The Journal of Animal & Plant Sciences, 29(5): 2019, Page: 1501-1518
ISSN: 1018-7081

MITOCHONDRIAL COI GENE BASED MOLECULAR IDENTIFICATION AND PHYLOGENETIC ANALYSIS IN EXOTIC FISH (*OREOCHROMIS MOSSAMBICUS*) OF PAKISTAN

A. Yaqub[1]*, M. Kamran[1,2], N. Malkani[1], K. M. Anjum[3], M. Faheem[1], M. Iqbal[1] and R. U. Khan[1]

[1]Department of Zoology, Government College University, Lahore, Pakistan
[2]Fisheries and aquaculture lab, Department of Animal Sciences, Quid-i-Azam, University, Islamabad, Pakistan
[3]Department of Wildlife and Ecology, University of Veterinary and Animal Sciences, Lahore, Pakistan
*Corresponding author: atif@gcu.edu.pk

ABSTRACT

Tilapia *Oreochromis* spp. are exotic fish introduced in Pakistan from Malaya in 1951. Due to its omnivorous feeding habits and adaptability to harsh environmental conditions, such as salinity and temperature, is considered suitable for aquaculture in Pakistan. However, to date, classification and phylogenetic relationship among the tilapia cultured in Pakistan are poorly understood. In the present study, samples of tilapia were collected from different aquatic systems of Pakistan and Cytochrome c Oxidase subunit I (COI) of mitochondrial genes were investigated to elucidate molecular characterization and find out phylogenetic relationships. DNA extraction was performed from the fin tissue of collected specimens using modified salt extraction method and COI gene of mtDNA was amplified using universal primers for fish and finally the amplified products were sequenced. Sequencing results showed that specimens belonged to species *O. mossambicus*, of family Cichlidae. Sequence analysis was done by the MEGA 7 software, and cladogram were constructed. The sequence analysis of the genes also showed that there was no significant divergence among all the specimen studied. The species of *O. mossambicus* was not only confirmed but also separated from other species of family Cichlidae with the help of molecular approach using mtCOI sequence.

Key words: *Oreochromis mossambicus*, Exotic, COI, mtDNA, molecular identification, phylogenetic analysis.

INTRODUCTION

The exotic species are important because of the role they play in biological communities especially in aquatic ecosystems. (Carey and Wahl, 2010; Strayer, 2010; Kornis *et al.*, 2013; Gu *et al.*, 2016). Exotic fish species can alter the local community in aquatic environment and reduce biodiversity. Sometimes, it adverse effects if the inhabiting aquatic ecosystem can be seen (Meador *et al.*, 2003; Zambrano *et al.*, 2006; Strayer, 2010; Giannetto *et al.*, 2012; Lowe *et al.*, 2012).

Among the eight exotic fish species found in Pakistan, tilapia is probably the most important due to its economic importance (Rafique and Khan, 2012). Tilapia belongs to African cichlids (D'amato *et al.*, 2007) which are local towards the Southwestern, Middle East and Africa (Bhassu *et al.*, 2004; Canonico *et al.*, 2005; Gu *et al.*, 2016). Tilapia species are euryhaline teleost, ideal candidates and most profitable in the aquaculture industry due to their adaptation to extreme environmental conditions including salinity, alkalinity, and high water temperature (Stickney, 2005; Lin *et al.*, 2016). They may be farmed in freshwater, brackish as well as in salt water. Tilapia species are opportunistic feeders, omnivores (Hassanien and Gilbey, 2005; Stickney, 2005; Briñez *et al.*, 2011). Tilapia exhibit robust reproduction habit; they can spawn about once a month if proper temperature is maintained. Their growth rate is higher as compared to other perciforms; they can attain maturity as early as 3 month after hatching. That is why they are easy to culture and their aquaculture is viable financially. Tilapia are second most farmed fish throughout the world (Stickney, 2005; Lacerda *et al.*, 2008; Briñez *et al.*, 2011; Gu *et al.*, 2016).

Tilapia were introduced to Pakistan from Malaysia in 1951 (Mirza, 1994; Canonico *et al.*, 2005). Three tilapia species have by far been reported in Pakistan including *O. mossambicus, O. aureus and O. niloticus* (Khan *et al.*, 2011). Whereas, *O. mossambicus* commonly, also known as Mozambique tilapia, have been popular in aquaculture in Pakistan and have been farmed in numerous localities of Pakistan; the same is also farmed in part of south China, where it represent 81% of the aggregate of tilapia production. Fillets of Mozambique Tilapia is favored for its white to reddish colour, flavorful taste, firm and succulent flesh (Josupeit, 2007).

Although numerous studies have done concerning Mozambique tilapia in Pakistan, but its molecular identification and phylogenetic analyses has never been done to date. Traditionally, morphological based identification is used to identify Mozambique tilapia. However, there is need that their characterization should done using molecular approaches which will also

help to establish phylogenetic relationship between different species. In this context, studies involving Cytochrome Oxidase type I (COI) Mt DNA, 16S ribosomal DNA (rDNA), 12S (rDNA), 18S (rDNA), and ribulose-biphosphate carboxylase are suitable (Hebert *et al.*, 2003; Ward *et al.*, 2009; Bhattacharya *et al.*, 2016).

Study of CO1 region of Mitochondrial DNA (mtDNA) is generally used to establish phylogenetic relationship in animals, microbes and plants (Stoneking and Soodyall, 1996; Chinnery, 2006; Knudsen *et al.*, 2006; Storey *et al.*, 2013; Kamran, 2017). This technique is reliable, simple and can trace even fast evolutionary rate (Curole and Kocher, 1999; Shin *et al.*, 2004; Wan *et al.*, 2004; Arif and Khan, 2009; Daravath *et al.*, 2013; Patwardhan *et al.*, 2014; Kamran, 2017). It also can be accessed in all stages of life from egg to adult (Ward *et al.*, 2009). Hence, COI sequences are reliable for the identification and phylogenetic investigations of animals (Avise, 2000; Abdel-Mawgood, 2012; Kamran, 2017).

It is important to retrieve phylogenetic analyses of alien species to regulate and control the species in a habitat (Gu *et al.*, 2016). The current study has been designed to investigate the COI gene sequences of mtDNA for the molecular identification and phylogenetic analysis of tilapia species (presumably Mozambique tilapia) found in the Pakistan.

MATERIALS AND METHODS

Sampling and isolation of DNA: Fin samples were collected from 12 Mozambique Tilapia specimen from three different localities (Table 01). Samples were taken from both, female and male. Genomic DNA was extracted by salt extraction method. Nano Drop 2000/2000c (Thermo scientific USA) was used to quantify the DNA samples.

PCR Amplification and Sequencing: Reported primers (Ward *et al.*, 2005) were used for the amplification of 655bp barcode region of the mitochondrial DNA. Primers were synthesized from MACROGEN Inc., Seoul, Korea.

Primer	Primer sequence (5'- 3')
Fish F1	TCAACCAACCACAAAGACATTGGCAC
Fish R1	TAGACTTCTGGGTGGCCAAAGAATCA

PCR reaction was carried out using Q cycler using following reaction conditions; an initial denaturing for 4 min at 94°C, 35 amplification cycles comprising of i) denaturation for 60 sec at 94°C, ii) annealing for 45 sec at 45°C, iii) extension for 60 sec at 72°C, and a final extension step at 72°C then storage at 4° C. PCR was followed by confirmation of product at 1.5 % agarose gel. PCR products, which yielded good quality bands, were selected for sequencing and sent to MACROGEN, Korea. Sequencing was done by using forward and reverse primers.

Bioinformatics analysis: Bioedit software and Clustal X1.83 was used to edit and align all the sequences respectively (Thompson *et al.*, 1997). For the purpose of phylogenic analyses, sequences of Tilapia spp. were obtained from BOLD, already reported in various parts of the world (Table 02) and maximum likelihood tree with 1000 bootstrap value was inferred on the basis of Kimura 2- parameter (K2P) model using MEGA version 7 (Molecular Evolutionary Genetics Analysis, MEGA Inc., Englewood, NJ) (Kumar *et al.*, 2016). Tree was constructed in BOLD (ID Accession No. YTC005-12.CYTB-) as an out-group. The COI based pairwise genetic distance values (K2P) as well as intraspecific distance were calculated using MEGA version 7 (Molecular Evolutionary Genetics Analysis, MEGA Inc., Englewood, NJ) (Kumar *et al.*, 2016).

RESULTS

Figure 01 shows the gel electrophoresis picture of 655 bp amplified PCR product of mtDNA COI gene region. mtDNA COI sequences with an aligned length of 655 bp were obtained from 12 specimens. No insertions and/or deletions were observed in all the obtained sequences. Eleven sequences of Mozambique Tilapia were obtained from BOLD representing 11 different countries, one sequence of *Homo sapiens* was used as an out-group.

All obtained sequences were compared with already reported *O. mossambicus* sequences based on mtDNA uploaded on BOLD (www.boldsystems.org) and NCBI (www.ncbi.nlm.nih.com), BLAST results showed that all the sequences belonged to tilapia species and sequences of studied specimen are already available in Gene Bank data. But there were no barcode records from Pakistan for Mozambique Tilapia in Gene Bank or BOLD.

The constructed Maximum Likelihood (ML) tree based on kimura 2-parameter (K2P) model for the twelve specimens of tilapia from Pakistan and eleven specimens from world. ML trees based on the DNA sequences of 23 Mozambique Tilapia groupings were built by utilizing K2P model. Figure 02 shows the ML tree. The ML tree uncovered indistinguishable phylogenetic relationship among all the specimens from world. All the 23 Mozambique Tilapia sequences belong to one species as shown in cluster. The ML tree demonstrates two groups. One cluster was among Tilapia species and other was showing out-group.

The COI sequences showed pairwise genetic distance and nucleotide sequence divergences from *O. mossambicus* (Table 03). The highest sequence divergence based on COI of studied sequences was

0.082% which was against the Tilapia sequence obtained from Egypt and lowest divergence was 0.003% against Indian Mozambique tilapia (Figure 03). Figure 4 shows the Clustal alignment of all the Tilapia species.

Table 01. Sampling details of *O. mossambicus* samples used in this study.

Sample Number	Location	Coordinates	
		Latitude	Longitude
04	Head Balloki	31.561920	74.348080
04	Trimmu Head works	31.028090	35.361351
04	Tawakkal Fish Hatchery Muzaffargarh	36.735640	-83.269190

Table 02. COI based sequence of *O. mossambicus* obtained from BOLD/GENE BANK and used for phylogenetic analysis with Exotic Fish (*O. mossambicus*) of Pakistan.

Country	BOLD Sequence ID	Gene bank accession Number	Coordinates	
			Latitude	Longitude
Indonesia	BIFD929-14.COI-5P	KU692686	-5.939	106.118
Canada	BNAF301-09.COI-5P	JN027866	NA	NA
Israel	FISH051-08.COI-5P	FJ348094	NA	NA
Philippines	FPHL196-16.COI-5P	KU565839	14.413	121.218
Egypt	GBGCA11622-15.COI-5P	LC052673	NA	NA
Australia	GBGCA7487-15.COI-5P	KF228005	-27.25	152.38
India	GDK592-13.COI-5P	NA	19.505	75.373
French_Polynesia	MBFB016-07.COI-5P	JQ431946	-17.483	-149.772
Mexico	MEFM196-05.COI-5P	EU751879	18.308	-95.775
Ontario	RFE283-05.COI-5P	EU752140	NA	NA
Zimbabwe	TSM015-14.COI-5P	KM438533	NA	NA

Table 03. Pairwise genetic distances (based on p distances) below the diagonal and Nucleotide sequence divergence (based on K2P model) above the diagonal in COI sequences derived from Tilapia species.

	INO	CAN	ISR	PHI	EGY	AUS	IND	FRN	MAX	ONT	ZIM	MZ-1	MZ-2	MZ-3	MZ-4	MZ-5	MZ-6	MZ-7	MZ-8	MZ-9	MZ-10	MZ-11	MZ-12
INO		-0.000	0.038	-0.000	0.078	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
CAN	0.00		0.038	-0.000	0.078	-0.00	-0.000	-0.000	-0.000	-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
ISR	0.036	0.036		0.038	0.090	0 0.038	0.038	0.038	0.038	0.038	0.038	0.045	0.043	0.043	0.041	0.043	0.043	0.043	0.005	0.043	0.043	0.050	0.041
PHI	0.00	0.00	0.036		0.078	-0.000	-0.000	-0.000	-0.000	-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.084	0.005	0.005	0.012	0.003
EGY	0.073	0.073	0.083	0.073		0.078	0.078	0.078	0.078	0.078	0.078	0.086	0.084	0.083	0.080	0.083	0.084	0.084	0.005	0.080	0.084	0.089	0.082
AUS	0.00	0.00	0.36	0.00	0.073		-0.000	-0.000	-0.000	-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
IND	0.00	0.00	0.036	0.00	0.073	0.00		-0.000	-0.000	-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
FRN	0.00	0.00	0.036	0.00	0.073	0.00	0.00		-0.000	-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
MAX	0.00	0.00	0.036	0.00	0.073	0.00	0.00	0.00		-0.000	-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
ONT	0.00	0.00	0.036	0.00	0.073	0.00	0.00	0.00	0.00		-0.000	0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.012	0.003
ZIMB	0.00	0.00	0.036	0.00	0.073	0.00	0.00	0.00	0.00	0.00		0.007	0.005	0.005	0.005	0.005	0.005	0.005	0.009	0.005	0.005	0.012	0.003
MZ-1	0.007	0.007	0.043	0.007	0.080	0.007	0.007	0.007	0.007	0.007	0.007		0.007	0.007	0.010	0.007	0.009	0.007	0.005	0.009	0.007	0.014	0.009
MZ-2	0.005	0.005	0.042	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.007		0.007	0.009	0.007	0.005	0.005	0.005	0.005	0.005	0.014	0.005
MZ-3	0.005	0.005	0.042	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.005	0.078		0.009	0.007	0.005	0.005	0.009	0.007	0.007	0.014	0.007
MZ-4	0.005	0.005	0.040	0.005	0.075	0.005	0.005	0.005	0.005	0.005	0.005	0.010	0.009	0.009		0.007	0.009	0.009	0.007	0.007	0.009	0.010	0.009
MZ-5	0.005	0.005	0.042	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.007	0.007	0.007		0.007	0.007	0.003	0.005	0.005	0.016	0.007
MZ-6	0.005	0.005	0.042	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.09	0.005	0.005	0.07	0.09		0.007	0.007	0.007	0.005	0.014	0.007
MZ-8	0.005	0.005	0.042	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.005	0.005	0.09	0.07	0.07			0.005	0.007	0.012	0.005
MZ-9	0.005	0.005	0.038	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.009	0.005	0.005	0.09	0.007	0.003	0.007		0.007	0.005	0.012	0.007
MZ-10	0.005	0.005	0.042	0.005	0.078	0.005	0.005	0.005	0.005	0.005	0.005	0.007	0.005	0.007	0.009	0.005	0.005	0.007	0.005		0.007	0.012	0.005
MZ-11	0.012	0.012	0.049	0.012	0.083	0.012	0.012	0.012	0.012	0.012	0.012	0.014	0.014	0.010	0.016	0.014	0.012	0.012	0.012	0.014		0.014	0.007
MZ-12	0.003	0.003	0.040	0.003	0.076	0.003	0.003	0.003	0.003	0.003	0.003	0.009	0.005	0.007	0.009	0.007	0.007	0.005	0.007	0.005	0.007		0.012

INO= Indonesia, CAN= Canada, ISR= Israel, PHI= Philippines, EGY= Egypt, AUS= Australia, IND= India, French_ FRN= Polynesia, MAX= Mexico, ONT= Ontario, ZIMB= Zimbabwe, MZ01-12= Samples from Pakistan

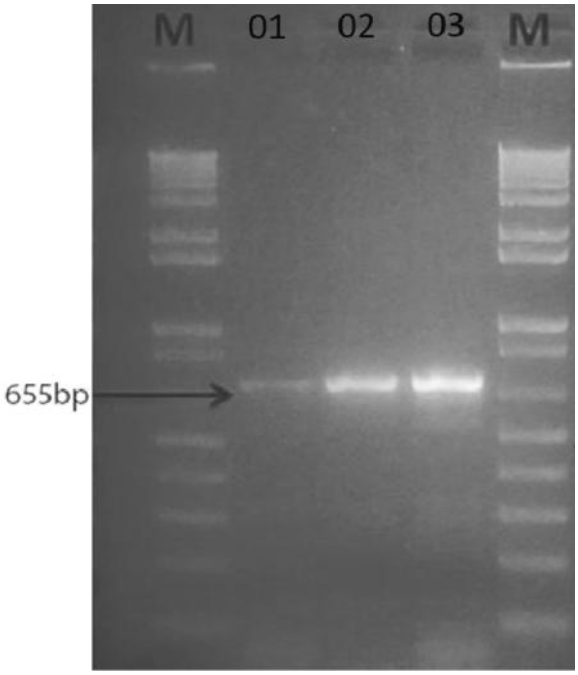

Figure 01. Agarose gel of PCR product of 655 bp fragment of COI gene. M: DNA Ladder; 01-03: Samples of *O. mossambicus*.

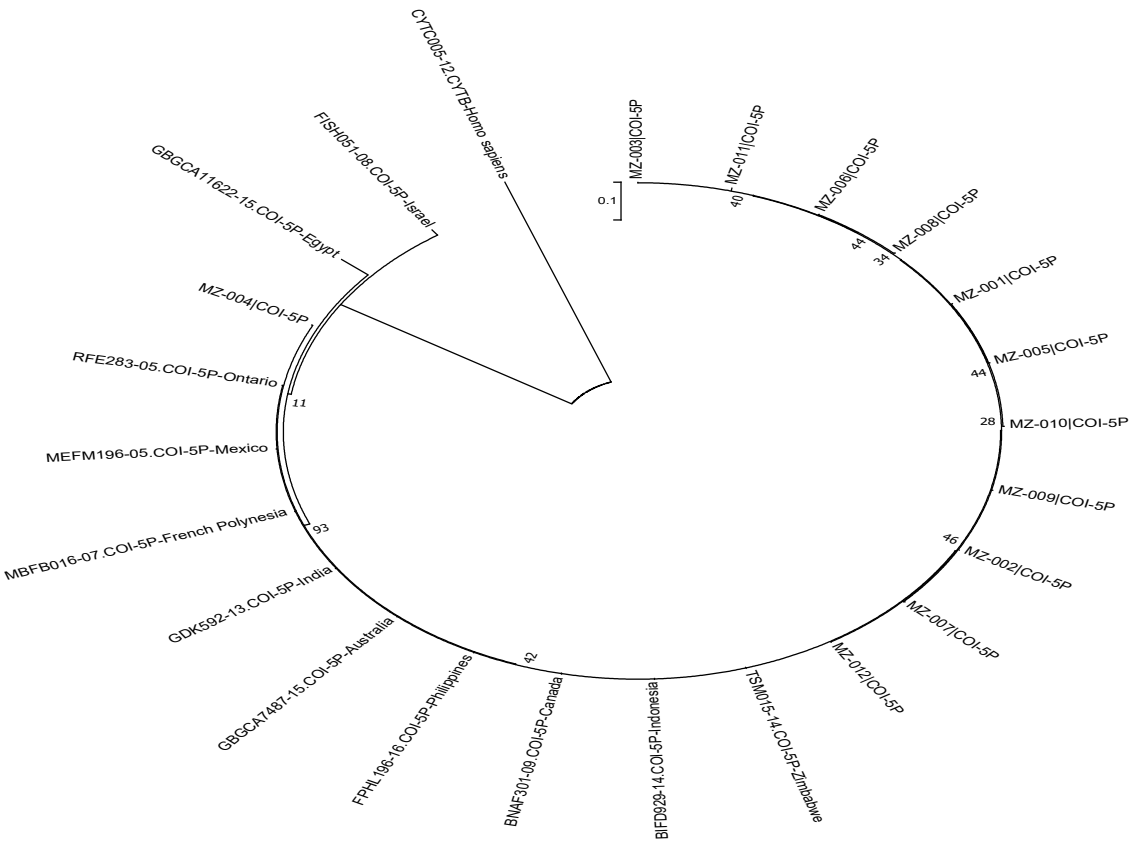

Figure 02. Maximum likelihood (ML) Molecular phylogenetic tree of *Oreochromis mossambicus* inferred from mtDNA sequences.

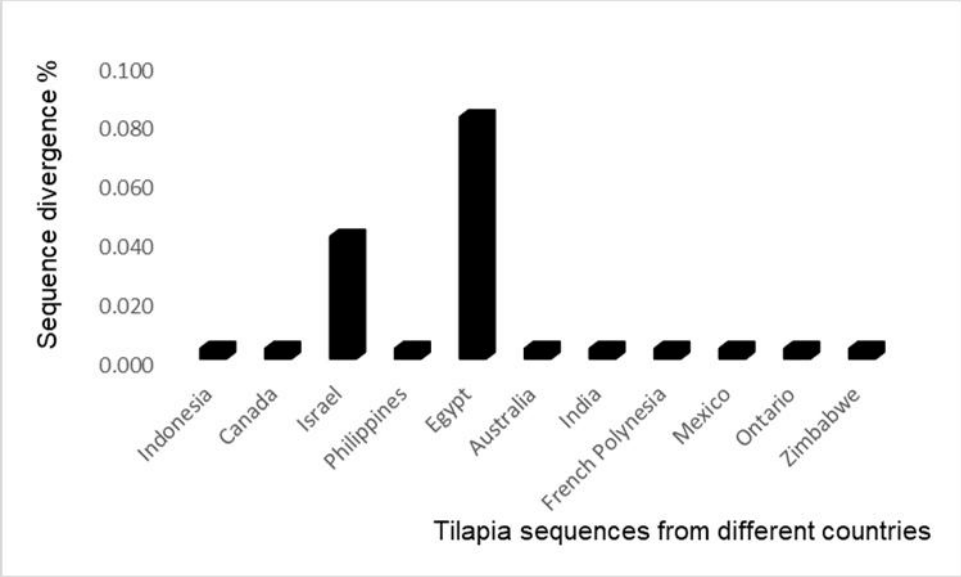

Figure 03. Pairwise sequence divergence among Tilapia from Pakistan and different countries.

```
GBGCA11622-15.COI-5P-Egypt          ------------------------------------------------------------  0
FISH051-08.COI-5P-Israel            ----------------------------------------GGAACTGCACTAAGTCTC  18
MZ-011|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-004|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-012|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
TSM015-14.COI-5P-Zimbabwe           ----------
TGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                                         40
GDK592-13.COI-5P-India              ------
CTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                           54
GBGCA7487-15.COI-5P-Australia-------------------------------ATAGTAGGAACTGCATTAAGCCTC            24
BIFD929-14.COI-5P-Indonesia         ----------
GCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                                             36
BNAF301-09.COI-5P-Canada            --
CACCCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                       58
FPHL196-16.COI-5P-Philippines
GGCACCCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                     60
MBFB016-07.COI-5P-French_Polynesia  ------
CTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                           54
MEFM196-05.COI-5P-Mexico            -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
RFE283-05.COI-5P-Ontario            -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-001|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-005|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-009|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-007|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
MZ-003|COI-5P                       -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC                                          55
```

```
MZ-002|COI-5P                   -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC       55
MZ-006|COI-5P                   -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC       55
MZ-008|COI-5P                   -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC       55
MZ-010|COI-5P                   -----
CCTCTATCTAGTATTTGGTGCTTGAGCCGGAATAGTAGGAACTGCATTAAGCCTC       55

GBGCA11622-15.COI-5P-Egypt      ---
ATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT     57
FISH051-08.COI-5P-Israel
CTTATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  78
MZ-011|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-004|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-012|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
TSM015-14.COI-5P-Zimbabwe
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  100
GDK592-13.COI-5P-India
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  114
GBGCA7487-15.COI-5P-Australia
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  84
BIFD929-14.COI-5P-Indonesia
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  96
BNAF301-09.COI-5P-Canada
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  118
FPHL196-16.COI-5P-Philippines
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  120
MBFB016-07.COI-5P-French_Polynesia
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  114
MEFM196-05.COI-5P-Mexico
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
RFE283-05.COI-5P-Ontario
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-001|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-005|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-009|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-007|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-003|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-002|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-006|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-008|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
MZ-010|COI-5P
CTAATTCGGGCAGAACTAAGCCAGCCCGGCTCTCTCCTCGGAGACGACCAGATTTATAAT  115
                              **********************************************************
```

```
GBGCA11622-15.COI-5P-Egypt
GTAATTGTTACAGCACATGCTTTTGTAATAATTTTCTTTATAGTAATGCCAATTATGATT 117
FISH051-08.COI-5P-Israel
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATACCAATTATAATT 138
MZ-011|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-004|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-012|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
TSM015-14.COI-5P-Zimbabwe
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 160
GDK592-13.COI-5P-India
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 174
GBGCA7487-15.COI-5P-Australia
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 144
BIFD929-14.COI-5P-Indonesia
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 156
BNAF301-09.COI-5P-Canada
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 178
FPHL196-16.COI-5P-Philippines
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 180
MBFB016-07.COI-5P-French_Polynesia
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 174
MEFM196-05.COI-5P-Mexico
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
RFE283-05.COI-5P-Ontario
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-001|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-005|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-009|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-007|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-003|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-002|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-006|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-008|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
MZ-010|COI-5P
GTAATTGTTACAGCACATGCTTTCGTAATAATTTTCTTTATAGTAATGCCAATTATAATT 175
                    ********************** ********************** ******** ***

GBGCA11622-15.COI-5P-Egypt
GGAGGCTTTGGAAACTGACTAGTACCACTCATGATTGGTGCCCCAGATATGGCCTTCCCT 177
FISH051-08.COI-5P-Israel
GGAGGTTTTGGAAACTGACTGGTGCCACTTATGATTGGGGCACCAGACATGGCCTTCCCT 198
MZ-011|COI-5P
GGAGGTGTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCGTGATTGGCCTTCCCT 235
MZ-004|COI-5P
GGAGGTATTGGAAACTGACTAGTGCCACTCATGATTGGTGCACCAGACATGGCCTTCCCT 235
```

```
MZ-012|COI-5P
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAAACATGGCCTTCCCT 235
TSM015-14.COI-5P-Zimbabwe
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 220
GDK592-13.COI-5P-India
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 234
GBGCA7487-15.COI-5P-Australia
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 204
BIFD929-14.COI-5P-Indonesia
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 216
BNAF301-09.COI-5P-Canada
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 238
FPHL196-16.COI-5P-Philippines
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 240
MBFB016-07.COI-5P-French_Polynesia
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 234
MEFM196-05.COI-5P-Mexico
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 235
RFE283-05.COI-5P-Ontario
GGAGGTTTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCAGACATGGCCTTCCCT 235
MZ-001|COI-5P
GGAGGTGTTGGAAACTGACTAGTGCCACCAATGATAGGTGCACCAGACATGGCCTTCCCT 235
MZ-005|COI-5P
GGAGGTATTGGAAACTGACTAGTGCCACTAAAGATTGGTGCACCAGACATGGCCTTCCCT 235
MZ-009|COI-5P
GGAGGTATTGGAAACTGACTAGTGCCACTAATGATTGGTGCCCCAGACATGGCCTTCCCT 235
MZ-007|COI-5P
GGAGGTGTTGGAAACTGACTAGTGCCACTAATGATTCGTGCACCAGACATGGCCTTCCCT 235
MZ-003|COI-5P
GGAGGTGTTGGAAACTGACTAGTGCCACTAATGTTTGGTGCACCAGACATGGCCTTCCCT 235
MZ-002|COI-5P
GGAGGTCTTGGAAACTGACTAGTGCCACGAATGATTGGTGCACCAGACATGGCCTTCCCT 235
MZ-006|COI-5P
GGAGGTCTTGGAAACTGACTAGTGCCACTAATGACTGGTGCACCAGACATGGCCTTCCCT 235
MZ-008|COI-5P
GGAGGTCTTGGAAACTGACTAGTGCCACTAATGATTGGGGCACCAGACATGGCCTTCCCT 235
MZ-010|COI-5P
GGAGGTCTTGGAAACTGACTAGTGCCACTAATGATTGGTGCACCCGACATGGCCTTCCCT 235
                ***** ************ ** **** * *  * ** **   **********

GBGCA11622-15.COI-5P-Egypt
CGAATGAACAACATGAGTTTCTGACTCCTCCCTCCCTCATTCCTCCTCCTCCTCGCCTCA 237
FISH051-08.COI-5P-Israel
CGAATAAATAACATGAGTTTTTGACTCCTTCCCCCCTCATTTCTCCTTCTCCTCGCCTCC 258
MZ-011|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-004|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-012|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
TSM015-14.COI-5P-Zimbabwe
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 280
GDK592-13.COI-5P-India
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 294
GBGCA7487-15.COI-5P-Australia
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 264
```

BIFD929-14.COI-5P-Indonesia
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 276
BNAF301-09.COI-5P-Canada
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 298
FPHL196-16.COI-5P-Philippines
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 300
MBFB016-07.COI-5P-French_Polynesia
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 294
MEFM196-05.COI-5P-Mexico
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
RFE283-05.COI-5P-Ontario
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-001|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-005|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-009|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-007|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-003|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-002|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-006|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-008|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
MZ-010|COI-5P
CGAATAAATAACATGAGTTTTTGACTCCTCCCCCCCTCATTTCTCCTTCTCCTCGCCTCA 295
***** ** ********** ******** ** ******** ***** **********

GBGCA11622-15.COI-5P-Egypt
TCTGGAGTCGAAGCAGGTGCCGGCACAGGGTGAACTGTTTACCCCCCGCTCGCAGGCAAT 297
FISH051-08.COI-5P-Israel
TCCGGAGTCGAAGCAGGAGCCGGTACAGGATGAACTGTTTATCCTCCCCTCGCAGGCAAT 318
MZ-011|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-004|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-012|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
TSM015-14.COI-5P-Zimbabwe
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 340
GDK592-13.COI-5P-India
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 354
GBGCA7487-15.COI-5P-Australia
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 324
BIFD929-14.COI-5P-Indonesia
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 336
BNAF301-09.COI-5P-Canada
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 358
FPHL196-16.COI-5P-Philippines
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 360
MBFB016-07.COI-5P-French_Polynesia
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 354

```
MEFM196-05.COI-5P-Mexico
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
RFE283-05.COI-5P-Ontario
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-001|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-005|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-009|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-007|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-003|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-002|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-006|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-008|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
MZ-010|COI-5P
TCCGGGGTCGAAGCAGGGGCCGGTACAGGATGGACTGTTTATCCCCCACTCGCAGGCAAT 355
                ** ** ********** ***** ***** ** ******** ** ** ***********

GBGCA11622-15.COI-5P-Egypt
CTTGCCCATGCTGGACCTTCTGTCGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTA 357
FISH051-08.COI-5P-Israel
CTCGCCCACGCTGGGCCTTCTGTTGACTTAACCATCTTCTCCCTCCACTTAGCCGGGGTG 378
MZ-011|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-004|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-012|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
TSM015-14.COI-5P-Zimbabwe
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 400
GDK592-13.COI-5P-India
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 414
GBGCA7487-15.COI-5P-Australia
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 384
BIFD929-14.COI-5P-Indonesia
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 396
BNAF301-09.COI-5P-Canada
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 418
FPHL196-16.COI-5P-Philippines
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 420
MBFB016-07.COI-5P-French_Polynesia
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 414
MEFM196-05.COI-5P-Mexico
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
RFE283-05.COI-5P-Ontario
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-001|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-005|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
```

```
MZ-009|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-007|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-003|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-002|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-006|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-008|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
MZ-010|COI-5P
CTCGCCCATGCTGGGCCTTCCGTTGACTTAACCATCTTCTCCCTCCACTTGGCCGGGGTG 415
                ** ***** ***** ***** ** ************************ ********

GBGCA11622-15.COI-5P-Egypt
TCATCTATTTTAGGCGCAATTAATTTCATTACAACAATCATTAACATGAAACCCCCCGCC 417
FISH051-08.COI-5P-Israel
TCATCTATTTTAGGTGCAATTAATTTTATCACAACCATCATTAACATAAAACCCCCTGCC 438
MZ-011|COI-5P
TCATCTATTTTAGGTGCAATTAATATTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-004|COI-5P
TCATCTATTTTAGGTGCAATTAATTCTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-012|COI-5P
TCATCTATTTTAGGTGCAATTAATCTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
TSM015-14.COI-5P-Zimbabwe
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 460
GDK592-13.COI-5P-India
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 474
GBGCA7487-15.COI-5P-Australia
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 444
BIFD929-14.COI-5P-Indonesia
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 456
BNAF301-09.COI-5P-Canada
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 478
FPHL196-16.COI-5P-Philippines
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 480
MBFB016-07.COI-5P-French_Polynesia
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 474
MEFM196-05.COI-5P-Mexico
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
RFE283-05.COI-5P-Ontario
TCATCTATTTTAGGTGCAATTAATTTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-001|COI-5P
TCATCTATTTTAGGTGCAATTAATGTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-005|COI-5P
TCATCTATTTTAGGTGCAATTAATGTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-009|COI-5P
TCATCTATTTTAGGTGCAATTAATCTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-007|COI-5P
TCATCTATTTTAGGTGCAATTAATCTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-003|COI-5P
TCATCTATTTTAGGTGCAATTAATATTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-002|COI-5P
TCATCTATTTTAGGTGCAATTAATCTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
```

```
MZ-006|COI-5P
TCATCTATTTTAGGTGCAATTAATATTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-008|COI-5P
TCATCTATTTTAGGTGCAATTAATATTATTACAACCATTATTAACATAAAACCCCCTGCC 475
MZ-010|COI-5P
TCATCTATTTTAGGTGCAATTAATGTTATTACAACCATTATTAACATAAAACCCCCTGCC 475
************* *********  ** ***** ** ******** ******** ***

GBGCA11622-15.COI-5P-Egypt
ATCTCTCAATATCAGACACCCCTATTTGTATGGTCCGTTCTAATTACAGCAGTATTACTT 477
FISH051-08.COI-5P-Israel
ATCTCTCAATATCAAACACCCCTCTTTGTGTGATCCGTTCTAATTACCGCAGTATTACTC 498
MZ-011|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-004|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-012|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
TSM015-14.COI-5P-Zimbabwe
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 520
GDK592-13.COI-5P-India
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 534
GBGCA7487-15.COI-5P-Australia
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 504
BIFD929-14.COI-5P-Indonesia
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 516
BNAF301-09.COI-5P-Canada
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 538
FPHL196-16.COI-5P-Philippines
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 540
MBFB016-07.COI-5P-French_Polynesia
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 534
MEFM196-05.COI-5P-Mexico
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
RFE283-05.COI-5P-Ontario
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-001|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-005|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-009|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-007|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-003|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-002|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-006|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-008|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
MZ-010|COI-5P
ATCTCCCAATATCAAACACCCCTCTTTGTATGATCCGTTCTAATTACCGCAGTACTACTC 535
***** ******** ******** ***** ** ************** ****** ****
```

GBGCA11622-15.COI-5P-Egypt
CTTCTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTCACAGACCGAAACCTA 537
FISH051-08.COI-5P-Israel
CTACTATCCCTGCCCGTTCTTGCCGCCGGTATCACAATACTTCTAACAGACCGAAACCTA 558
MZ-011|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-004|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-012|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
TSM015-14.COI-5P-Zimbabwe
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 580
GDK592-13.COI-5P-India
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 594
GBGCA7487-15.COI-5P-Australia
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 564
BIFD929-14.COI-5P-Indonesia
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 576
BNAF301-09.COI-5P-Canada
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 598
FPHL196-16.COI-5P-Philippines
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 600
MBFB016-07.COI-5P-French_Polynesia
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 594
MEFM196-05.COI-5P-Mexico
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
RFE283-05.COI-5P-Ontario
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-001|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-005|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-009|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-007|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-003|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-002|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-006|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-008|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
MZ-010|COI-5P
CTACTATCCCTACCCGTTCTTGCCGCCGGCATCACAATACTTCTAACAGACCGAAACCTA 595
** ******** ********** ********** **********

GBGCA11622-15.COI-5P-Egypt
AACACAACCTTCTTTGATCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 597
FISH051-08.COI-5P-Israel
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 618
MZ-011|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-004|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655

```
MZ-012|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
TSM015-14.COI-5P-Zimbabwe
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 640
GDK592-13.COI-5P-India AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCC 633
GBGCA7487-15.COI-5P-Australia
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 624
BIFD929-14.COI-5P-Indonesia
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTA--- 633
BNAF301-09.COI-5P-Canada
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 658
FPHL196-16.COI-5P-Philippines
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 660
MBFB016-07.COI-5P-French_Polynesia
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTA--- 651
MEFM196-05.COI-5P-Mexico
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTA--- 652
RFE283-05.COI-5P-Ontario
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTA--- 652
MZ-001|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-005|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-009|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-007|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-003|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-002|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-006|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-008|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
MZ-010|COI-5P
AACACAACCTTCTTTGACCCTGCCGGAGGAGGAGACCCCATCCTTTACCAACACTTATTC 655
                  ********** **********

GBGCA11622-15.COI-5P-Egypt TGATTCTTAG---------- 607
FISH051-08.COI-5P-Israel T---------- 619
MZ-011|COI-5P ---------- 655
MZ-004|COI-5P ---------- 655
MZ-012|COI-5P ---------- 655
TSM015-14.COI-5P-Zimbabwe TGATTCTTCGG---------- 651
GDK592-13.COI-5P-India ---------- 633
GBGCA7487-15.COI-5P-Australia TGA---------- 627
BIFD929-14.COI-5P-Indonesia ---------- 633
BNAF301-09.COI-5P-Canada ---------- 658
FPHL196-16.COI-5P-Philippines TGATTCTTCGGTCACCCTGAAGTGT 685
MBFB016-07.COI-5P-French_Polynesia ---------- 651
MEFM196-05.COI-5P-Mexico ---------- 652
RFE283-05.COI-5P-Ontario ---------- 652
MZ-001|COI-5P ---------- 655
MZ-005|COI-5P ---------- 655
MZ-009|COI-5P ---------- 655
```

MZ-007|COI-5P ---------- 655
MZ-003|COI-5P ---------- 655
MZ-002|COI-5P ---------- 655
MZ-006|COI-5P ---------- 655
MZ-008|COI-5P ---------- 655
MZ-010|COI-5P ---------- 655

Figure 04. Clustal alignments of twenty-three sequences of *Oreochromis mossambicus*.

DISCUSSION

In Pakistan, no validated data about phylogeny of Mozambique Tilapia species was reported before; ours is the first in this regard. We also report phylogenetic relationship between the exotic cultured fish Tilapia in Pakistan. The precise and quick fish identification is vital for logical studies as well as for sustainable aquaculture. Previously, identification key for freshwater fish species of Pakistan is available (Mirza, 1994; Rafique and Khan, 2012), which is based on morphological characters only. There have been limitations to this identification key. For the species identification, mtCOI gene offers the open door for an institutionalized arrangement and scientific research (Hebert *et al.*, 2004; Ward *et al.*, 2009; Hebert *et al.*, 2003; Ward *et al.*, 2005). A variety of Tilapia species are available in Pakistan, and their identification based on morphological characteristics is very equivocal and not applicable to every life stage (Zhu *et al.*, 2013; Gu *et al.*, 2016). Therefore, this study was the first in Pakistan to identify the Mozambique Tilapia specie by using molecular tools. Phylogenetic analyses of Mozambique Tilapia species are very important especially which are found in Pakistan because this information will be useful for administration and management of alien tilapia species in the country.

Our results indicate that the molecular approach using sequences of COI gene could be effectively used for the purpose of identification and phylogenetic analysis of the exotic fish species *O. mossambicus* of Pakistan. In our investigation, there were no significant variations found among all the Mozambique Tilapia sequences, indicating that the mtDNA COI gene can be used for phylogenetic analyses. Phylogenetic analysis showed that *O. mossambicus* from Pakistan showed highest genetic distance and lowest genetic distance against Egypt and India respectively.

Conclusion: mtDNA COI gene is an effective barcoding marker for the precise identification and phylogeny of exotic Mozambique tilapia. The phylogenetic relations appeared in this examination are helpful for broadening the Linnaean framework and can be utilized to enhance the sustainability of Mozambique Tilapia species in aquaculture industry of Pakistan.

REFERENCES

Abdel-Mawgood, A. L. (2012). DNA based techniques for studying genetic diversity. In: Genet. Div. Mico. pp 95-122.

Arif, I. and H. A. Khan, (2009). Molecular markers for biodiversity analysis of wildlife animals: a brief review. Anim. Biodiv. Conserv. 32(1): 9-17.

Avise, J. C. (2000). Phylogeography: the history and foration of species. Harvard university press, 135 p.

Bhassu, S., K. Yusoff, J. M. Panandam, W. K. Embong, S. Oyyan, and S. G. Tan. (2004). The genetic structure of *Oreochromis* spp. (Tilapia) populations in Malaysia as revealed by microsatellite DNA analysis. Biochem. Genet. 42(7-8): 217-229.

Bhattacharya, M., A. R. Sharma, B. C. Patra, G. Sharma, E. M. Seo, J.S. Nam, C. Chakraborty, and S.-S. Lee. (2016). DNA barcoding to fishes: current status and future directions. Mitochondr DNA. 27(4): 2744-2752.

Briñez, R., O. Caraballo, and V. Salazar. (2011). Genetic diversity of six populations of red hybrid tilapia, using microsatellites genetic markers. Rev. Mvz. Cordoba. 16(2): 2491-2498.

Canonico, G. C., A. Arthington, J. K. Mccrary, and M. L. Thieme, (2005). The effects of introduced tilapias on native biodiversity. Aquat. Conserv. 15(5): 463-483.

Carey, M. P., and D. H. Wahl, (2010). Native fish diversity alters the effects of an invasive species on food webs. Ecology. 91(10): 2965-2974.

Chinnery, P. F. (2006). Mitochondrial DNA in Homo sapiens. In: Human Mitochondrial DNA and the Evolution of *Homo sapiens.* Springer, pp. 3-15.

Curole, J. P. and T. D. Kocher. (1999). Mitogenomics: digging deeper with complete mitochondrial genomes. Trends. Ecol. Evol. 14(10): 394-398.

D'amato, M. E., M. M. Esterhuyse, B. C. Van Der Waal, D. Brink, and F. A. Volckaert. (2007). Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. Conserv. Genet. 8(2): 475-488.

Daravath, S., M. M. Yadav, P. Chakrapani, and B. R. Naik. (2013). Molecular Identification of Aedesalbopictus (Diptera: Culicidae) and Quantitative Analysis of COI Gene in South-Indian Species. Int. J. Curr. Microbiol. App. Sci. 2: 102-109.

Giannetto, D., A. Carosi, E. Franchi, L. Ghetti, G. Pedicillo, L. Pompei, and M. Lorenzoni. (2012). Assessing the impact of non-native freshwater fishes on native species using relative weight. Knowl. Manag. Aquat. Ec. (404): 03.

Gu, D. E., X. D. Mu, M. Xu, D. Luo, H. Wei, Y. Y. Li, Y. J. Zhu, J. R. Luo, and Y. C. Hu. (2016). Identification of wild tilapia species in the main rivers of south China using mitochondrial control region sequence and morphology. Biochem. Syst. Ecol. 65: 100-107.

Hassanien, H. A. and J. Gilbey. (2005). Genetic diversity and differentiation of Nile tilapia (Oreochromis niloticus) revealed by DNA microsatellites. Aquac. Res. 36(14): 1450-1457.

Hebert, P. D., A. Cywinska, and S. L. Ball. (2003). Biological identifications through DNA barcodes. Proc. R. Soc. Ser. B-Bio. 270(1512): 313-321.

Hebert, P. D., E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly Astraptes fulgerator. P. Natl. Acad. Sci. Usa. 101(41): 14812-14817.

Josupeit, H. (2007). World tilapia trade. Globefish. org.

Kamran, M. (2017). Cytochrome oxidase-i based identification of fish (family channidae) from River Ravi. B.Sc (Hons) thesis (unpublished). Zool. Deptt., GC Univ., Lahore.

Khan, A. M., Z. Ali, S. Y. Shelly, Z. Ahmad, and M. R. Mirza. (2011). Aliens; a catastrophe for native fresh water fish diversity in Pakistan. J. Anim. Plant Sci. 21(2 Suppl): 435-440.

Knudsen, B., A. B. Kohn, B. Nahir, C. S. Mcfadden, and L. L. Moroz. (2006). Complete DNA sequence of the mitochondrial genome of the sea-slug, Aplysia californica: conservation of the gene order in Euthyneura. Mol. Phylogenet. Evol. 38(2): 459-469.

Kornis, M. S., S. Sharma, and M. Jake Vander Zanden. (2013). Invasion success and impact of an invasive fish, round goby, in Great Lakes tributaries. Divers. Distrib. 19(2): 184-198.

Kumar, S., G. Stecher, and K. Tamura. (2016). MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. Mol. Biol. Evol. 33(7): 1870-1874.

Lacerda, S., S. R. Batlouni, L. H. Assis, F. M. Resende, S. M. Campos-Silva, R. Campos-Silva, T. M. Segatelli, and L. R. Franca. (2008). Germ cell transplantation in tilapia (*Oreochromis niloticus*). Cybium. 32(2): 115-118.

Lin, C.-H., W. C. Kuan, B. K. Liao, A. N. Deng, D. Y. Tseng, and P.-P. Hwang. (2016). Environmental and cortisol-mediated control of Ca2+ uptake in tilapia (*Oreochromis mossambicus*). J. Comp. Physiol. B. 186(3): 323-332.

Lowe, M. R., W. Wu, M. S. Peterson, N. J. Brown-Peterson, W. T. Slack, and P. J. Schofield. (2012). Survival, growth and reproduction of non-native Nile tilapia II: fundamental niche projections and invasion potential in the northern Gulf of Mexico. PLoS. One. 7(7): e41580.

Meador, M. R., L. R. Brown, and T. Short. (2003). Relations between introduced fish and environmental conditions at large geographic scales. Ecol. Indic. 3(2): 81-92.

Mirza, M. (1994). Geographical distribution of freshwater fishes in Pakistan: A review. Pnj. Univ. J. Zool. 9: 93-108.

Patwardhan, A., S. Ray, and A. Roy. (2014). Molecular markers in phylogenetic studies-a review. J. Phylogenetics. Evol. Biol. 2014.

Rafique, M. and N. U. H. Khan. (2012). Distribution and status of significant freshwater fishes of Pakistan. Rec. Zool. Surv. Pakistan. 21: 90-95.

Shin, M. G., S. Kajigaya, M. Tarnowka, J. P. Mccoy, B. C. Levin, and N. S. Young. (2004). Mitochondrial DNA sequence heterogeneity in circulating normal human CD34 cells and granulocytes. Blood. 103(12): 4466-4477.

Stickney, R. R. (2005). Aquaculture. Springer; Netherlands.

Stoneking, M. and H. Soodyall. (1996). Human evolution and the mitochondrial genome. Curr. Opin. Genet. Dev. 6(6): 731-736.

Storey, A. A., A. C. Clarke, T. Ladefoged, J. Robins, and E. Matisoo-Smith. (2013). DNA and Pacific commensal models: Applications, construction, limitations, and future prospects. J. Isl. Coast. Archaeol. 8(1): 37-65.

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. Freshwater. Biol. 55(s1): 152-174.

Thompson, J. D., T. J. Gibson, F. Plewniak, F. Jeanmougin, and D. G. Higgins. (1997). The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic. Acids. Res. 25(24): 4876-4882.

Wan, Q. H., H. Wu, T. Fujihara, and S. G. Fang. (2004). Which genetic marker for which conservation

genetics issue? Electrophoresis. 25(14): 2165-2176.

Ward, R. D., R. Hanner, and P. D. Hebert. (2009). The campaign to DNA barcode all fishes, FISH-BOL. J. Fish. Biol. 74(2): 329-356.

Ward, R. D., T. S. Zemlak, B. H. Innes, P. R. Last, and P. D. Hebert. (2005). DNA barcoding Australia's fish species. Philos. T. Roy. Soc. B. 360(1462): 1847-1857.

Zambrano, L., E. Martínez-Meyer, N. Menezes, and A. T. Peterson. (2006). Invasive potential of common carp (Cyprinus carpio) and Nile tilapia (Oreochromis niloticus) in American freshwater systems. Can. J. Fish. Aquat. Sci. 63(9): 1903-1910.

Zhu, S.-R., J. J. Fu, Q. Wang, and J. L. Li. (2013). Identification of Channa species using the partial cytochrome c oxidase subunit I (COI) gene as a DNA barcoding marker. Biochem. Syst. Ecol. 51: 117-122.