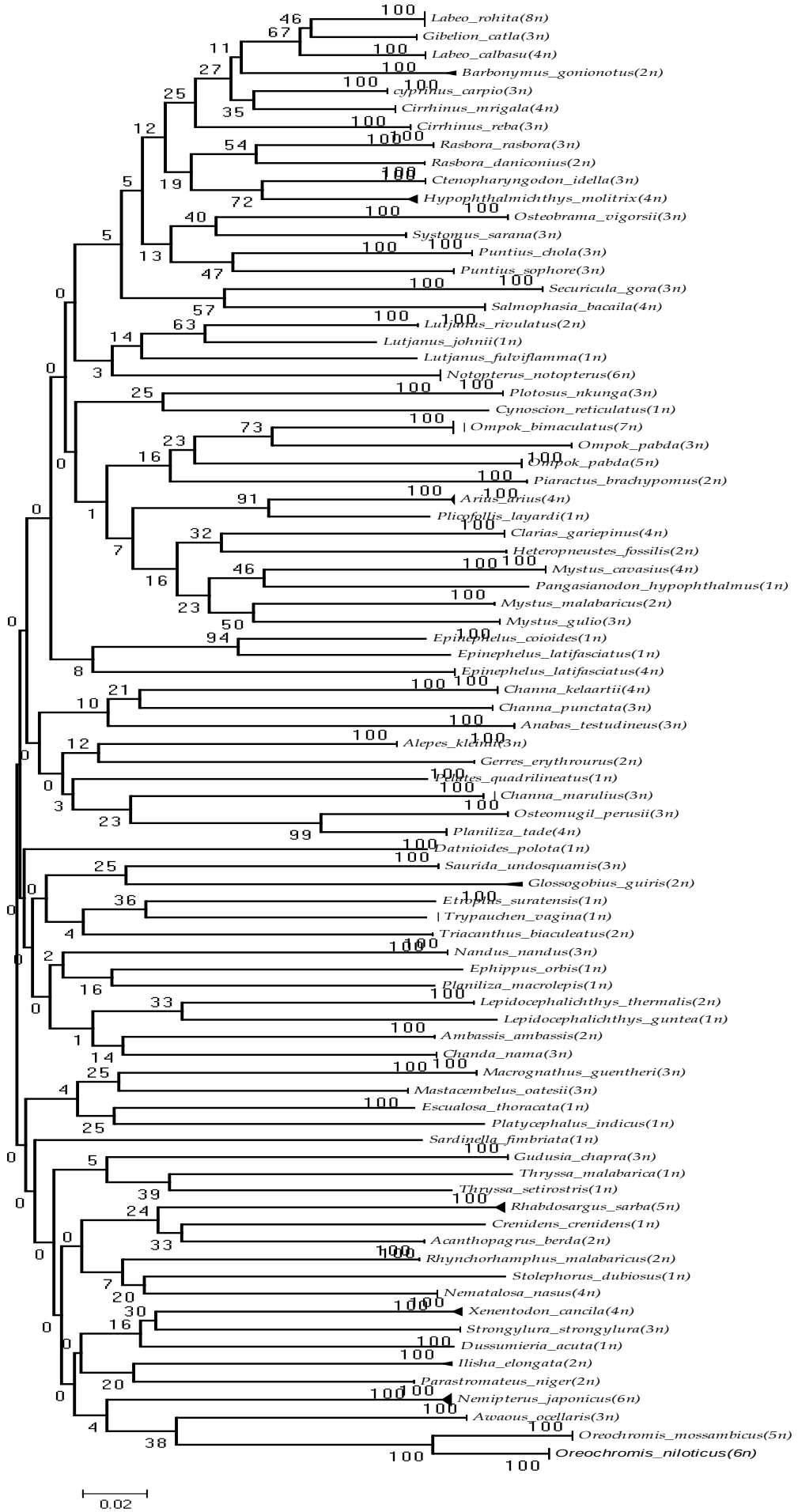

Figure 1. Neighbour-Joining (NJ) tree of 226 COI barcodes and scale bar indicates percent divergence calculated under the K2P model. *The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 8.26533783 is shown.

Table 5. The mean and maximum intraspecific values for each species, compared to the nearest neighbour distance.

Order	Family	Species	Mean Intra-Sp	Max Intra-Sp	Nearest Species	Nearest Neighbour	Distance to NN
Acanthuriformes	Sciaenidae	*Cynoscion reticulatus*	N/A	0	*Pelates quadrilineatus*	MAHAN2206-19	21.28
Anabantiformes	Anabantidae	*Anabas testudineus*	0.7	1.9	*Macrognathus guentheri*	MAHAN1896-18	19.17
Anabantiformes	Channidae	*Channa kelaartii*	0	0	*Channa punctata*	MAHAN916-15	21.23
Anabantiformes	Channidae	*Channa marulius*	0	0	*Channa punctata*	MAHAN915-15	19.23
Anabantiformes	Channidae	*Channa punctata*	0	0	*Channa marulius*	MAHAN1268-16	19.23
Anabantiformes	Nandidae	*Nandus nandus*	0	0	*Acanthopagrus berda*	MAHAN1140-16	20.82
Aulopiformes	Synodontidae	*Saurida undosquamis*	0.13	0.19	*Lutjanus fulviflamma*	MAHAN1992-18	20.96
Beloniformes	Belonidae	*Strongylura strongylura*	0	0	*Xenentodon cancila*	MAHAN1079-16	17.36
Beloniformes	Belonidae	*Xenentodon cancila*	0.24	0.4	*Strongylura strongylura*	MAHAN2208-19	17.36
Beloniformes	Hemiramphidae	*Rhynchorhamphus malabaricus*	0.54	0.54	*Nemipterus japonicus*	MAHAN1182-16	17.92
Carangiformes	Carangidae	*Alepes kleinii*	0	0	*Parastromateus niger*	MAHAN1601-18	14.73
Carangiformes	Carangidae	*Parastromateus niger*	0.77	0.77	*Alepes kleinii*	MAHAN1461-18	14.73
Characiformes	Serrasalmidae	*Piaractus brachypomus*	0.23	0.23	*Ompok bimaculatus*	MAHAN185-15	18.45
Cichliformes	Cichlidae	*Etroplus suratensis*	N/A	0	*Lutjanus johnii*	MAHAN1460-18	20.2
Cichliformes	Cichlidae	*Oreochromis mossambicus*	0	0	*Oreochromis niloticus*	MAHAN878-15	5.7
Cichliformes	Cichlidae	*Oreochromis niloticus*	0.04	0.2	*Oreochromis mossambicus*	MAHAN963-15	5.7
Clupeiformes	Clupeidae	*Dussumieria acuta*	N/A	0	*Ilisha elongata*	MAHAN1918-18	19.88
Clupeiformes	Clupeidae	*Escualosa thoracata*	N/A	0	*Acanthopagrus berda*	MAHAN1140-16	22.68
Clupeiformes	Clupeidae	*Gudusia chapra*	0	0	*Ilisha elongata*	MAHAN1917-18	19.4
Clupeiformes	Clupeidae	*Nematalosa nasus*	0	0	*Cirrhinus reba*	MAHAN1938-18	19.85
Clupeiformes	Clupeidae	*Sardinella fimbriata*	N/A	0	*Nematalosa nasus*	MAHAN1960-18	20.47
Clupeiformes	Engraulidae	*Stolephorus dubiosus*	N/A	0	*Thryssa malabarica*	MAHAN616-15	21.95
Clupeiformes	Engraulidae	*Thryssa malabarica*	N/A	0	*Thryssa setirostris*	MAHAN1256-16	19.47
Clupeiformes	Engraulidae	*Thryssa setirostris*	N/A	0	*Thryssa malabarica*	MAHAN616-15	19.47
Clupeiformes	Pristigasteridae	*Ilisha elongata*	0.25	0.25	*Rasbora daniconius*	MAHAN155-15	18.79
Cypriniformes	Cobitidae	*Lepidocephalichthys sp. MT-2015*	N/A	0	*Lepidocephalichthys thermalis*	MAHAN153-15	18.62
Cypriniformes	Cobitidae	*Lepidocephalichthys thermalis*	0.2	0.2	*Lepidocephalichthys sp. MT-2015*	MAHAN1760-18	18.62
Cypriniformes	Cyprinidae	*Barbonymus gonionotus*	0.56	0.56	*Cyprinus carpio*	MAHAN130-15	12.69
Cypriniformes	Cyprinidae	*Cirrhinus mrigala*	0	0	*Cyprinus carpio*	MAHAN130-15	11.21
Cypriniformes	Cyprinidae	*Cirrhinus reba*	0.36	0.55	*Labeo calbasu*	MAHAN188-15	11.99
Cypriniformes	Cyprinidae	*Ctenopharyngodon idella*	0	0	*Hypophthalmichthys molitrix*	MAHAN1038-15	10.32
Cypriniformes	Cyprinidae	*Cyprinus carpio*	0	0	*Cirrhinus mrigala*	MAHAN914-15	11.21
Cypriniformes	Cyprinidae	*Gibelion catla*	0	0	*Labeo calbasu*	MAHAN188-15	6.85
Cypriniformes	Cyprinidae	*Hypophthalmichthys molitrix*	0.5	0.81	*Ctenopharyngodon idella*	MAHAN1362-16	10.32
Cypriniformes	Cyprinidae	*Labeo calbasu*	0	0	*Gibelion catla*	MAHAN1209-16	6.85
Cypriniformes	Cyprinidae	*Labeo rohita*	0	0	*Gibelion catla*	MAHAN1209-16	6.92
Cypriniformes	Cyprinidae	*Osteobrama vigorsii*	0	0	*Systomus sarana*	MAHAN927-15	15.37
Cypriniformes	Cyprinidae	*Puntius chola*	0	0	*Puntius sophore*	MAHAN2192-19	12.08
Cypriniformes	Cyprinidae	*Puntius sophore*	0	0	*Puntius chola*	MAHAN233-15	12.08
Cypriniformes	Cyprinidae	*Rasbora daniconius*	0.79	0.79	*Rasbora rasbora*	MAHAN685-15	11.2
Cypriniformes	Cyprinidae	*Rasbora rasbora*	0	0	*Rasbora daniconius*	MAHAN670-15	11.2

Table 5. *Continued*

Cypriniformes	Cyprinidae	*Salmophasia bacaila*	0.12	0.18	*Hypophthalmichthys molitrix*	MAHAN1038-15	16.15
Cypriniformes	Cyprinidae	*Securicula gora*	0.13	0.2	*Salmophasia bacaila*	MAHAN1517-18	17.43
Cypriniformes	Cyprinidae	*Systomus sarana*	0	0	*Labeo rohita*	MAHAN1239-16	13.59
Gobiiformes	Gobiidae	*Glossogobius guiris*	0.62	0.62	*Plicofollis layardi*	MAHAN2093-18	19.75
Gobiiformes	Oxudercidae	*Awaous ocellaris*	0	0	*Oreochromis niloticus*	MAHAN947-15	18.85
Gobiiformes	Oxudercidae	*Trypauchen vagina*	N/A	0	*Lutjanus fulviflamma*	MAHAN1992-18	18.18
Moroniformes	Ephippidae	*Ephippus orbis*	N/A	0	*Planiliza sp. H_JDD*	MAHAN2196-19	20.93
Mugiliformes	Mugilidae	*Osteomugil perusii*	0	0	*Planiliza tade*	MAHAN907-15	10.62
Mugiliformes	Mugilidae	*Planiliza sp. H_JDD*	N/A	0	*Osteomugil perusii*	MAHAN1242-16	17.77
Mugiliformes	Mugilidae	*Planiliza tade*	0.19	0.37	*Osteomugil perusii*	MAHAN1217-16	10.62
Osteoglossiformes	Notopteridae	*Notopterus notopterus*	0.07	0.2	*Lutjanus rivulatus*	MAHAN1183-16	19.53
Ovalentaria	Ambassidae	*Ambassis ambassis*	0	0	*Plicofollis layardi*	MAHAN2093-18	18.64
Ovalentaria	Ambassidae	*Chanda nama*	0.16	0.25	*Parastromateus niger*	MAHAN1951-18	19.04
Perciformes	Gerreidae	*Gerres erythrourus*	0	0	*Lutjanus fulviflamma*	MAHAN1992-18	21.5
Perciformes	Lutjanidae	*Lutjanus fulviflamma*	N/A	0	*Lutjanus rivulatus*	MAHAN1915-18	16.49
Perciformes	Lutjanidae	*Lutjanus johnii*	N/A	0	*Lutjanus rivulatus*	MAHAN1915-18	14.04
Perciformes	Lutjanidae	*Lutjanus rivulatus*	0	0	*Lutjanus johnii*	MAHAN1460-18	14.04
Perciformes	Serranidae	*Epinephelus coioides*	N/A	0	*Epinephelus latifasciatus*	MAHAN2035-18	10.21
Perciformes	Serranidae	*Epinephelus latifasciatus*	N/A	0	*Epinephelus coioides*	MAHAN1221-16	10.21
Perciformes	Terapontidae	*Pelates quadrilineatus*	N/A	0	*Planiliza sp. H_JDD*	MAHAN2196-19	19.48
Scombriformes	Stromateidae	*Pampus chinensis*	0.17	0.35	*Lutjanus johnii*	MAHAN1460-18	18.83
Scorpaeniformes	Platycephalidae	*Platycephalus indicus*	N/A	0	*Lutjanus johnii*	MAHAN1460-18	20.27
Siluriformes	Ariidae	*Arius arius*	0.11	0.26	*Plicofollis layardi*	MAHAN2093-18	10.27
Siluriformes	Ariidae	*Plicofollis layardi*	N/A	0	*Arius arius*	MAHAN1255-16	10.27
Siluriformes	Bagridae	*Mystus cavasius*	0.08	0.16	*Mystus gulio*	MAHAN2201-19	15.61
Siluriformes	Bagridae	*Mystus gulio*	0.97	1.1	*Mystus malabaricus*	MAHAN1645-18	15.31
Siluriformes	Bagridae	*Mystus malabaricus*	0	0	*Mystus gulio*	MAHAN2201-19	15.31
Siluriformes	Clariidae	*Clarias gariepinus*	0	0	*Heteropneustes fossilis*	MAHAN1935-18	15.34
Siluriformes	Heteropneustidae	*Heteropneustes fossilis*	0	0	*Clarias gariepinus*	MAHAN918-15	15.34
Siluriformes	Pangasiidae	*Pangasianodon hypophthalmus*	N/A	0	*Plicofollis layardi*	MAHAN2093-18	14.42
Siluriformes	Plotosidae	*Plotosus nkunga*	0.27	0.41	*Pangasianodon hypophthalmus*	MAHAN1246-16	19.32
Siluriformes	Siluridae	*Ompok bimaculatus*	0	0	*Ompok pabda*	MAHAN1851-18	14.27
Siluriformes	Siluridae	*Ompok pabda*	0	0	*Ompok bimaculatus*	MAHAN185-15	14.27
Siluriformes	Siluridae	*Wallago attu*	0	0	*Ompok bimaculatus*	MAHAN185-15	14.84
Spariformes	Lobotidae	*Datnioides polota*	N/A	0	*Lutjanus rivulatus*	MAHAN1183-16	18.5
Spariformes	Nemipteridae	*Nemipterus japonicus*	0.32	0.69	*Rhynchorhamphus malabaricus*	MAHAN620-15	17.92
Spariformes	Sparidae	*Acanthopagrus berda*	0	0	*Rhabdosargus sarba*	MAHAN621-15	14.28
Spariformes	Sparidae	*Crenidens crenidens*	N/A	0	*Acanthopagrus berda*	MAHAN1140-16	16.85
Spariformes	Sparidae	*Rhabdosargus sarba*	0.08	0.19	*Acanthopagrus berda*	MAHAN1140-16	14.28
Synbranchiformes	Mastacembelidae	*Macrognathus guentheri*	0	0	*Mastacembelus oatesii*	MAHAN1225-16	14.58
Synbranchiformes	Mastacembelidae	*Mastacembelus oatesii*	0	0	*Macrognathus guentheri*	MAHAN1896-18	14.58
Tetraodontiformes	Triacanthidae	*Triacanthus biaculeatus*	0.36	0.36	*Lutjanus fulviflamma*	MAHAN1992-18	18.42

operational taxonomic count (83 OTUs) for initial partition, while the recursive partition produced a higher OTU count (83-89 OTUs) ranging with prior intraspecific values (P) from 0.0001 to 0.04641 for JC69, K2P and Simple distance metrics respectively is shown in Table 6.

The range varied from 1 to 7 specimens in some species. Analysis of mitochondrial COI barcodes for 226 specimens belonging to 83 species, 67 genera, 39 families and 21 orders. The average number of specimens analysed was three individuals per species. The average genetic distance between species is 0.3% in BOLD (Barcode of Life Data System) for fish databases, and congeneric distance should be 30-fold greater than conspecific distances (Zhang & Hanner, 2011).

Conservation Status of Fish

Briefly, the conservation status for each of the 83 barcoded species from the Chilika Lagoon does not raise

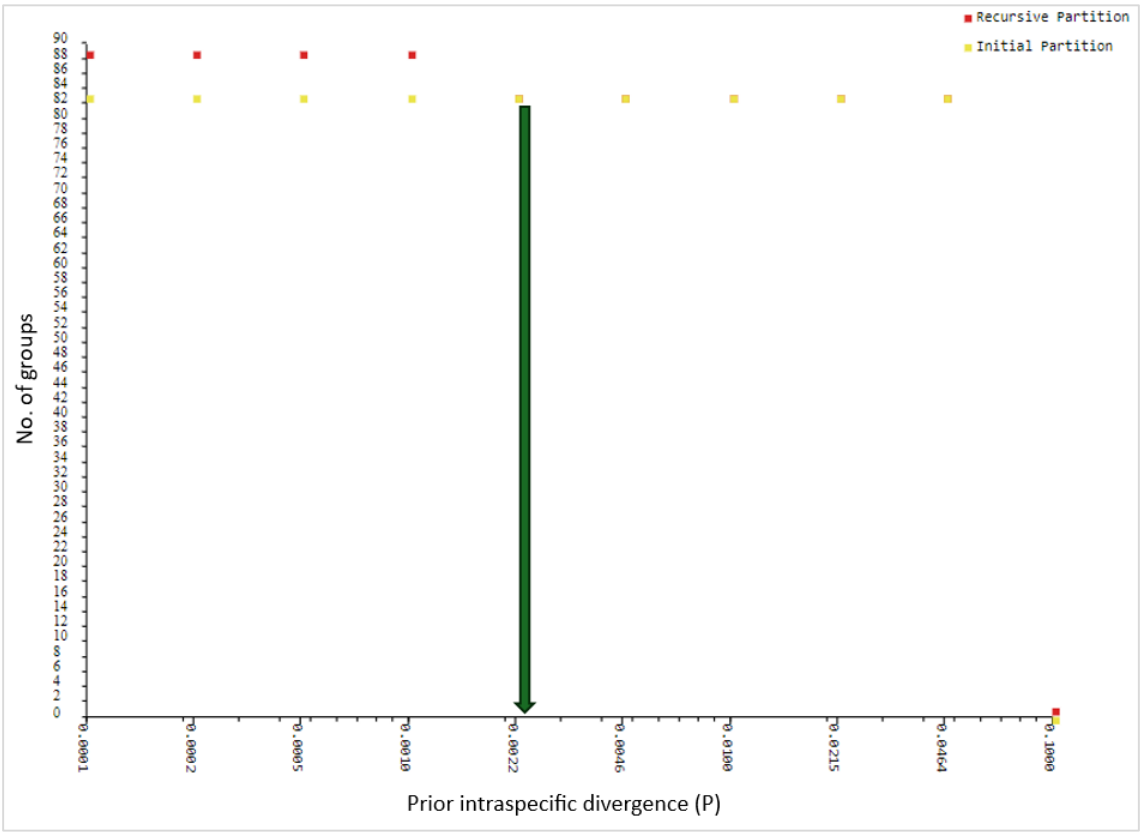

Figure 2. The number of genetically distinct OTUs according to the prior intraspecific divergence value generated by ABGD based on K2P. [* In concordance with Barcoding Gap Analysis, ABGD also generated 83 operational taxonomic units (OTUs) with the initial partition at a prior intraspecific divergence (P) (P=0.0022–0.0465).]

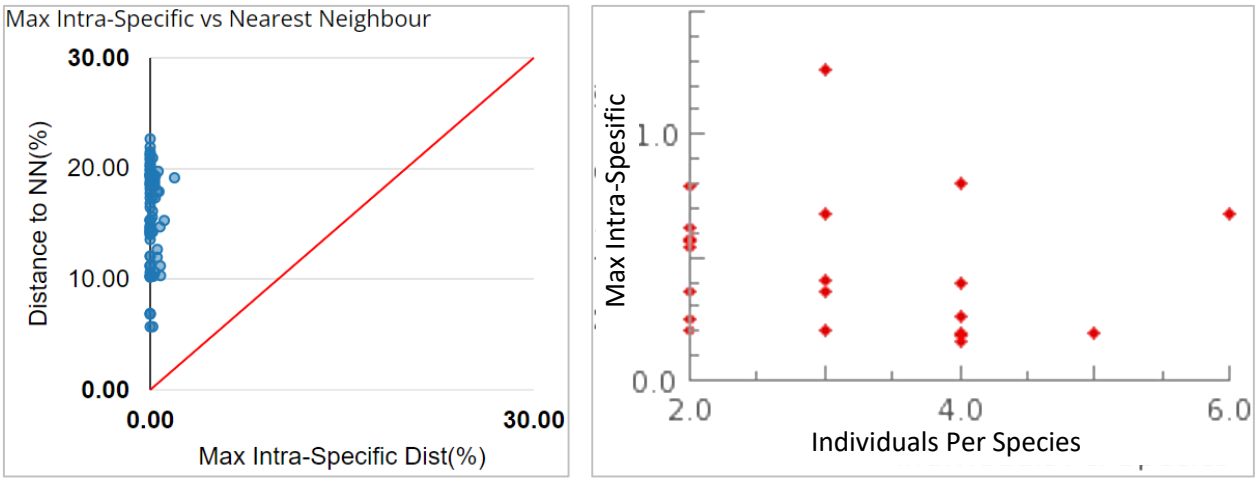

Figure 3. Scatterplots show the overlap of the max intra-specific distances' vs the inter-specific (nearest neighbour) distances and relationship between maximum intraspecific divergence (% K2P) at COI plotted against the number of individuals barcoded per species.

any cause for alarm with 76%, 8%, 6%, 4%, 4% and 2% categorized into LC, NE, NT, DD, VU and NN respectively shown in Figure 4. The majority (LC) do not appear to require any additional protection as required for Endangered and Vulnerable categories (IUCN, 2012) However, urgent attention is needed for the exploiting the species listed in NE and DD categories as these have no or limited biological., ecological or distributional information based on the IUCN criteria (Sadovy de Mitcheson et al., 2013). Cyprinidae was the most abundant family found in the Chilika lagoon landing containing 17 species (21%), followed by Clupiedae has 5 species (6%) is shown in the Figure 5.

Chilika Lagoon is a junction of marine, brackish and freshwater ecosystems, on the east coast of India is a serve as Ramsar site and a biodiversity hotspot. In total, 255 collections (178 collections of previously recorded species and 77 that represent newly recorded species) were made during a post-restoration survey accounting for 80.44% retrieval of historically documented species.

The conservation status for the species based on the information from the IUCN Red List (IUCN, 2014) as well as Ponniah (1993), Molur & Walker, (1998), Menon (2004), Barman, et al., (2007) and Lakra, et al., (2010). The discrimination potential of DNA barcoding is evaluated based on genetic distance between species and within the species. (Lievens et al., 2001). Finally, the identification success rate of DNA barcoding was evaluated based on the sequencing success rate and species discrimination rate (Kress, et al., 2009).

Our study indicates that marine species (M), brackish species (B), freshwater species (F), marine and brackish water (MB), brackish and freshwater (BF) and Marine, Brackish and Freshwater species (MBF) constitute 12%, 1%, 33%, 25%, 23% and 6% respectively are shown in Figure 6 and Table 7.

Chilika Lagoon is a junction of marine, brackish and freshwater ecosystems, on the east coast of India is a serve as Ramsar site and a biodiversity hotspot. In total, 255 collections (178 collections of previously recorded species and 77 that represent newly recorded species) were made during a post-restoration survey accounting for 80.44% retrieval of historically documented species.

Table 6. Recognizing subsequent OTU's from 226 COI sequences through automatic barcode gap discovery (ABGD) using substitution model

Substitution Model	Partition	Prior intraspecific divergence (P)								
		0.0001	0.000215	0.00046	0.001	0.00215	0.00464	0.010000	0.02154	0.04641
Jukes Cantor (JC)	Initial	83	83	83	83	83	83	83	83	83
	Recursive	89	89	89	89	89	89	89	89	89
Kimura 2 parameter (K2P)	Initial	83	83	83	83	83	83	83	83	83
	Recursive	89	89	89	89	89	89	89	89	89
p-Distance (simple)	Initial	83	83	83	83	83	83	83	83	83
	Recursive	83	83	83	83	83	83	83	83	83

*For ABGD, initial partition with P values from 0.0001 to 0.04641 for JC69, K2P and Simple distance metrics with relative gap width (X) 1.0 are included.

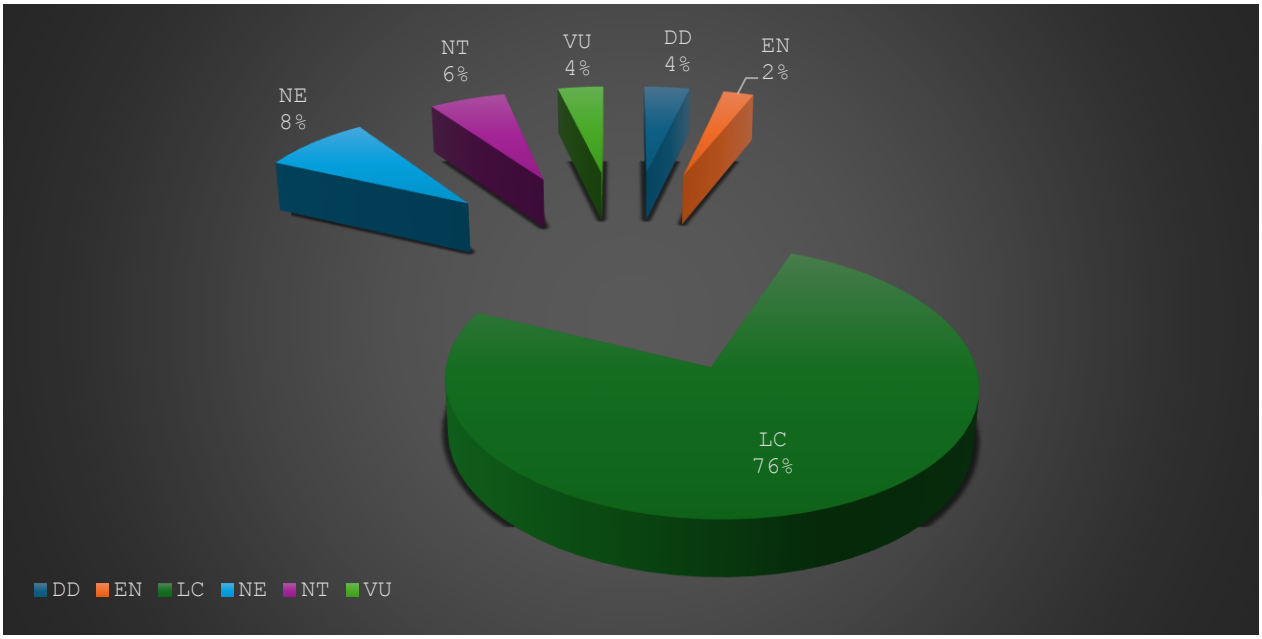

Figure 4. Conservation status of the fish species caught as Chilika Lake of India. The classifications were obtained from the IUCN Red List of Threatened Species (https://www.iucnredlist.org/).

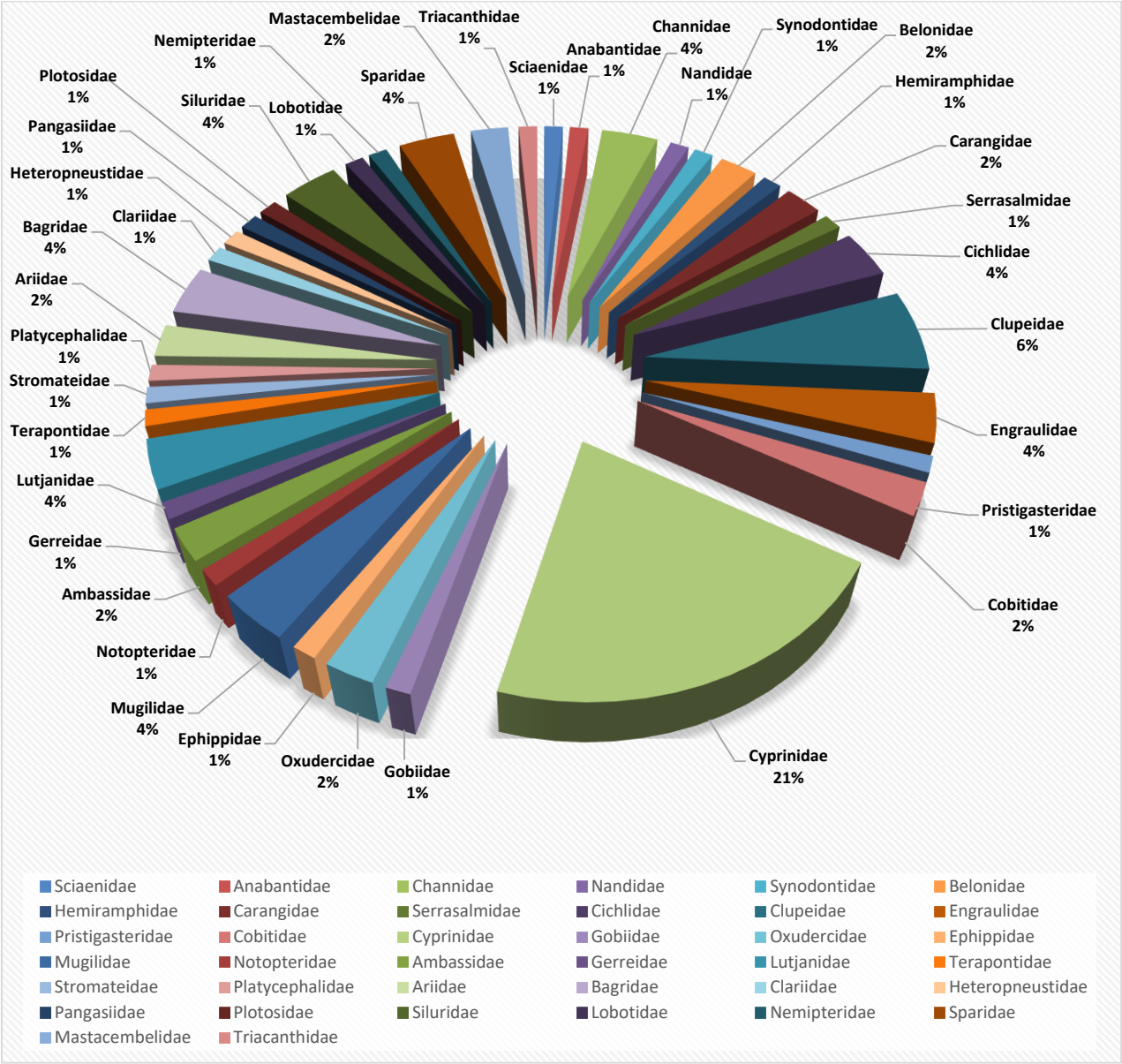

Figure 5. The composition (family) of fishes found in the Chilika Lake.

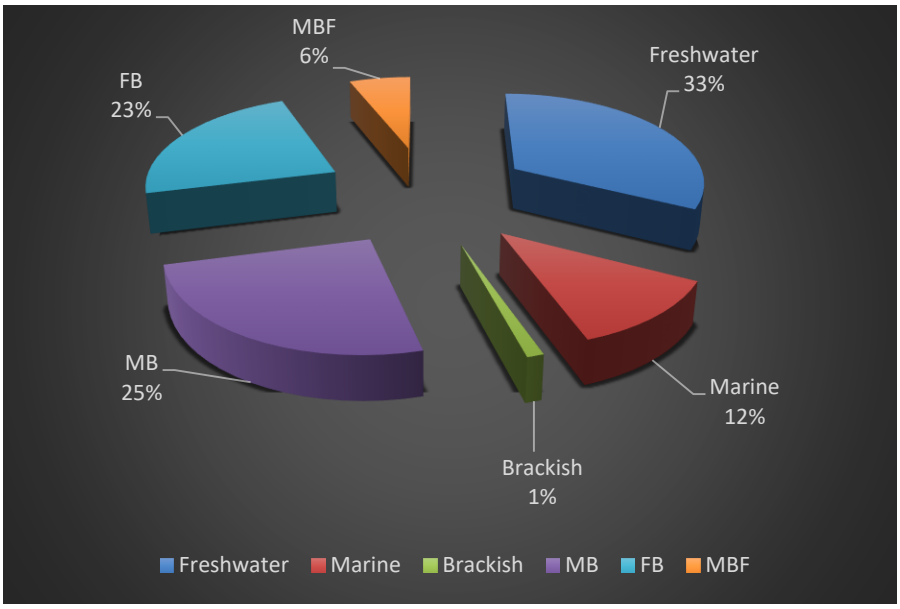

Figure 6. Ichthyofaunal composition of marine, brackish and freshwater species found in Chilika Lake.

Table 7. Evaluation of biodiversity status and their habitat of Chilika lagoon fishes

Order	Family	Genus	Species	Conservative Status	Fish Habitat
Spariformes	Sparidae	Acanthopagrus	*Acanthopagrus berda*	NE	MB
Carangiformes	Carangidae	Alepes	*Alepes kleinii*	NE	M
Ovalentaria	Ambassidae	Ambassis	*Ambassis ambassis*	LC	FB
Anabantiformes	Anabantidae	Anabas	*Anabas testudineus*	DD	FB
Siluriformes	Ariidae	Arius	*Arius arius*	LC	B
Gobiiformes	Oxudercidae	Awaous	*Awaous ocellaris*	LC	MBF
Cypriniformes	Cyprinidae	Barbonymus	*Barbonymus gonionotus*	LC	F
Ovalentaria	Ambassidae	Chanda	*Chanda nama*	LC	FB
Anabantiformes	Channidae	Channa	*Channa kelaartii*	NT	F
Anabantiformes	Channidae	Channa	*Channa marulius*	LC	F
Anabantiformes	Channidae	Channa	*Channa punctata*	LC	F
Cypriniformes	Cyprinidae	Cirrhinus	*Cirrhinus mrigala*	LC	F
Cypriniformes	Cyprinidae	Cirrhinus	*Cirrhinus reba*	LC	F
Siluriformes	Clariidae	Clarias	*Clarias gariepinus*	LC	F
Spariformes	Sparidae	Crenidens	*Crenidens crenidens*	LC	M
Cypriniformes	Cyprinidae	Ctenopharyngodon	*Ctenopharyngodon idella*	LC	FB
Acanthuriformes	Sciaenidae	Cynoscion	*Cynoscion reticulatus*	LC	MB
Cypriniformes	Cyprinidae	Cyprinus	*Cyprinus carpio*	VU	FB
Spariformes	Lobotidae	Datnioides	*Datnioides polota*	LC	FB
Clupeiformes	Clupeidae	Dussumieria	*Dussumieria acuta*	LC	M
Moroniformes	Ephippidae	Ephippus	*Ephippus orbis*	LC	M
Perciformes	Serranidae	Epinephelus	*Epinephelus coioides*	LC	MB
Perciformes	Serranidae	Epinephelus	*Epinephelus latifasciatus*	LC	M
Clupeiformes	Clupeidae	Escualosa	*Escualosa thoracata*	LC	M
Cichliformes	Cichlidae	Etroplus	*Etroplus suratensis*	LC	BF
Perciformes	Gerreidae	Gerres	*Gerres erythrourus*	LC	MB
Cypriniformes	Cyprinidae	Gibelion	*Gibelion catla*	LC	F
Gobiiformes	Gobiidae	Glossogobius	*Glossogobius guiris*	LC	MBF
Clupeiformes	Clupeidae	Gudusia	*Gudusia chapra*	LC	F
Siluriformes	Heteropneustidae	Heteropneustes	*Heteropneustes fossilis*	LC	F
Cypriniformes	Cyprinidae	Hypophthalmichthys	*Hypophthalmichthys molitrix*	NT	FB
Clupeiformes	Pristigasteridae	Ilisha	*Ilisha elongata*	LC	BM
Cypriniformes	Cyprinidae	Labeo	*Labeo calbasu*	LC	F
Cypriniformes	Cyprinidae	Labeo	*Labeo rohita*	LC	F
Cypriniformes	Cobitidae	Lepidocephalichthys	*Lepidocephalichthys guntea*	LC	F
Cypriniformes	Cobitidae	Lepidocephalichthys	*Lepidocephalichthys thermalis*	LC	F
Perciformes	Lutjanidae	Lutjanus	*Lutjanus fulviflamma*	LC	MB
Perciformes	Lutjanidae	Lutjanus	*Lutjanus johnii*	LC	MB
Perciformes	Lutjanidae	Lutjanus	*Lutjanus rivulatus*	LC	M
Synbranchiformes	Mastacembelidae	Macrognathus	*Macrognathus guentheri*	LC	F
Synbranchiformes	Mastacembelidae	Mastacembelus	*Mastacembelus oatesii*	EN	F
Siluriformes	Bagridae	Mystus	*Mystus cavasius*	LC	FB
Siluriformes	Bagridae	Mystus	*Mystus gulio*	LC	FB
Siluriformes	Bagridae	Mystus	*Mystus malabaricus*	NT	FB
Anabantiformes	Nandidae	Nandus	*Nandus nandus*	LC	FB
Clupeiformes	Clupeidae	Nematalosa	*Nematalosa nasus*	LC	BM
Spariformes	Nemipteridae	Nemipterus	*Nemipterus japonicus*	LC	M
Osteoglossiformes	Notopteridae	Notopterus	*Notopterus notopterus*	LC	FB
Siluriformes	Siluridae	Ompok	*Ompok bimaculatus*	NT	F
Siluriformes	Siluridae	Ompok	*Ompok pabda*	NT	F
Cichliformes	Cichlidae	Oreochromis	*Oreochromis mossambicus*	VU	FB
Cichliformes	Cichlidae	Oreochromis	*Oreochromis niloticus*	LC	FB
Cypriniformes	Cyprinidae	Osteobrama	*Osteobrama vigorsii*	LC	F
Mugiliformes	Mugilidae	Osteomugil	*Osteomugil perusii*	LC	MB
Scombriformes	Stromateidae	Pampus	*Pampus chinensis*	NE	MB
Siluriformes	Pangasiidae	Pangasianodon	*Pangasianodon hypophthalmus*	EN	F
Carangiformes	Carangidae	Parastromateus	*Parastromateus niger*	LC	MB
Perciformes	Terapontidae	Pelates	*Pelates quadrilineatus*	LC	MB
Characiformes	Serrasalmidae	Piaractus	*Piaractus brachypomus*	NE	F
Mugiliformes	Mugilidae	Planiliza	*Planiliza macrolepis*	LC	MBF
Mugiliformes	Mugilidae	Planiliza	*Planiliza tade*	DD	MBF
Scorpaeniformes	Platycephalidae	Platycephalus	*Platycephalus indicus*	LC	MB
Siluriformes	Ariidae	Plicofollis	*Plicofollis layardi*	NE	MB
Siluriformes	Plotosidae	Plotosus	*Plotosus nkunga*	NE	MBF
Cypriniformes	Cyprinidae	Puntius	*Puntius chola*	LC	F
Cypriniformes	Cyprinidae	Puntius	*Puntius sophore*	LC	F
Cypriniformes	Cyprinidae	Rasbora	*Rasbora daniconius*	LC	F
Cypriniformes	Cyprinidae	Rasbora	*Rasbora rasbora*	LC	F
Spariformes	Sparidae	Rhabdosargus	*Rhabdosargus sarba*	LC	MB
Beloniformes	Hemiramphidae	Rhynchorhamphus	*Rhynchorhamphus malabaricus*	NE	M
Cypriniformes	Cyprinidae	Salmophasia	*Salmophasia bacaila*	LC	FB
Clupeiformes	Clupeidae	Sardinella	*Sardinella fimbriata*	LC	MB
Aulopiformes	Synodontidae	Saurida	*Saurida undosquamis*	LC	M
Cypriniformes	Cyprinidae	Securicula	*Securicula gora*	LC	F
Clupeiformes	Engraulidae	Stolephorus	*Stolephorus dubiosus*	LC	MB
Beloniformes	Belonidae	Strongylura	*Strongylura strongylura*	LC	FB
Cypriniformes	Cyprinidae	Systomus	*Systomus sarana*	LC	F
Clupeiformes	Engraulidae	Thryssa	*Thryssa malabarica*	DD	MB
Clupeiformes	Engraulidae	Thryssa	*Thryssa setirostris*	LC	MB
Tetraodontiformes	Triacanthidae	Triacanthus	*Triacanthus biaculeatus*	LC	MB
Gobiiformes	Oxudercidae	Trypauchen	*Trypauchen vagina*	LC	MB
Siluriformes	Siluridae	Wallago	*Wallago attu*	VU	FB
Beloniformes	Belonidae	Xenentodon	*Xenentodon cancila*	LC	FB

*Note:- VU-Vulnerable; NT-Near Threatened; LC-List Concern; DD-Data Deficient; M Menon (2004); B Barman et al. (2007) NE-Not Evaluated; EN/E- Endangered; F- Fresh water, M- Marine, B- Brackish water, BM- Brackish water, Marine water FB- Fresh water, Brackish water, MBF- Marine, Brackish water, Freshwater.

Discussion

This is the first molecular based study, in which COI barcodes have been used to analysed fishes from the Chilika lagoon of India, one of the world's biodiversity hotspots (Mohanty, et al., 2015). The Chilika lagoon shows unique and phylogenetically diverse, endemic, and exotic fish diversity, contributing a total of 355 freshwater fish species (Mohanty, et al., 2015). We generated 226 COI barcodes for 83 species the sequences analysed exhibit mean intraspecific genetic distance of 0.10% (SE=0) and interspecific distance 13.57% (SE=0.03) which is somewhat similar with the previously studied fishes (Hubert, et al., 2008, Lakra, et al 2011, Cu-ban Lara, et al., 2012, Chakraborty & Ghosh 2014, Khedkar, et al., 2014, Ward, et al., 2015, Bingpeng, et al., 2018, Suryawanshi et al., 2024). The Chilika lagoon shows unique and phylogenetically diverse, endemic, and exotic fish diversity, contributing a total of 355 freshwater fish species (Mohanty, et al., 2015). Utilization of the species delimitation methods. BIN analysis of the present study delineated a total of 83 OTUs corresponding to concordant and singleton BINs. We found about 73.49% BINs to be taxonomically concordant; this reflected the proofread of our dataset, 26.50% were singleton BIN, absence of discordant BINs in our dataset. ABGD generated consistent OTUs having overlapped with the results obtained from BIN. ABGD analysis exhibited 83 compel OTUs in the initial partition by default value of P=0.01 based on JC69, K2P and Simple distance metrics, while recursive partition generated inconsistent OTUs (84-89). The OTUs generated in ABGD from initial partition were concordant with morphological identification. This may reflect that the absence of barcode gap resulting in merged OTUs (Puillandre, et al., 2012). In the present study, we can consider the OTUs produced by recursive partition indicated the independent taxon because they are morphologically congruent (Kekkonen & Hebert, 2014).

Establishment of DNA Barcode Library for Chilika Lagoon Fishes

This study has demonstrated the utility of DNA barcoding to complement the morphological identification of 83 fish species from Chilika lagoon. The discrimination power of proposed DNA barcodes made formation of reliable DNA barcode reference records for fishes in Chilika lagoon has been initiated. The obtained DNA barcodes data is critical for the future fisheries management on this Ramsar Site. Database sequence similarity and genetic distance comparisons with voucher reference supported the accurate identification of the 83 putative species. The exact or near matches (97% to 100%) identity with reference DNA libraries both in BLAST and the BOLD Identification System (IDS) has found to be great evidence in the success of the DNA barcoding approach (Bhattacharjee et al., 2012; Ratnasingham & Hebert, 2007; Ward, 2009; Zhang & Hanner, 2012). Further species delineation method ABGD and the monophyletic cluster generated on the NJ tree distinctly recognized species. The mean genetic distances between individuals within species, genera, and families were 0.10%, 13.57%, and 17.33%, respectively and the current study results are very similar to the previous studies. For example, the mean genetic distances of Australian fishes within families, genera and species were 15.46%, 9.93% and 0.39% respectively (Ward, et al., 2005); intra-species distance of South Africa fishes were 0.21% and Australia fishes were 0.28%, (Zemlak, et al., 2009); Conspecific distance of Indian marine fishes were 0.30% while 6.60% 9.91% congeneric and confamilial respectively (Lakra, et al., 2011); divergence of IndoPacific coral reef fishes was 1.06% (Hubert, et al., 2012).

Categorization of Marine, Estuarine and Freshwater Fish Species

Chilika lagoon is one of the important hotspots for the biodiversity of South Asia having international importance. A modified form of the widely accepted categorization by Elliot et al., (2007) is followed by to categorize the fishes into marine, brackish and freshwater species. In India two most common threats to the biodiversity of fishes are anthropogenic and natural stressors (Das, et al., 2004, Kurup & Radhakrishnan, 2006, Rout, et al., 2007), also been observed for fishes in Chilika lagoon, some traditional methods like destructive fishing practices and traditional illegal large pen culture units. The global fish diversity has impact on the natural limiting factors to the native fish species. Furthermore, the negative impact of introduced species is also increasing (Crivelli, A J, 1995, Rainbow, P, 1998, Balestrieri et al., 2013). At the same time, the negative impact of anthropogenic factors on the fish biodiversity of freshwater basins is also growing (Tickner, et al., 2020). Moreover, biologically species reducing rate enhancing day by day therefore, DNA barcoding is becoming a popular tool that can be used to assess fish biodiversity, monitor and their conservation, and manage fishery resources, processed food, ecosystem management and conservation, seafood forensics, biosecurity, and invasive species detection, predator-prey relationship, and seed recognition (Pavan-Kumar, et al., 2018, Takahara, et al., 2013, Muchlisin, et al., 2017, Ran, et al., 2020, Gilbey, J et al., 2021). Moreover, DNA barcoding also has several limitations like it need a reliable DNA library for comparing the newly generated sequence with the existing database (Taylor & Harris, 2012). Co-amplification of Nuclear mitochondrial pseudogenes (numts) with mtDNA when using conserved universal PCR primers could lead to species diversity with error (Song, et al., 2008). The COI gene is of mitochondrial origin, typically inherited from the mother, for this reason, any hybrid fish will be identified as its maternal

species (Bhattacharya, et al., 2016; Linacre, et al., 2011). Furthermore, to allow the accurate identification it need to employ additionally nuclear markers. (Bhattacharya, et al., 2016; Kochzius, et al., 2010; Linacre, et al., 2011, Dudu, et al., 2016). Broad range of teleost fishes, both fresh water (Scribner, Page & Bartron, 2000) and marine (Montanari, et al., 2012; Qu et al., 2018; Yaakub, et al., 2006) showing the presence of hybridization. Finally, results of the current study have significantly contributed records for the molecular taxonomy of fishes from the Chilika Lagoon. DNA barcodes sequences have been deposited in the BOLD Systems and listed the fishes from the Chilika lagoon as well as Indian fish in general. Furthermore, current data could facilitate improved monitoring, conservation, and management of fisheries in this area. Needs more funds for the more intensive studies with a wider coverage and through direct sampling.

Conclusion

Establishment of a reliable DNA barcodes library using a single locus mitochondrial COI gene when analysed with various species delimitation methods BIN and ABGD were efficient for delineating fish species of fish fauna in the Chilika lagoon for accurate identification of fishes that may facilitate ichthyological research, including taxonomy, fishery, and biodiversity management. Furthermore, we utilized integrated approach of molecular and morphological taxonomy, our study highlights and accelerate the poorly identified fishes. Consequence was able to recognize cryptic fish diversity in the Chilika lagoon of India in the form of genetically diverged and generically significant putative species. The establishment of a DNA barcode reference library of the Chilika Lagoon fishes has been achieved through this study. In total, 226 specimens from 83 species belonging to 32 genera in 17 families were barcoded. No overlapping has been found between conspecific and interspecific comparisons. Cyprinidae were the dominant family followed by Clupiedae, Siluridae, Engraulidae, Cichlidae. We are contributing to the DNA barcode library of Chilika Lagoon fishes and the worldwide barcode entries in general.

Ethical Statement

We declare that all individuals were obtained from the lagoon nearest market, they were already dead Many fish are routinely caught by professional fisherman and sold as a food fish in Indian markets. In India there is no need to take the permission for fish caught and no in vitro study was conducted the fish.

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Author Contribution

First Author: Conceptualization, Writing -review and editing,
Second Author: Data Curation, Methodology, Visualization and Writing,
Third Author: Writing review and editing,
Fourth Author: Writing review and editing,
Fifth Author: References alignment.

Conflict of Interest

The authors declare that they have no conflict of interest.

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