GENOMIC DNA FINGERPRINTING OF GROUPER FROM SABAH, MALAYSIA

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DECLARATION

I hereby declare that this dissertation is based on my original work, except for quotations and summaries, each of which has been fully acknowledged.

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21 May 2011
VERIFICATION

Signature

SUPERVISOR
(DR. JULIAN RANSANGAN)
ACKNOWLEDGEMENT

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ABSTRACT

Genetic relatedness between species can be applied to determine the viability of intraspecific hybrids. A study was carried out to determine the genetic relatedness between *Epinephelus lanceolatus*, *E. fuscoguttatus*, *E. coioides* and *E. coralligcola* using randomly selected single locus DNA markers. A total of 27 DNA markers from *E. lanceolatus* were able to cross-amplify 15 and 7 loci in *E. coralligcola* and *E. coioides* respectively indicating that *E. lanceolatus* is genetically related to *E. coralligcola*. On the other hand 27 markers derived from *E. fuscoguttatus* failed to amplify in *E. coralligcola* and only one locus amplified in *E. coioides*. This provides a strong indication of the fact that *E. fuscoguttatus* does not share a genetic similarity with these two species. These results of this study provide a basis for developing F1 hybrids from *E. fuscoguttatus* and *E. coralligcola* as well as *E. coioides* due to their large genetic distance.
GENOMIK DNA CAP JARI IKAN KERAPU DARI SABAH, MALAYSIA

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<td>tris-borate-EDTA</td>
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<tr>
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<td>ribonucleic acid</td>
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<tr>
<td>NaCl</td>
<td>Sodium chloride</td>
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CHAPTER 1

INTRODUCTION

1.1 Diversity of groupers

Groupers are divided into five subfamilies which are Serraninae, Anthiinae, Niphoninae, Grammistinae and Epinephelinae. Grouper with order of Perciformes, the families contains about 500 species of Serranides and subfamily, contains approximately 159 species of Epinephelinae (Heemstra and Randall, 1993). Groupers of family Serranidae are one of the top predatory fish taxa found on coral reefs. They target a range of prey, from crustaceans and cephalopods to reef fish including damselfish (Pomacentridae), parrotfish (Scaridae), blennies (Blenniidae) and wrasse (Labridae). They have also been shown to prey on small pelagic fish groups that venture onto the reef slope, including sardines (Clupeidae) and anchovies (Engraulidae). Predation is an important process in any ecosystem, it helps drive biodiversity by maintaining prey populations below their carrying capacity, thus preventing niche saturation. However, they themselves rely on sufficient prey items to survive, and therefore are indicative of a healthy reef associated fish community. Due to their ecological importance, groupers are heavily exploited. They are an important target species for fishing due to their market value, and are also widely exploited for the living fish trade. However, because of their slow growth rates and long lifespan, they are vulnerable to overfishing and thus their management is often a priority for conservation organizations.
Among Epinephelinae species that are commercially important is *Epinephelus fuscoguttatus* and this species is cultured intensely in Kuwait, Indonesia, Malaysia, Thailand, the Philippines, Hong Kong, Taiwan, The Republic of China, Japan, and Mexico (INFOFISH, 1989; Tookiwinas, 1989; Bombeo-Tuburan et al., 2001; Lin and Shiau, 2003). Throughout the most warm and temperate marine regions, Serranids are highly valued for food, and some kept in aquaria.

The most consistently and abundantly species captured for culture proposes and also reared in hatcheries are *Epinephelus coioides* (Hamilton, 1822) (frequently misidentified either as *Epinephelus tauvina* (Forsskal, 1775) or *Epinephelus malabaricus* (Bloch and Schneider, 1801)) and *Epinephelus fuscogutattus* (Forsskal, 1775). Other important species include *Epinephelus bleekeri* (Vaillant, 1878), *Epinephelus akaara* (Temminck and Schlegel, 1842), *Epinephelus awoara* (Temminck and Schlegel, 1842) and *Epinephelus areolatus* (Forsskal, 1775). Also cultured in small numbers are *Epinephelus amblycephalus* (Bleeker, 1857), *Epinephelus malabaricus* (Bloch and Schneider, 1801), *Epinephelus lanceolatus* (Boch, 1790), *Epinephelus sexfasciatus* (Valenciennes, 1828), *Epinephelus trimaculatus* (Valenciennes, 1828), *Epinephelus quoyanus* (Valenciennes, 1830), *Epinephelus bruneus* (Boch, 1793), *Epinephelus polyphkeadion* (Bleeker, 1849), *Epinephelus coralicola* (Valenciennes, 1828), *Cromileptes altivelis* (Valenciennes, 1828) [*Epinephelus altivelis* after (Ding et al., 2006)], *Plectropomus leopardus* (Lacepede, 1802) and *Plectropomus maculates* (Bloch, 1790) (Ottolenghi et al., 2004).

1.2 Demand for groupers by the population

There is a high demand for groupers because of threats from human and environment itself, groupers resources are decreasing. For example, *Epinephelus fuscoguttatus* is decreasing in the open ocean, particularly caused by overfishing and water pollution.
International Union for Conservation Union for Conservation of Nature and Natural Resources (IUCN) status *E. fuscoguttatus* as ‘being near threatened’ because of the extensive collection from the wild since every stages or size has its own value (Cornish, 2004). From there, people are trying to culture groupers to produce more high productivity. Aquaculture of groupers is now practiced in many South-East Asian Countries, including Indonesia, Malaysia, the Philippines, Taiwan, Thailand, Hong Kong, the south-east of China, and Vietnam, and in other parts of tropics, for example, In south-eastern USA and the Caribbean. Grouper also cultured but much lesser extent in India, Sri Lanka, Saudi Arabia, Korea and Australia (Ottolenghi *et al.*, 2004). Groupers that cultured are very expensive, especially for live food fish restaurant trade (LFFRT) in Hong Kong, Singapore and China, leading to concerns about the decreasing wild caught product as a result of over fishing (Sadovy *et al.*, 2003). The LFFRT is almost the only way limit the selected South-East Asian nations, often with a group of ethnic Chinese.

LFFRT usually dominated by reef fishes, like groupers (Family Serranidae), snappers (Family Lutjanidae) and some wrasses (Family Labridae). After a period, the LFFRT has aware to the attention of conservationists with regard to the depletion of resources and destruction of habitats (Pet, 1997; Mous *et al.*, 2000; Pet-Soede *et al.*, 2004). So, in order to cater to LFFRT, it has been suggested to have a spread through a considerable proportional of the Indian and Pacific Oceans (Sadovy *et al.*, 2003).

### 1.3 Genetic studies on groupers

Historically, the effort to explain the evolutionary relationships among groupers have been difficult by the large number of species, a global distribution and lack of morphological specializations traditionally used in ichthyological classification.
But recently, several studies have used molecular markers to address this issue. The studies conducted in the Asia – Pacific region using mitochondrial DNA (mtDNA) cytochrome b partial gene sequences (Ding et al., 2006), microsatellite markers (Koedprang et al., 2007) and random amplified polymorphic DNA (RAPD) markers (Govindaraju and Jayasankar, 2004) all point to the fact that there is great diversity among grouper species, but equally that there is significant confusion in taxonomy. However, the existing confusion on taxonomy is genetically resolvable (Koedprang et al., 2007).

1.4 Objectives

Objectives for this study are to differentiate cross-amplification patterns against different grouper species and also to identify specific molecular markers to differentiate between Epinephelus lanceolatus, Epinephelus fuscoguttatus, Epinephelus coioides and Epinephelus coralicola.
CHAPTER 2

LITERATURE REVIEW

2.1 Taxonomy of *Epinephelus* spp.

Taxonomy is a classification of organisms into groups based on similarities of structure or origin. The taxonomy of four species is shown on the table 2.1. Table 2.1 showed the taxonomy of *Epinephelus* spp. Belongs to the order of Perciformes of extensive tribe of fishes including Percidae, Centrarrchidae, Sciaenoidae, Sparadae, Serranidae and some other related families. As presently understood, the order Perciformes contains >9000 species arranged in 18 suborders and 148 families. Although no synapomorphies have been proposed to unite Perciform fishes, general characters in most include 1 spine and 5 or fewer rays in pelvic fin, lack of an adipose fin, presence of 17 or fewer hypural bones (plus the parhypural), presence of 7 or fewer branchiostegal rays and 4 gill arches. Johnson (1983) has proposed that the Serranidae comprises three subfamilies Serraninae, Anthiinae and Epinephelinae into 5 tribes include Niphonini, Epinophelini, Diploprionini, Lioproporini and Grammistini. Family Serranidae is now divided into five subfamilies include Serraninae, Anthiinae, Niphoninae, Epinephelinae and Grammistinae. Then the Grammistinae includes the tribes Grammistini, Diploprionini and Liopropomini. Epinephelinae consist genus of Aethaloperca, Alphesres, Anyperodon, Cephalopholis, Cromileptes, Dermatolepis, Ephinephelus, Gonioplectrus, Gracila, Mycteroperca, Paranthias, Plectropomus, Saloptia, Triso and Variola according to Heemstra and Randall (1993). In the family Serranidae contains about 500 species with approximately 159 species belong to Epinephelinae (Heemstra and Randall, 1993).
Table 2.1 showed the taxonomy of *Epinephelus* spp

<table>
<thead>
<tr>
<th>Domain</th>
<th>Eukaryota (Whittaker and Margulis, 1978)</th>
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<tbody>
<tr>
<td>Kingdom</td>
<td>Animalia (Linnaeus, 1758)</td>
</tr>
<tr>
<td>Phylum</td>
<td>Chordata (Bateson, 1885)</td>
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<tr>
<td>Subphylum</td>
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</tr>
<tr>
<td>Superclass</td>
<td>Osteichthyes (Huxley, 1880)</td>
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<tr>
<td>Class</td>
<td>Actinopterygii (Huxley, 1880)</td>
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<tr>
<td>Infraclass</td>
<td>Teleostei</td>
</tr>
<tr>
<td>Superorder</td>
<td>Acanthopterygii</td>
</tr>
<tr>
<td>Order</td>
<td>Perciformes</td>
</tr>
<tr>
<td>Suborder</td>
<td>Percoidei</td>
</tr>
<tr>
<td>Family</td>
<td>Serranidae</td>
</tr>
<tr>
<td>Subfamily</td>
<td>Epinephelinae</td>
</tr>
<tr>
<td>Tribe</td>
<td>Epinephelini</td>
</tr>
<tr>
<td>Genus</td>
<td>Epinephelus (Bloch, 1793)</td>
</tr>
<tr>
<td>Species</td>
<td><em>Epinephelus lanceolatus</em> (Bloch, 1790)</td>
</tr>
<tr>
<td></td>
<td><em>Epinephelus fuscoguttatus</em> (Forsskal, 1775)</td>
</tr>
<tr>
<td></td>
<td><em>Epinephelus coioides</em> (Hamilton, 1822)</td>
</tr>
<tr>
<td></td>
<td><em>Epinephelus coralicola</em> (Valenciennes in Cuvier and Valenciennes, 1828)</td>
</tr>
</tbody>
</table>

FAO English name for *Epinephelus coioides* is coral grouper. Head and body of coral grouper more likely to be tan dorsally, shading to whitish vertically, numerous small brownish orange or reddish brown spots on head, body and median fin; body with 5 faint, irregular, oblique, dark bars which bifurcate ventrally; first dark bar below anterior dorsal-fin spines, last bar on caudal peduncle; 2 dark spots on interopercle and another 1 or 2 at junction of sub- and interopercles. It is known from continental shores and large islands. It is often found in estuaries, also taken offshore to depths of 100m. Geographical distribution occurs from Red Sea South to at least Durban and east to the western pacific, where it ranges from Ryukyu Islands to Australia and eastwards to Palau and Fiji. Other localities include the Persian Gulf, India, Reunion, Mauritius, Andaman Islands, Singapore, Hong Kong, Taiwan and Philippines. FAO English name for *Epinephelus coralicola* is Coral grouper. Their head and body are
brownish to greenish grey; widely spaced small black spots over all over head, body and fins; 3 dark blotches on body at base of dorsal fin and dark saddle blotch on caudal peduncle. Mostly is founded in the shallow silty reefs and estuarine areas. Geographical distribution occurs from Western Pacific from Thailand, Hong Kong and Taiwan to Australia (Western Australia, Northern Territory, Queensland and New South Wales) and eastward to the Solomon and Mariana Islands, including Indonesia, Singapore, Philippines, Papua New Guinea and Belau.

FAO English name for *Epinephelus lanceolatus* is Giant grouper. Small juveniles of giant grouper (12 cm standard length) is yellow with irregular broad black bars on body, the first from spinous dorsal fin to belly and chest and extending onto head, the second from base of soft dorsal fin to anal fin and the last at base of caudal fin. As for adult (80-150 cm standard length) is dark brown with faint mottling, the fins with numerous small black spots while large adult (160-230 cm standard length), it is dark brown in color and the fins darker. Giant grouper has been caught at the depths of 100m but it is more often in shallow water. Specimen more than a meter long has been caught from the shore and in harbors (Heemstra and Randall, 1993). It is commonly seen in caves on coral reefs and around wrecks and estuaries. Giant grouper is the most widely distributed grouper in the world. It is occurs throughout the Indo-Pacific region from the Red Sea to Algoa Bay, South Africa and eastward to the Hawaiian and Pitcairn Islands. In western Pacific, giant grouper ranges northward to Southern Japan and Southward to Australia (from northern western Australia to Northern New South Wales and Kailola and Jones (1981) reported a 212 cm total length specimen from South Australia. FAO English name for *Epinephelus fuscoguttatus* is brown-marbled grouper. The color of the brown-marbled grouper is pale yellowish brown, with 5 vertical series of dark brown blotches that are very irregular in outline. The head, body and fins covered with close set small brown spots, those on the dark blotches much darker than spots in-between blotches. Geographical distribution widely distributed in the Indo-Pacific region, including the Red Sea, but not known from the Persian Gulf, Hawaii or French Polynesia. It occurs at most of all tropical islands of the Indian and west-central Pacific Oceans (east to Samoa and the Phoenix Islands) along the east coast of Africa to Mozambique and it has also been reported from Madagascar, India,
Thailand, Indonesia, tropical coast of Australia, Japan, Philippines, New Guinea and New Caledonia.

2.2 Morphology of grouper

Groupers are identified by their morphological characters including the body shape and size of fins. Besides that, grouper also identified by the shape and relative size of head and number of fins rays, scales and gill rakers. For example, long, numerous gill rakers and are thus adapted for plankton feeding. Lastly, they also identified by their colour pattern, but there is a different color pattern between juvenile and adult. Unfortunately, studies show that species identification of grouper is problematic because morphological traits overlap among species, and variations of body coloration during different life stages can make it difficult to correctly classify species (Koedprang et al., 2007). Figure 2.1 showed the external morphology and measurements of groupers and Figure 2.2 showed the color marking according to Heemstra and Randall (1993).

Following are the terminology terms for figure 2.1 and 2.2. Caudal peduncle: the part of the tail that joins the caudal fin to the body. Caudal-peduncle length: the horizontal distance from the rear end of the anal fin base to a vertical at the caudal-fin base. Dorsal fin: the median fin on the upper part of the body. Head length: The straight-line measurements from the front of the upper lip at the midline (with upper jaw retracted) to the posterior most point of the operculum. Lateral line: the series of pored or tubed scales that run from the upper end of the gill cavity to the caudal fin. Nape: the posterior part of the top of the head. Snout: the part of the head in front of the eye. Standard length: the straight line distance from the tip of the snout to a vertical line passing through the base of the caudal fin (taken to be the point of flexure of the caudal fin). Total length: The straight-line distance from the tip of the anterior most jaw to a vertical line passing through the posterior tip of the longest caudal-fin ray.
Figure 2.1 show external morphology and measurements (Heemstra and Randall, 1993).

**Band:** an elongate color marking, approximately 1 or 2 eye diameters in width. **Bar:** an elongate color mark with vertical or near vertical orientation. **Blotch:** a color mark, larger than the eye and usually with irregular or indistinct borders. **Dot:** a color mark about the size of a nostril. **Saddle blotch:** a dark saddle-shaped blotch on the dorsal part of the body or caudal peduncle. **Spot:** a color mark which is eye-sized or smaller, but larger than the size of the nostrils and usually with a regular or distinct margin. **Stripe:** a narrow, oblique or horizontal color marking, less than an eye diameter in width.
Figure 2.2 Color markings (Heemstra and Randall, 1993).

2.3 Previous Research on *Epinephelus* spp.

The identification of grouper species was a problematic issue since the morphological traits overlap among the species itself, and variations of body coloration during different life stages can make it difficult to correctly classify the species. Although there is an identification guides based on morphological characteristics for the identification of almost all groupers species in the world (Heemstra and Randall, 1993), misidentification between species is still common. For an example, in one grouper species *Epinephelus coiodes* which is cultured in South-East Asia is often misreported as *Epinephelus tauvina* (Leong, 1998) and the cases of misidentification between *Epinephelus coiodes* and *Epinephelus malabaricus* are also common.

Molecular genetic markers have been used to resolve taxonomic ambiguity in many taxa (Frankham *et al.*, 2000) including fishes (Avise, 1994). Further more, species specific genetic markers serve as an efficient tool for precise species identification and detection of introgression between species (Senanan *et al.*, 2004; Na-Nakorn *et al.*, 2004). Several types of molecular markers have been used to
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