DEVELOPMENT OF SINGLE LOCUS MOLECULAR MARKERS FOR *Epinephelus fuscoguttatus*

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BIOTECHNOLOGY PROGRAMME

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THIS DISSERTATION IS SUBMITTED AS PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF BACHELOR OF SCIENCE WITH HONOURS

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DECLARATION

I hereby declare that the material in this dissertation is my own work except for the quotations, excerpt, equation, summaries and references which have been fully acknowledged.

_____

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10th MAY 2012

VERIFICATION

Signature

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ABSTRACT

Epinephelus fuscoguttatus is a commercially important marine fish species in Southeast Asia and has been declared as near-threatened marine species by International Union for Conservation of Nature and Natural Resources. Based on single locus, molecular markers can be applied in the population studies as well as for the development of genomic databases which very useful in conservation programs. This project focused on the development of single locus molecular markers for Epinephelus fuscoguttatus. Epinephelus fuscoguttatus samples were obtained from Borneo Marine Research Centre of University Malaysia Sabah, Sabah. DNA from lateral fin was extracted by using modified salt extraction method. The extracted DNA was amplified by polymerase chain reaction (PCR) and the PCR products were ligated onto pUC19 cloning vector. This was followed by sequencing and development of six markers that can be used to amplified in the Epinephelus fuscoguttatus. A total of 20 sequences were isolated, 18 primers were designed and six primers amplified in the target species. These primers were used in the cross amplification process among the grouper species as well as the F1 hybrid grouper. These markers have a potential application in detection of the inheritance pattern among the grouper species as well as in the F1 grouper hybrids. Hence, through this application, these markers may be useful in the conservation and breeding programs of the grouper species.

PEMBINAAN LOKUS TUNGGAL BAGI SPECIES EPINEPHELUS FUSCOGUTTATUS

ABSTRAK

Epinephelus fuscoguttatus adalah salah satu spesis marin yang komersial di Asia Tenggara dan telah diisytiharkan sebagai spesis marin yang hampir terancam oleh Kesatuan Antarabangsa bagi Pemuliharaan Alam dan Sumber Asli. Berdasarkan dari lokus tunggal, penanda molekul boleh digunakan dalam kajian populasi serta di dalam pembinaan pangkalan data genomik yang mana ia sangat berguna terutamanya dalam program pemuliharaan. Projek ini adalah untuk membina penanda lokus tunggal bagi spesis *Epinephelus fuscoguttatus*. Sampel *Epinephelus fuscoguttatus* diperolehi daripada Pusal Penyelidikan Marin Borneo, Universiti Malaysia Sabah, Sabah. DNA dari sirip sisi ikan dan diekstrak menggunakan kaedah 'pengekstrakan garam' yang telah diubah suai. DNA yang telah diekstrak telah diamplifikasikan dengan menggunakan tindak balas rantai polymerase (PCR) dan produk PCR tersebut dilekatkan ke dalam vektor pengklonan pUC19. Langkah ini diikuti dengan proses penjujukan dan pembinaan enam penanda molekul yang boleh digunakan untuk mengesan spesis *Epinephelus fuscoguttatus* . Terdapat 20 jujukan DNA bagi *Epinephelus fuscoguttatus* yang telah dipencilkan, 18 primer atau penanda molekul telah dibina dan hanya 6 primer yang boleh digunakan. Keenam-enam primer tersebut digunakan dalam proses amplifikasi silang antara spesis kerapu and F1 hibrid kerapu. Penanda molekul ini mempunyai potensi dimana ianya boleh diaplikasikan untuk mengesan corak pewarisan dalam kalangan spesis kerapu dan juga F1 hibrid kerapu. Oleh itu, melalui aplikasi ini, keenam-enam penanda molekul ini mempunyai potensi untuk digunakan dalam program pemuliharaan spesis kerapu.

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LISTS OF SYMBOLS, ABBREVIATIONS AND UNITS

cm	Centimeter
m	Meter
M	Molar
%	Percent
mM	milimolar
TBE	Tris-borate EDTA
DNA	Deoxyribonucleic acid
RNA	Ribonucleic acid
NaCl	Sodium chloride
$MgCl_2$	Magnesium chloride
dNTP	dinucleotide triphosphate
L	Liter
mL	milliliter
mg	microgram
µL	microliter
°C	Degree celcius
g	Gram
kg	kilogram

UV	ultra violet
rpm	Rotation per minute
kb	kilobase
volt	Voltan
PCR	Polymerase chain reaction
E.coli	*Escherichia coli*
SDS	Sodium dodecyl sulphate
$Mg2^{+}$	Magnesium ion
mtDNA	Mitochondrial DNA
mt	Metric tone
US$	U.S dolar
LB	Luria Bertani

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CHAPTER 1

INTRODUCTION

Epinephelus fuscoguttatus, which also called as the brown-marbled grouper or tiger grouper, is one of a grouper species under the subfamily of Serranidae. *Epinephelus fuscoguttatus* is a commercially important marine fish species in Southeast Asia (Moktar *et al.*, 2011) and has been declared as near-threatened marine species by International Union for Conservation of Nature and Natural Resources (IUCN, 2004). This is because in the Southeast Asia the overfishing of this species is too much as the demand of the grouper is high in Southeast Asia. Hong Kong traders are actively looking for sources of wild caught groupers species such as *E. polyphekadion, E. fuscoguttatus, and Plectropomus spp.* (Sadovy and Vincent, 2002). Beside, water pollution also affects the distribution of this species (Moktar *et al.*, 2011). They are normally found tropical coral waters between 35°N - 27°S and 39°E - 171°W, with the depth between 1 to 60 meters.

A molecular marker is a DNA sequence used to "mark" or track a particular location or locus on a particular chromosome (Ibrahim and Yilmaz, 2003). Molecular markers have wide applications in marine and agricultural studies. One of the applications is for the conservation studies. Molecular markers can be used in conservation studies as it can be used either to identify the species itself of study for the evolutionary of the species. In this study, partial single locus molecular markers had been developed for *E. fuscoguttatus*. It can be used to detect the *E. fuscoguttatus* species since this species had been declared as a near threatening species. Single locus molecular marker is a PCR-based co-dominant marker where it

can easily be transferred to other populations and shared between research laboratories (Pugh *et al.*, 2004).

This study aims to develop single locus molecular markers for *E. fuscoguttatus*. The objectives for this study are stated as below:

a) To construct partial genomic library for the *Epinephelus fuscoguttatus* species.
b) To develop a single locus molecular markers for *Epinephelus fuscoguttatus* species.
c) To determine the cross amplification pattern against other groupers and F1 hybrid grouper species.

CHAPTER 2

LITERATURE REVIEW

2.1 Sample Study – *Ephinephelus fuscoguttatus*

E. fuscoguttatus (figure 2.1) is one of the grouper species from the family of Serranidae which the total length can reach up to 120 centimeter (cm) and maximum weight up to 11 kilogram (kg). *E. fuscoguttatus* species is reef associated which occurs in lagoon pinnacles, outer reef slopes, and in coral rich area with deep clear water at depth range from 1 to 60 meters. Where as, the juveniles of *E. fuscoguttatus* often occur in seagrass beds. In addition, *E. fuscoguttatus* species feeds on other fishes, crabs and cephalopods.

Figure 2.1 *E. fuscoguttatus* species (redOrbit,2008)

E. fuscoguttatus is one of the grouper species that relatively long-lived which the lifespan can exceed up to 40 years. According to the Society for the Conservation of Reef Fish Aggregation (SCRFA), the sexual pattern of *E. fuscoguttatus* appears to be monandric protogeny where the juveniles species mature and function as adult females before changing to the male sex pattern even though the confirmation of sex change is still required. Figure 2.2 shows the reproduction and life cycle for most of the grouper species.

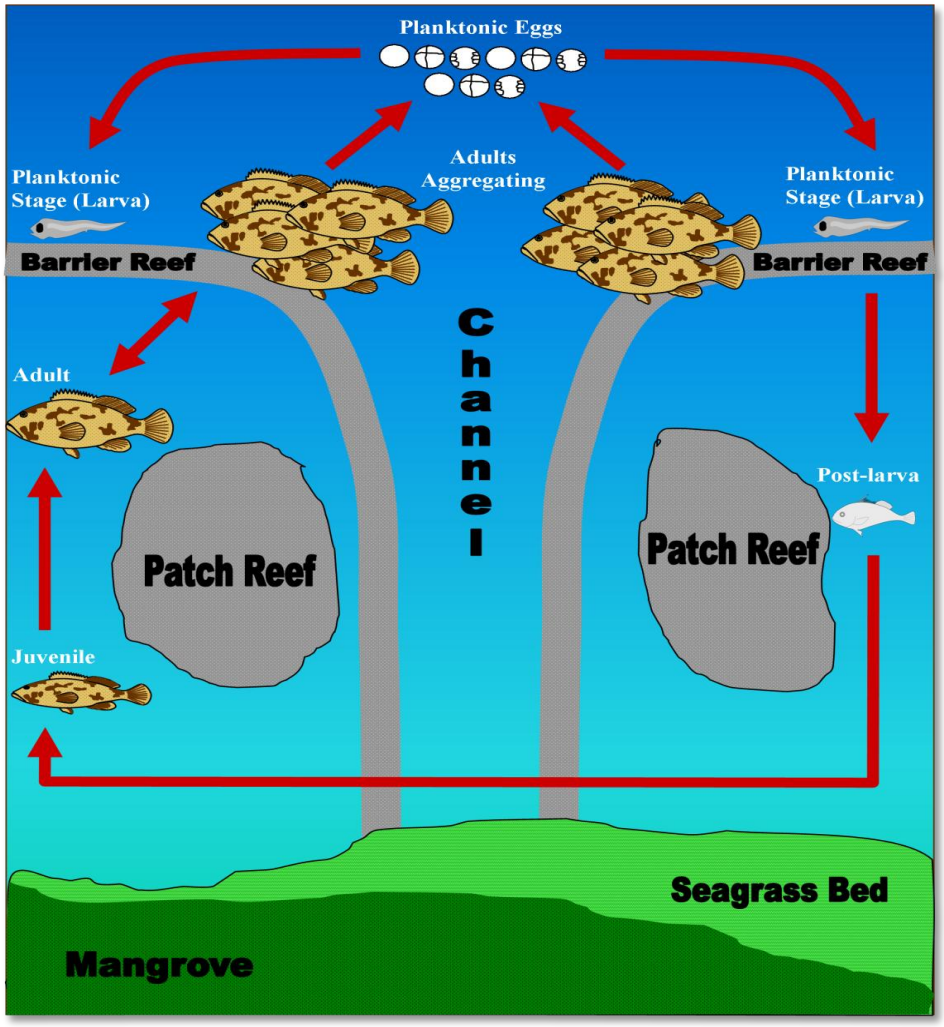

Figure 2.2 Reproduction and life cycle for most of the grouper species (source, http://www.scrfa.org/)

According to SCRFA also, the postulate life history strategy is one of the energy which the energy is primarily invested in body growth for 9-10 years, then the growth slows and energy still invested in reproduction. Large female has big reproductive contribution when they reach more than 30 year of reproductive lifespan. Approximately, 50% effective maturity is calculated from the percentage of females that were sexually active during the spawning season.

E. fuscoguttatus is the only known grouper species that spawn in aggregation. According to the Sudaryato and Mous (2004), *E. fuscoguttatus* spawn in pairs but different pair would often spawn at the same time which giving the effect of a 'group spawning'. The male displayed or 'danced' to establish a pairing with chosen females that lay inactive on the cage floor. And once the pair was established, the spawning behavior started with the pair swimming together up from the cage floor to the water surface, and then both fish released their eggs and sperm. Besides that, the spawning also was concentrated in the fourth quarter of the moon, when 97% of all spawning event were observed. Female *E. fuscoguttatus* spawn more than once during particular aggregation period. Other study also reveals that the *E. fuscoguttatus* was considered a better potential species than other grouper species for large-scales spawning in net cages where it exhibit few characteristics which are, higher egg production and all year round spawning capabilities, higher fertilization rate, higher percentage in buoyant eggs, faster larval development and higher larval survival (Lim *et al*., 1990; Chao *et al.*, 1993).

E. fuscoguttatus species can be found in Australia, Bangladesh, British Indian Ocean Territory, Brunei, Cambodia, Cocos (Keeling) Islands, Comoros, Djibouti, Egypt, Eritrea, Fiji, Guam, Hong Kong, India, Indonesia, Israel, Japan, Jordan, Kenya, Kiribati, Madagascar, Malaysia, Maldives, Marshall Islands, Mauritius, Micronesia, Mozambique, Myanmar, New Caledonia, Northern Mariana Islands, Pakistan, Palau, Papua New Guinea, the Philippines, Samoa, Saudi Arabia, Seychelles, Singapore, the Solomon Islands, Somalia, Sri Lanka, Sudan, Taiwan, Tanzania, Thailand, Vanuatu, Vietnam, and Yemen (redOrbit,2008).

Figure 2.3 below shows the distribution of the *E. fuscoguttatus* species in some of the geographical area.

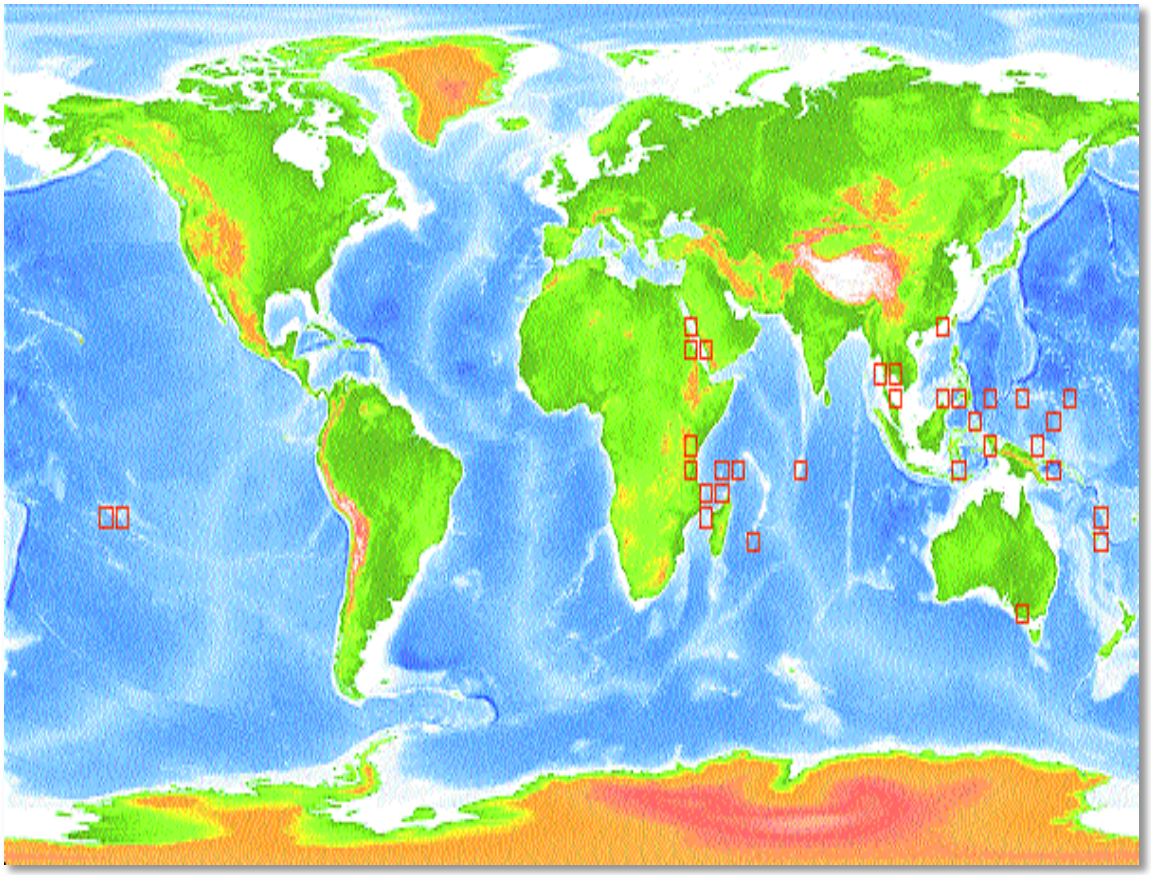

Figure 2.3 Zoological sites sampled for geographic distribution (indicated by the red Squares) of *E. fuscoguttatus (*redOrbit,2008).

As mention before, *E. fuscoguttatus* has been declared as near-threatened by International Union for Conservation of Nature and Natural Resources (IUCN). This is due to its late maturation, aggregation-spawning when the aggregations are targeted and because of it higher value in the market. The aggregation-spawning had become overfishing when people targeting to catch large volume of *E. fuscoguttatus* which it consist large *E. fuscoguttatus* species. Thus, the number of the new progeny of the *E. fuscoguttatus* species had decline. The degree of protection for *E. fuscoguttatus*

spawning aggregation varies widely. One of the approach to protect this species is to introduced to people which area from particular aggregation site that suitable for fishing this species instead to understand when the maturity and spawning period for *E. fuscoguttatus* species. Many acts and laws that had been establish in order to protect the marine life from extinction which involved *E. fuscoguttatus* species. For example, the Pohnpei State Government had implementing measures to reduce fishing effort on grouper. In addition, Marine Protection Act of 1994 also intended to prohibit any capture of grouper even for subsistence purpose during the closed period. Minimum capture size limit of 35cm also has been introduced in Australia on order to protect any of marine species from extinction.

There are several measures preventing the extinction that have been taken in grouper fisheries. These measures include limiting the number of the fishers through the fishing license, limiting the amount of fish caught by giving the specific quota for each species, and restricting the type of gear used. Besides that, there also have options for the community-based management which the prohibiting the usage of a gill nets as the usage of the gill nets during the spawning aggregation, it will reduce the number of the breeding fish. In addition, other approach that can be taken is ban on fishing during the peak of the spawning season which may involve several short closures at monthly intervals as some of the grouper species appear to aggregate at particular times in the moon cycle.

2.2 Taxonomy of *Ephinephelus fuscoguttatus*

Table 2.1 shows the taxonomy of *E. fuscoguttatus. E. fuscoguttatus* belongs to family of Serranidae and known with few local name such as black-tipped cod,blotch grouper,blotchy grouper,blotchy rockcod,brown-marbled grouper,flower cod,flowery cod,grouper,kawakawa,marble grouper,tiger grouper, and black rockcod.

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