# Genetic Diversity in Farmed Tiger Grouper Broodstock Inferred by Partial Cytochrome b Gene

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***Abstract*—**A study was conducted to determine the genetic diversity of a farmed tiger grouper *Epinephelus fuscoguttatus* broodstock population located in Tanjung Demong, Terengganu using Cytochrome b (Cyt-b) gene. A total of 51 broodstock were collected and subjected to DNA extraction. PCR amplification of partial Cyt-b was conducted using a universal Cyt-b primer. The fragments were then purified, sequenced and the genetic relationship among individuals was analyzed. A total of 402 bp length of partial Cyt-b gene was obtained from each sample. Genetic distance among individuals ranged between 0.002 – 0.018. Seven haplotypes were found within the population with 2 haplotypes (Hap01 and Hap02) are commonly shared by most individuals. Phylogenetic analysis showed broodstocks is divided into two major groups. However, 47 individuals were clustered in 1 group with shallow genetic divergence. These findings indicate the tiger grouper broodstock have low genetic diversity and most of the individuals might be originating from a single population. Genetic diversity of mtDNA cyt-b of *E. fuscoguttatus* would be useful in tiger grouper broodstock management in breeding program for seed production.

***Keywords*—** Broodstock, *Epinephelus fuscoguttatus*, partial Cyt-b, Tiger grouper

## I. Introduction

GROUPERS are marine species that have high economic value comprising 5180.3 tonnes of production in Malaysian mariculture sector [1]. In Malaysia, several farmers and government agencies reported a decline in growth performance and survival of tiger grouper with increase occurrence of deformities during seed production. This occurs in seed obtained from both local and imported stock. Several steps had been taken by farmers in the aspect of water quality and nutrition, to overcome this problem, however the situation still persist [2]. In seed production, it has been reported that most farmers use broodstock with unknown genetic background [3] and also produced from small founder broodstock [4]. There is a possibility such practices might occur in the local tiger grouper production. If this case is not given attention, it may lead to reproduction among individuals that are closely related thus causing inbreeding depression which is particularly common in aquaculture industry [5]. The effect of inbreeding has been seen in terms of reduction in genetic variation [6] causing the deterioration of growth rate, body weight, and food conversion efficiency [7] [8]. Reference [3] reported that this phenomenon is partially caused by environmental factors and genetic deterioration. However, genetic study to address this issue in Malaysia has not been properly elucidated. Genetic study on farmed broodstock had been conducted on several species and loss of genetic diversity had been reported in turbot [9], common carp [10], Nile tilapia [11] and barramundi [12]. Currently, genetic diversity and breeding performance of cultured tiger grouper broodstock in Malaysia is not well studied. Hence, effort should be done to determine the genetic status of these cultured tiger grouper broodstock in Malaysia. The study would enable researcher to get a better understanding regarding the genetic relationship and population structure among the tiger grouper broodstock in Malaysia. Finding also can assist farmers in broodstock management to increase production as well as producing high quality seeds. In addition, it can also lead to the implementation of strategic breeding programs for tiger grouper seed production.

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## II. Methodology

### A. Sample Collection

Fin sample of 51 tiger grouper broodstock was taken from a farm located at Tanjung Demong Setiu, Terengganu. The samples were preserved in 95% ethanol and kept at -20ºC..

### B. Polymerase Chain Reaction (PCR) Amplification and Purification

The PCR amplification was done using CytB universal

primer Cytb28f (5'CGAACGTTGATATGAAAAACCATCGTTG3') and Cytb34r (5'AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA 3') [13]. A 25ul PCR mixture was prepared containing 2.5 µl of 10× PCR buffer; 0.6 µl of 100 pmol of both primers; 0.3 µl of 5U Taq DNA polymerase (Transgen, China); 1.0 of 10,000 uM dNTP; and 1.0 µl of DNA sample. The amplification was done in a Thermal Cycler (Eppendorf AG, Germany) with a cycling profile beginning with the initial step 95ºC for 5 minutes, followed by 25 cycles of denaturation at 94ºC for 40 seconds, annealing at 54ºC for 45 seconds and extension at 72ºC for 60 seconds and finally subjected to the final extension at 72ºC for 7 minutes. The amplified product (3 µl) was then examined through 1.5% agarose gels. Prior to sequencing, the PCR products were purified using a DNA purification kit (Bioteke, China) according to the manufacturer instruction. All the purified PCR products were then sequenced bi-directionally using the same primers for PCR amplification by First Base Laboratories Sdn. Bhd. Malaysia.

### *C. Sequence Alignment and Phylogenetic Analysis*

The obtained sequences were edited using chromas and bioedit to remove any unwanted sequences, noise and gaps to validate the sequence. All sequences were identified using Basic Local Alignment Search Tool (BLAST) [14] at the National Center of Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov). The edited sequences were aligned and compared together with the sequences of other *Epinephelus* sp. available in the GenBank database using the CLUSTAL W. *E. coiodes* and *Thunnus tonggol* were used as outgroup. Phylogenetic trees were constructed using Neighbour-Joining and Maximum-Likelihood to determine the genetic relationship among individual. Identification of haplotype were done after alignment using CLUSTER W. Mitochondrial genetic diversity variation, haplotype and nucleotide variation between the samples was estimated using DnaSP (Version 5.1, Universitat de Barcelona) to determine the level of genetic variation within phylogenetic clades. The calculation was based on Tajima's test of neutrality.

## III. Result

The amplification of partial Cyt-b gene was performed on all E. fuscoguttatus broodstock samples collected from a farm in Terengganu. Sequence analysis of the amplified fragment showed a total length of 402 bp with all the 51 sequences having 100% similarity with the mtDNA Cyt-b sequence of *E. fuscoguttatus* in the Genbank Database (Accession no.: HQ174879.1). Seven haplotypes were identified from all 51 individuals (Table 1). Genetic relationship amongst haplotype for all samples is shown in Table 2. Hap01 and Hap02 were shared among seventeen individual each with the highest relative frequency of 0.3333. The other five haplotypes showed distinctive unique composite nucleotides with relatively low frequency of 0.1961 (hap 03), 0.0196 (hap 04 and hap 06) and 0.0784 (hap 07) respectively. The haplotype diversity and nucleotide diversity was 0.747 and 0.00414 respectively with genetic distance among individuals ranged between 0.002 to 0.018.

Phylogenetic tree analysis showed that all individuals were clustered into two major groups (group 1 : Hap01 – Hap06; group 2 : Hap07) indicating that the individuals might originate from two different populations (Figure 1). NJ tree on haplotype showed evidence of multiple shallow haplotype divergent within the clade suggesting the individuals might originate from several founder broodstocks within the same population in the past.

TABLE I
NUMBERS OF HAPLOTYPE AND RELATIVE FREQUENCY

| Samples Terengganu | Haplotype | Frequency |
|---|---|---|
| KHT22, KHT24, KHT29, KHT30, KHTG2, KHTG3, KHT6, KHTG7, KHT10, KHT12, KHT13, KHT15, KHT18, KHT20, KHT21, KHT36, KHT41 | Hap01 | 0.3333 |
| KHT25, KHT27, KHT28, KHT1, KHT5, KHT9, KHT14, KHT17, KHT32, KHT40, KHT44, KHT46, KHT47, KHT48, KHT49, KHT50, KHT51 | Hap02 | 0.3333 |
| KHT23, KHT31, KHT8, KHT16, KHT19, KHT33, KHT38, KHT39, KHT42, KHT45 | Hap03 | 0.1961 |
| KHT4 | Hap04 | 0.0196 |
| KHT11 | Hap05 | 0.0196 |
| KHT26 | Hap06 | 0.0196 |
| KHT34, KHT35, KHT37, KHT43 | Hap07 | 0.0784 |

Represented haplotypes of partial Cyt-b gene towards the individuals of *E. fuscoguttatus*. The Hap01 and Hap02 are represented to 17 samples with the highest frequency, whereas the other five haplotypes represent low frequency.

TABLE II
GENETIC DISTANCE OF TIGER GROUPER

| | Hap 01 | Hap 02 | Hap 03 | Hap 04 | Hap 05 | Hap 06 | Hap 07 |
|---|---|---|---|---|---|---|---|
| Hap 01 | | | | | | | |
| Hap 02 | 0.002 | | | | | | |
| Hap 03 | 0.002 | 0.005 | | | | | |
| Hap 04 | 0.005 | 0.003 | 0.008 | | | | |
| Hap 05 | 0.005 | 0.002 | 0.008 | 0.005 | | | |
| Hap 06 | 0.005 | 0.003 | 0.008 | 0.005 | 0.005 | | |
| Hap 07 | 0.013 | 0.015 | 0.015 | 0.018 | 0.018 | 0.018 | |

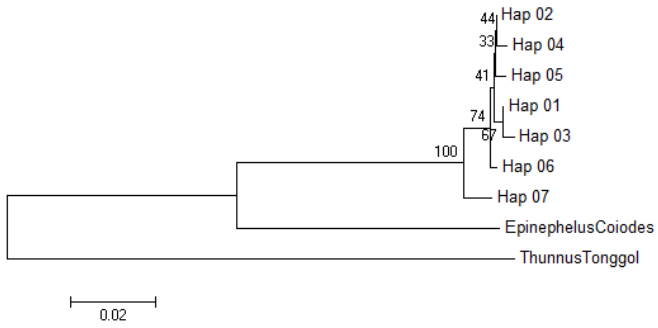


Fig. 1 Neighbour-Joining (NJ) phylogenetic tree of *E. fuscoguttatus* Cyt-b haplotypes and *E. Coiodes* and *T.tonggol* as outgroup.

## IV. Discussion

The study was done to access the genetic variation among broodstock Tiger grouper using mtDNA Cyt-b gene. The use of mtDNA markers offer several advantages such as maternal mode of inheritance, relatively limited recombination and they are highly conserved with definitive functions which makes them suitable in phylogenetics analysis, genetic population and variation studies [15]-[17].

Result showed that the individuals among tiger grouper broodstock have low genetic diversity. Even though the genetic distance of the population ranges between (0.002-0.018), however, 47 out of 51 individuals showed low genetic distance (0.002) with Hap01 and Hap02 are shared by majority of the individuals (Hap01:17 individuals; Hap02:17 individuals). Apart from the 2 common haplotypes (Hap01 and Hap02), 1 haplotype is only shared by 4 individuals and 4 unique haplotypes are only represented by one individual. The shallow divergent between Hap01 to Hap06 which is grouped within the same clade might indicate that the individuals are produced from small number of founder broodstocks within the same population. In aquaculture, reference [18] reported that limited facilities coupled with the need to breed only from individuals of superior genetic merit may result in selected lines of small population size. Small population size causes inbreeding and genetic drift leading to low genetic diversity. The use of limited number of broodstock has been reported occur in Oreochromis niloticus [19] and bay scallop, Argopecten irradians [4] and also in Seabass [12]. In this study, low genetic diversity might also originated from the use of limited number of parent broodstock to produce the current tiger grouper broodstock. Since hap01 and hap02 consist of 17 broodstock respectively, they is a possibility that this broodstock might be coming from closely related parent.

Genetic diversity of *E. fuscoguttatus* population was also determined based on haplotype and nucleotide diversity. Result of the study showed high haplotype diversity (Hd = 0.747) and low nucleotide diversity ($\pi = 0.00414$) indicating a low level of genetic diversity in tiger grouper population. Similar result indicating low genetic diversity has been reported in Indian Salmon (*Elutheronema tetradactylum*) [20]. Reference [20] found high haplotype diversity (0.5793-0.8230) and low nucleotype diversity (0.001514-0.002780) in all of four Indian Salmon population. High haplotype diversity and low nucleotide diversity may indicate shallow divergence and has also been reported in yellow croacker [21], Silver pomfret [22] and red tilefish [23]. Currently, genetic information regarding *E. fuscuguttatus* broodstock in Malaysia is unavailable. Most farmers in Terengganu area are small scale operators. There is not enough capacity to maintain a large number of broodstock. Hence there is a possibility that a small number of broodstock is used for breeding which can lead to loss of genetic diversity. Loss of genetic variation among hatchery population has been reported in turbot [9], common carp [10], Nile tilapia [11], barramundi [12], Japanese scallop [24] and bay scallop [4]. Loss of genetic variability in most hatchery stock may possibly results in the loss of disease resistance or in the reduction of populations' capability to adapt to new environment [25].

To increase genetic variation, reference [26] suggested that wild or genetically divergent populations should be introduced. Reference [27] has reported increase performance when crossbreeding conducted between genetically differentiated introduced captive stocks of Pacific blue shrimp. Similar result was also reported in African catfish (*C. gariepinus*) [3]. Hence, diversity of population can be expanded by taking broodstock from different population with known genetic information. Reference [28] stated that improving the genetic management can minimize the genetic impact for hatchery strains by monitoring the genetic variability and estimating precise effective population size. Due to this, farmers should be aware regarding the potential losses of genetic diversity and an understanding of where this genetic diversity may be lost through the hatchery production cycle could be used to develop improved hatchery techniques in aquaculture production [12]. Further study needs to be done to identify the genetic relationship of *E. fuscoguttatus* in other population.

## V. Conclusion

The study had managed to determine the genetic diversity of tiger grouper broodstock population in one tiger grouper Terengganu farm. The study showed low genetic diversity in the broodstock population. As genetic information of tiger grouper broodstock in Malaysia is not well documented, further study should be done to determine their genetic diversity on other farms.

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