STUDIES ON GENETIC VARIABILITY OF GROUPERS (*EPINEPHELUS* SPP.) FROM INDO-MALAYSIAN WATERS USING PCR-RAPD ANALYSIS

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A SPECIAL DEDICATION ...

To my daughter and my son

ANDI DHIYA AQILAH PARASETIA
ANDI MUHAMMAD ATAILLAH ASYRAF

Who were born during my study period

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science.

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The serranid subfamily (*Epinephelus* genus), popularly known as grouper, is one of the most economically important fishes in Southeast Asian countries. In this study, RAPD in association with PCR was employed to determine the genetic variability and to establish the RAPD fingerprinting of groupers (*Epinephelus* spp.). A total of 100 individuals of *E. tauvina* from six populations taken from Indo-Malaysian waters and 36 individuals of *E. merra* and *E. areolatus* taken from Makassar Strait, South Sulawesi were studied. Three tissue preservatives and four DNA extraction techniques were employed to find the best preservative and DNA extraction technique for grouper. A series of optimization experiments of PCR-RAPD protocol were conducted. Out of the 34 screened random primers, ten primers were selected for further analysis.

The results of this study indicated that the TNES-Urea buffer was the best for tissue preservation and Phenol-Chloroform method was the excellent DNA extraction

technique for grouper. The optimal concentration of genomic DNA, $MgCl_2$, taq DNA polymerase, dNTP-mix and primer for PCR requirements were 50 ng, 3.5-4.5 mM, 2 units, 0.4 mM and 0.4 µM, respectively. The GeneAmp PCR System 2400 produced clear RAPD banding patterns with an optimal annealing temperature of 36°C for 45 cycles. The DNA purity values obtained in this study were 2.19±0.22 for *E. tauvina*, 2.07±0.13 for *E. merra* and 2.00±0.14 for *E. areolatus*. The similarity indices among individuals were 0.80+0.11 for *E. tauvina*, 0.58±0.11 for *E. merra* and 0.62±0.07 for *E. areolatus*.

Ten RAPD primers generated a total of 403 fragments with 205 polymorphic fragments (50.9%) and their size range was between 250bp and 2500bp. Genetic distance among populations of *E. tauvina* varied from 0.20 to 0.41. The dendrogram constructed from these values revealed two main clusters. The cluster from Pulau Pinang and Terengganu population was genetically separated from that of Tanjung Pinang, Pare-Pare, Makassar and Bone. Eight species-specific markers for *E. tauvina* were established, namely 700pb (OPA-02), 900bp (OPA-06), 550bp (OPA-10), 650bp (OPA-16), 850bp (OPA-17), 950bp (OPA-18), 750bp (OPA-19) and 650bp (CA-05).

The total number of fragments generated by ten primers in the *Epinephelus* genus was 75 fragments in *E. tauvina*, 72 fragments in *E. merra* and 75 fragments in *E. areolatus*. Number of genotypes detected for each primer ranged from 3 to 5 for *E. tauvina* and *E. areolatus*; and 3 to 6 for *E. merra*. Percentage of polymorphic band was 37.5-75.0%, 33.3-85.6% and 37.5-62.5% for *E. tauvina, E. merra* and *E.*

areolatus, respectively. The proportion of polymorphic fragments was high in *E. tauvina* (60.0%), followed by *E. merra* (56.9%) and *E. areolatus* (52.0%). A total of 11 diagnostic markers were detected to be present in both species, *E. merra* and *E. areolatus*, but not in *E. tauvina*. Five fragments (950bp in OPA-02; 550bp and 700bp in OPA16; 600bp in OPA-08; 650bp in OPA-17) were identified as the genus specific markers. Genetic distance among individuals of *E. tauvina* was 0.02-0.30, while that of *E. merra* and *E. areolatus* were 0.14-0.50 and 0.19-0.42, respectively. The genetic relatedness between *E. merra* and *E. areolatus* (0.52) was closer compared with *E. merra* and *E. tauvina* (0.67).