



Origin of Invasive Fish Species, Peacock Bass *Cichla* Species in Lake Telabak Malaysia Revealed by Mitochondrial DNA Barcoding

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ABSTRACT

Peacock bass (Perciformes, Cichlidae, *Cichla*) are multi-coloured and highly predatory fish originated from Amazonian region. The species was deliberately introduced into Malaysia freshwater bodies by anglers in the early 1990's for sport fisheries. In this recent study, we found the population of peacock bass in Lake Telabak, a man-made lake in Besut, Terengganu. Using mitochondrial DNA analysis approach, the origin and taxonomy of peacock bass in the lake were clarify. A total of forty fishes were sampled from Lake Telabak for the analysis. Haplotype was detected among all samples. The current study revealed that *Cichla* spp. in Lake Telabak are closer to *Cichla ocellaris* (Bloch and Schneider, 1801) with a sequence similarity of 99.72% as blasted at the National Center for Biotechnology Information (NCBI) database. The evolutionary history inferred using the maximum likelihood method in the molecular phylogenetic analysis also confirmed the species as *C. ocellaris*, but no record found concerning their origin. The Tasik Telabak peacock bass also match with the *Cichla temensis* which is traced to the Amazonas and Parà states of Brazil (Manaus, Novo Airão, Tapajós and Tefé).

INTRODUCTION

Human interference in the movement of living organisms across different continents of the world has caused profound changes in both the ecology of guest species and the native communities (Callaway and Maron, 2006; Yong *et al.*, 2014; Havel *et al.*, 2015; Fazhan *et al.*, 2017). The rate of increase and the measure of these relocation is quickly becoming a major concern for ecologists, resource managers and conservation biologists around the globe largely because biological invasion or introduction of non-native fishes may cause danger and create additional threats to native biodiversity (Dudgeon, 2003; Rahim *et al.*, 2013; Gubiani *et al.*, 2018; Reid *et al.*, 2018).

Malaysia is well known for its large number of flora and fauna mega diversity (Nafis *et al.*, 2001; Chong *et al.*, 2010; Paknia *et al.*, 2015). However, like other countries of the globe, Malaysia has suffered from negligent introduction of invasive species, primarily for agriculture and livestock production (Department of Fisheries Malaysia, 2010; Rahim *et al.*, 2013). *Cichla* spp. popularly known as peacock bass, are voracious predator fish with wide prey range, threatening the native fishes including endemic species (Novaes *et al.*, 2004; Agostinho *et al.*, 2005; Reiss *et al.*, 2012; Marques *et al.*, 2016). According to Khairul Adha (2006), peacock bass is an invasive species that was intentionally introduced and released by some anglers into lake in the early 1990s for sport fishing in Peninsular Malaysia. Recently, a few specimens identified as peacock bass were discovered for the first time in the Lake Telabak, located in Besut, Terengganu, East Coast of Peninsular Malaysia. The lake is important for socioeconomic activities where the fish cage culture being introduced, as well as water sources for rice field activity. Furthermore, the lake become the recreational spot for fishing and family activities. The origin, taxonomy and introduction of *Cichla* spp. in Lake Telabak still remain unclear.

Analysis of mitochondrial DNA is a useful tool for molecular genetic studies with various applications in genetic diversity (Ahmad-Syazni *et al.*, 2017; Ha *et al.*, 2017; Khaleel *et al.*, 2019) and species identification (Li *et al.*, 2019; Golani *et al.*, 2019). Genetic information and specific taxonomy is essential for controlling introduced *Cichla* spp. populations, especially those that are now invasive and a continuous part of existing species in Lake Telabak. Due to the high complexity in the *Cichla* genus, there is a wide range of ambiguity regarding their taxonomic identifications and descriptions morphologically (Golani *et al.*, 2019). However, based on the idea of DNA barcoding, a short defined sequence can differentiate individual species due to the fact that genetic diversity between species surpassed within species (Hebert *et al.*, 2004). In addition, cytochrome c oxidase I (COI) of the mitochondrial gene with sequence diversity in a 650 base pair (bp) fraction was believed to generate powerful species-level resolution for various animal populations including birds, fish and insects (Hebert *et al.*, 2004; Ward *et al.*, 2005; Hajibabaei *et al.*, 2006; Golani *et al.*, 2019; Granato *et al.*, 2019). The aim of this study was to provide a scientific information regarding the origin, taxonomy and introduction of *Cichla* spp. in Lake Telabak by analysing phylogenetic relationship using COI fraction of mitochondrial DNA barcoding.

MATERIALS AND METHODS

General flowchart of the entire experimental methods carried out in current study including sampling, DNA extraction, polymerase chain reaction (PCR), sequencing, DNA barcoding and data analysis were given below (Figure 1).

Sampling

A total of forty samples of the peacock bass were successfully collected from Lake Telabak East Coast of Peninsular Malaysia (Figure 2) from September 2018 to February 2019. Approximately 1 cm of tail tissue was removed with scissors and preserved in a sterile 1.5 ml tube containing 95% ethanol.

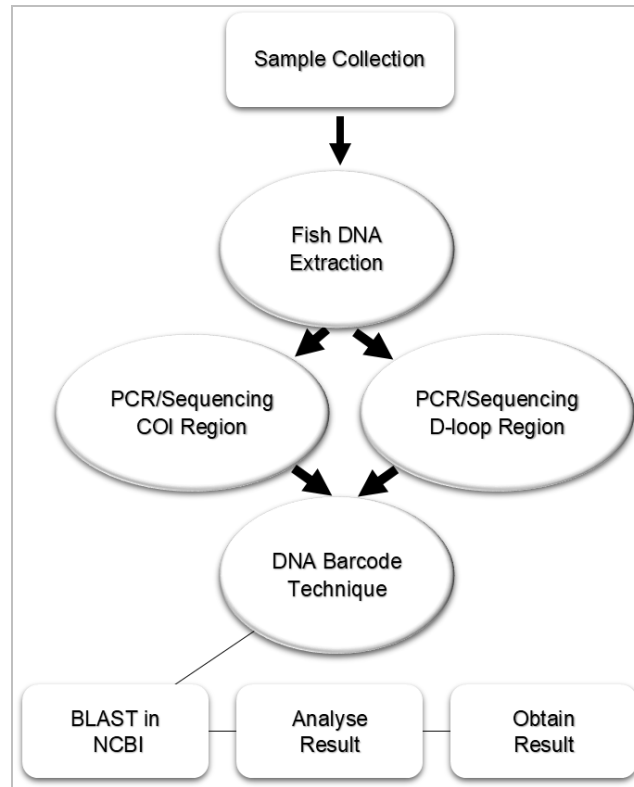


Figure 1. The overall summary of methodology flowchart

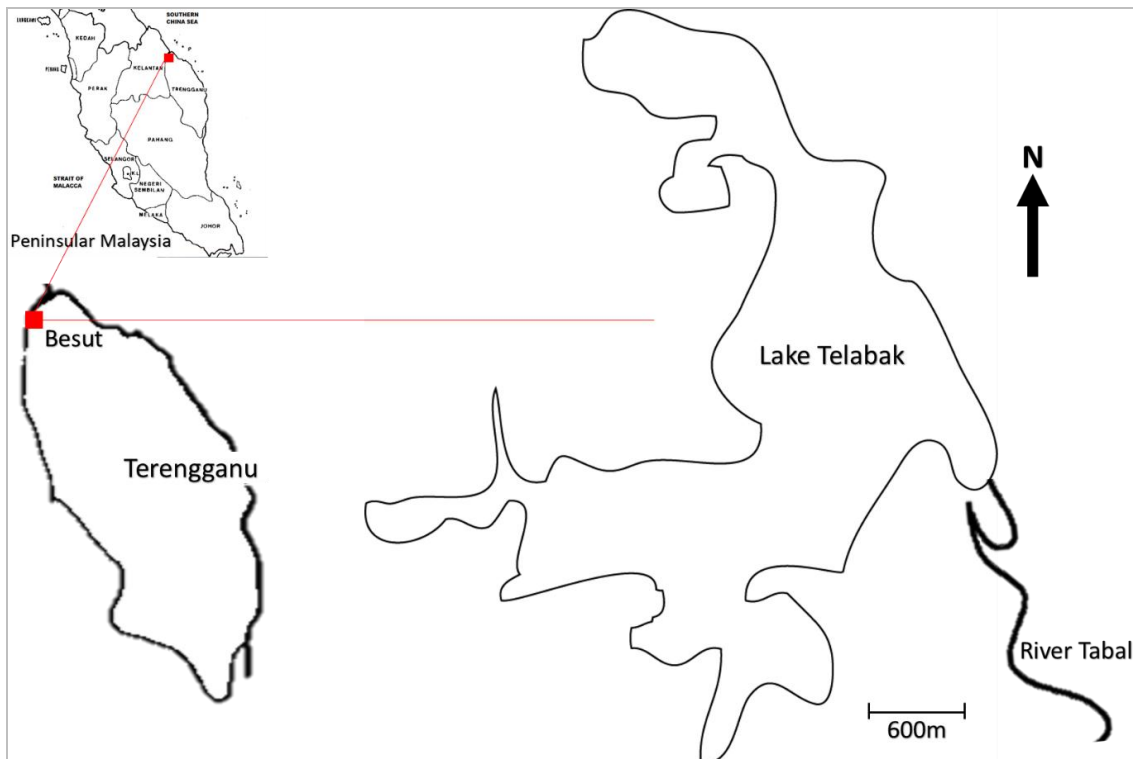


Figure 2. Location map showing the samples collection site of peacock bass in Lake Telabak, East Coast of Peninsular Malaysia ($5^{\circ}37'56.9''N$ $102^{\circ}28'24.5''E$)

DNA Extraction, Amplification and Sequencing

The total genomic DNA of peacock was isolated using Favorgen DNA extraction Kit (Favorgen Biotech Corp., Ping-Tung 908, Taiwan) following manufacturer's instructions. The partial COI gene of mitochondrial DNA was amplified by PCR using the universal primers COI-Fish2 F (5'TCGACTAATCATA AAGATATCGGCAC3') and COI-Fish2 R (5'ACTTCAGGGTGACCGAAGAATCAGAA 3') by **Ward *et al.* (2005)**. Another set of primer tPro2 (5'ACCCTAACTCCCAAAGC3') and HN-20 (3'GTGTTATGCTTTAGTTAAGC5') (**Lee *et al.*, 1995; Palumbi, 1996**) was used for the amplification of D-loop region. For both COI and D-loop region, the PCR was carried out in a 25 µl reaction volume containing 18.2 µl sterile distilled water, 2.5 µl Taq buffer, 2.0 µl dNTP Mix (2.5 mM), 0.5 µl of each primer (10 µM), 0.3 µl of 5 unit/µl Taq polymerase (TaKaRa) and 1 µl template DNA (1-50 ng/µl) on a thermal cycler PCR machine Veriti 96 Well Thermal Cycler (Applied Biosystem, California, USA), under the following thermal cycling conditions. Initial denaturation at 95 °C for 5 min; 35 cycles including denaturation at 95 °C for 30 sec, annealing at (50 °C, COI) and 52 °C, D-loop) for 30 sec and elongation at 72 °C for 10 min; followed by a final extension for 10 min at 72 °C and the PCR product was maintained at 4 °C. Sequencing was succeeded using BigDye Terminator v3.1 cycle sequencing kit (Applied Biosystems) following manufacturer's instructions, performed on an ABI Prism 3730xl Genetic Analyzer (Applied Biosystems).

Data Analysis

Forty COI and ten D-loop sequences (current study) were aligned and edited using ClustalW multiple sequence alignment program in MEGA 7 (**Kumar *et al.*, 2016**). DnaSP software was used to determine the variable sites among the sequence (**Librado and Rozas 2009**). To discover the origin of *Cichla* spp., the haplotype discovered in the present study was queried using basic local alignment search tool (BLAST) against NCBI nucleotide database. A top species match was identified with a sequence similarity of at least >99% to avoid false positives. Other haplotypes closely related to *Cichla* spp. were also downloaded from NCBI were included to compare sequence variation; and construct a phylogenetic analysis using Hasegawa-Kishino-Yano model HYK-1 (**Hasegawa *et al.*, 1985**) in MEGA 7 (**Kumar *et al.*, 2016**).

RESULTS

Sequence Alignment, Variation and Blast Search

A total of 617 bp of COI and 857 bp of D-loop mitochondrial DNA were obtained after deletion of low quality nucleotides at the 5' and 3' ends. Only one haplotype obtained among the forty COI sequences (T₁) and one haplotype from ten D-loop sequences (T₂) used in the current study. The T₁ and T₂ BLAST search sequence match results showed highest percentage identity with three *C. ocellaris* sequences as shown in Table 1 below. The result revealed extremely low sequence variation between T₁ and two *C. ocellaris* and one *C. temensis* with nucleotide variation of single base pair between each *C. ocellaris* as highlighted in Table 2. T₂ haplotype of D-loop also showed higher sequence variation with 3 gaps in nucleotide position when compared to the previous studies sequence haplotypes of *Cichla* spp. (Table 2).

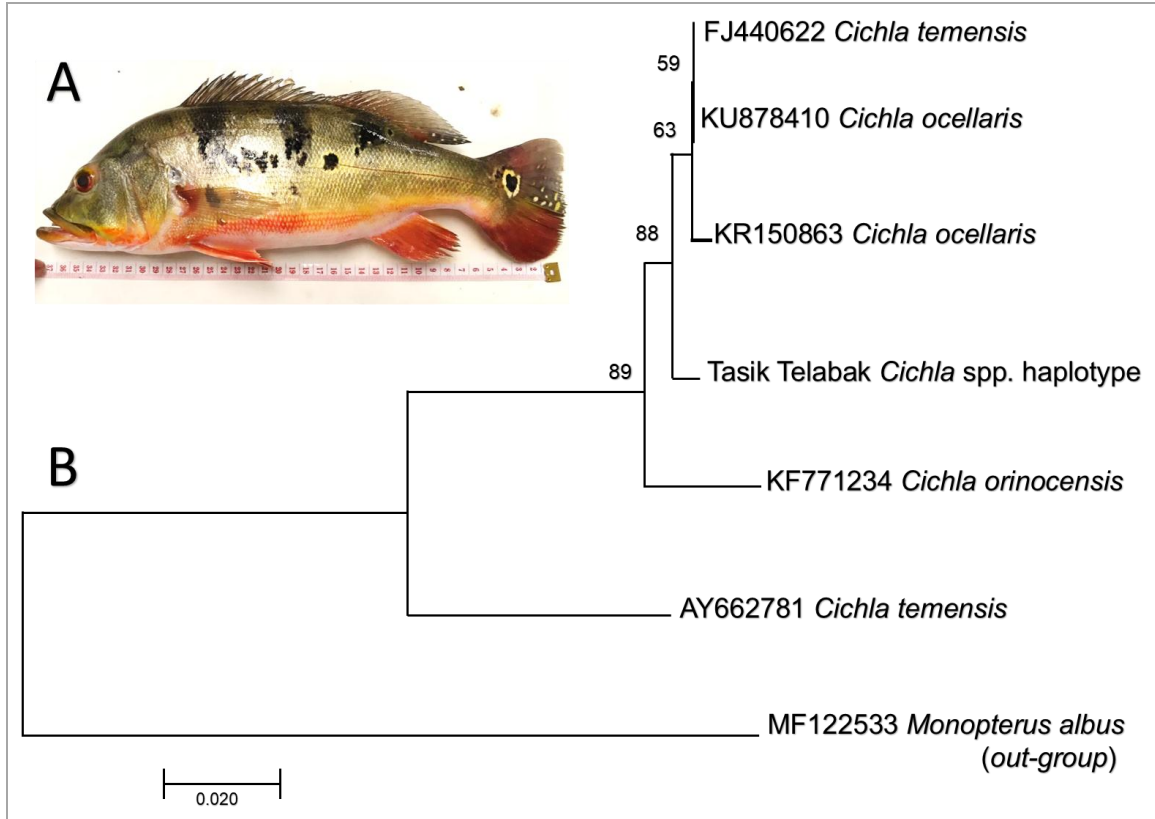


Figure 3. A: Sample of *Cichla* spp. collected from Lake Telabak **B:** Molecular phylogenetic analysis of COI gene of *Cichla* spp. by Maximum Likelihood method. The evolutionary history was inferred by using the Maximum Likelihood method based on the HKY model. The tree with the highest log likelihood (-1481.81) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

DISCUSSION

Lake Telabak serve a significant role for the people around the lake. For the serenity, diversity of aquatic life especially fishes, and the strategic location, the government has launch a special program to boost the economy of villagers through the development of the lake. Villagers are given subsidiary to start freshwater fish culture using cage system in the lake. Other than that, the lake was also being promoted as recreational spot where kayak and fishing become the main activities. From this study, we recognize the present of peacock bass from the lake, which is not native to Malaysia. Recent studies show that COI gene of mitochondrial DNA barcoding method is a very useful way to identify and traced the taxonomy and origin of some invasive species (Granato *et al.*, 2019; Golani *et al.*, 2019). Single haplotype (T1 COI and T2 D-loop) obtained in this recent study is a clear indication that *Cichla* spp. in Lake Telabak has a single maternal origin and introduction. This finding might not reflected the mechanism and channel for peacock bass introduction. However, from personal communication with

the villagers, they claimed that anglers come to Lake Telabak to fish Asian snakehead fish, *Channa argus*, which are among the main fish species present in the lake. Eventually their desire for more fishing excitement has encourage them to introduce peacock bass into the lake. **Chong et al. (2010)** and **Barroco et al. (2017)** described peacock bass as a good sport fish. The modus operandi of introduction of peacock bass was also similar in Malaysia like in Raban Lake (**Desa and Aidi, 2013**), Kapal Tujuh Lake (**Saat et al., 2014**), Temenggor Lake (**Yap et al., 2016**), Kampar River (**Tan and Sze, 2017; Ng et al., 2018**) and Timah Tasoh Dam (**Zulkifli, 2017**) in West Coast of Peninsular Malaysia.

Phylogenetic relationship and BLAST results of the current study confirm that *Cichla* spp. populations introduced to Lake Telabak belong to the *C. ocellaris* (KU878410 and KR150863). Hence, the origin of these two sequences are unknown as they were directly submitted to the genebank. Moreover, current study sequence also matched with *C. temensis* (FJ440622) that were originated and sampled from Manaus, Novo Airão, Tapajós and Tefé of Amazonas and Parà states of Brazil (**Ardura et al., 2010**). Knowledge about the origin and taxonomy of invasive species could help in understanding the biology of these species. Discovery of *C. ocellaris* in the current study is a milestone for careful investigation on the negative effects and consequences of this species based on the previously invaded habitat around the globe with their general role in biodiversity. For decade, studies on introduction *C. ocellaris* indicated a negative effects on local fish species in environments where it invaded (**Pinto-Coelho et al., 2008; Pelicice and Agostinho, 2009; Rahim et al., 2013**). Previous discovery of the fish population in Lake Redonda from 1989 to 1990, documented that many local fish species such as *Metynnis cf. roosevelt*, *Cheirodon* spp., *Crenicichla lepidota*, *Astyanax bimaculatus*, *Hoplias malabaricus*, and *Cichlasoma bimaculatum* extinct after the introduction of *C. ocellaris* (**Molina et al., 1996**). Recently, **Menezes et al. (2012)** reported that introduction of *C. ocellaris* in the coastal Lakes of Rio Grande do Norte is reducing native fish abundance with a negative impact on their diversity.

For now, it is difficult to measure the impact of *C. ocellaris* in Lake Telabak since there were no published records on species biodiversity in the lake before this. However, previous studies in Brazil and Puerto Rico reported how invasive *Cichla* spp. established and their continuous impact on reducing the native fishes population (**Pelicice and Agostinho, 2009; Britton and Orsi, 2012; Sharp et al., 2017**). This shows how these species becoming wide spread and more successful in occupying the newly habitat around the world. These could be a reasons that knowledge of genetic diversity and taxonomy is necessary for monitoring invasive *Cichla* population (**Oliviera et al., 2006; Golani et al., 2019**). Also, *C. ocellaris* are highly prolific and are associated with multiple spawning producing 2000-3000 eggs per brood (**Gamero and Braga, 2004; GISD, 2019**). As such, they wipe out small sized invertebrate-feeding fishes resulting in accumulation of large number of mosquito larvae around the lake and causing mosquito related diseases (**Zaret and Paine, 1973**). This could affect the fishing activities in the lake especially during the late and early hours of the day. Furthermore, studying *C. ocellaris* regarding prey, feeding and reproduction behaviour in their natural habitat might aid in discovering a common prey in Lake Telabak that might be at higher risk and first target.

CONCLUSION

Our research revealed that invasive species peacock bass population in Lake Telabak were highly identified as *C. ocellaris*, with single introduction pattern. No record found regarding the origin of these sequence matched, but origin might be traced to *C. temensis* that originated from Manaus, Novo Airão, Tapajós and Tefé of Amazonas and Parà states of Brazil. This findings will contribute and enhance better understanding of *Cichla* spp. In order to preserve and guarantee the safety of biodiversity in the lake, it is highly recommended that further study should focus on the continuous diversity documentation and observation of native aquatic species in the lake. This will aid for better understanding on the impact of *Cichla* spp. introduction in the lake.

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