

Genetic Characterization of GI Macro and Freshwater Prawns from Makassar-Sulawesi, Pangkalanbun-Kalimantan, Jambi-Sumatera, Sukabumi-Java using mtDNA CO-I Markers⁴

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Introduction

The giant freshwater prawn (*Macrobrachium rosenbergii*) is a widely distributed indigeneous species in Indonesia. The giant freshwater prawn from Pangkalanbun-Kalimantan has a big head, long claws and is yellow-green in color. On the other hand, those from Kuala Tungkal-Jambi has a small head, short claws and is gold in color (Sabar and Ali, 2001).

The giant freshwater prawn is an important commodity that has been successfully farmed in Indonesia. It has been produced in several areas in West Java, i.e. Ciamis (Tambaksari, Parigi, Rancah and Pasir Nagara) and Tasikmalaya. Some commercial hatcheries (a local government hatchery, and seven private hatcheries) are found in Jogjakarta in East Java, freshwater prawns are cultured in brackishwater ponds. Freshwater prawn culture has also spread to some areas in the island of Bali, e.g. Gianyar, Klungkung, Buleleng and Tabanan, and in Riau, South Sulawesi and South Sumatera.

Several natural populations of freshwater prawns are unique to Indonesia. To date, about 19 species are found in almost all of the islands in the archipelago (Holthuis, 1980). However, this potential genetic resource has not yet been fully used in freshwater aquaculture. Moreover, inspite the fact that the freshwater prawn culture technology has been developed and adopted in Indonesia, some problems are still plaguing the industry. To solve the problems of poor growth rate, disease susceptibility and small meat yield or edible portion, the Research Institute for Freshwater Aquaculture (formerly RIFF) started numerous research programs since 1996 to improve growth rate and the size of the edible portion of cultured prawns.

The GI Macro, a selected strain of the giant freshwater prawn was developed by RIFA and has been distributed to farmers in Java. Varying results were obtained from growth trials conducted in different locations, hence another phase of selection is necessary to construct a wider and more improved base population using additional wild stocks. An assessment of the genetic background of the wild stocks is an important pre-requisite before the new selection program is initiated. Genetic variation is an important parameter to consider to enable the evaluation of individual fitness of the stock in the short term and their survival in the long term (Ferguson *et al.*, 1995). In this study, genetic variation of giant freshwater prawns collected from Sulawesi, Kalimantan, Sumatera and Java were analyzed using molecular markers.

Materials and Methods

Samples of M. rosenbergii

Twenty-seven samples of giant freshwater prawns were collected from three wild stocks (Makassar-Sulawesi, Pangkalanbun-Kalimantan, Sukabumi-Jawa), an unselected hatchery stock (Jambi-Sumatera) and the genetically improved GI Macro.

Whole DNA extraction and amplification of mtDNA CO-I region

Whole DNA extracts were obtained from pleopods of freshwater prawns using the standard phenol-chloroform procedure as follows: 5-10 mg of pleopod was placed into a 1.5 ml vial containing 500 µl DNA



lysis solution and 120 μ l 0.5M EDTA (pH 8.0). Proteinase K (10 μ g/ml) was added onto the vial and then incubated at 37°C for 12 hours. At room temperature, chloroform (500 μ l) was added onto the mixture, after which the solution was kept on ice for five minutes. The solution was then centrifuged at 10,000 rpm for 10 minutes, after which the supernatant is removed and placed into a new 1.5 ml vial. Ammonium acetate (10 μ l) and ethanol (500 μ l) are then added onto the supernatant and the mixture gently vortexed. The DNA pellet was suspended by centrifugation (10 min at 10,000 rpm) and air-dried. Once dry, the pellet is re-suspended with 50-100 μ l Tris-EDTA (TE) buffer and kept at 4 °C before use.

The primers used to amplify the mitochondrial DNA cytochrome oxidase I segment were LCO-1490 (GGT CAA CAA ATC ATA AAG ATA TTG G) and HCO-2198 (TAA ACT TCA GGG TGA CCA AAA AAT CA). This CO-I region was PCR (Polymerase Chain Reaction)- amplified using the following reaction mixture: 10 μ g DNA template, 10 pmol of each primer and “pure Taq DNA ready to go” (Promega) for a total reaction volume of 25 μ l. PCR cycles consist of a denaturation cycle at 95 °C for 2 min, 35 cycles for annealing (95 °C for 1 min, 45 °C for 1 min and 72 °C for 2.5 min), followed by an extension cycle at 72 °C for 10 min. The amplified mtDNA CO-I regions were restricted by seven endonucleases (Hae III, Rsa I, Mbo I, Taq I, Alu I, Sac II and Hin6 I), and separated via electrophoresis on 2-3% agarose gel in Tris-Boric-EDTA (TBE) buffer. The resulting bands were subsequently observed under a UV illuminator.

Data Analysis

mtDNA variation in the five giant freshwater prawn stocks was evaluated by examining the observed haplotypes for each type of restriction endonuclease. These endonuclease-specific haplotypes were further used in scoring the composite haplotypes which comprised the data for statistical analysis. AMOVA (Analysis of Molecular Variance) and Fst value estimations were obtained using the TFPGA (Tools for Population Genetic Analysis) program. Haplotype and genetic diversities were calculated based on the method developed by Nei and Tajima (1981) in estimating levels of genetic variation.

Result and Discussion

The mtDNA CO-I region (700-1500 bp) of giant freshwater prawns can be PCR-amplified. Four restriction endonucleases (Hae III, Rsa I, Mbo I, and Taq I) successfully cleaved the amplified DNA products. Polymorphic patterns were detected using Hae III and Rsa I, while Mbo I and Taq I gave monomorphic results (Table 1). Six and three restriction morphs were obtained using Hae III and Rsa I, respectively. An example of a restriction pattern is shown in Figure 1. The length of the mtDNA CO-I sequence of giant freshwater prawn is similar to the D-loop sequence observed in fish species such as tilapia, kingfish, yellow tail and red sea bream (Nugroho, 2001).

Table 1. Type of enzyme and restriction site of mtDNA CO-I of giant freshwater prawn

Number	Enzyme Type	Restriction Site	Restriction Type
1	Hae III	+	Polymorphic
2	Rsa I	+	Polymorphic
3	Mbo I	+	Monomorphic
4	Taq I	+	Monomorphic
5	Alu I	-	-
6	Sac II	-	-
7	Hin6 I	-	-



Figure 1. Restriction pattern of mtDNA CO-I region using Hae III

Haplotype variation levels differed according to the source of the giant freshwater prawn stocks. In general, twelve composite haplotypes can be identified based on four endonucleases that cleaved the mtDNA CO-I region. Two to five composite haplotypes were observed in most of the stocks. Giant freshwater prawns collected from Makassar-Sulawesi showed two composite haplotypes only, while those from Sukabumi-Java and GI Macro had five composite haplotypes. Haplotype diversity ranged from 0.111 (Makassar-Sulawesi) to 0.280 (Sukabumi-Java, Table 2).

Genetic variability levels (based on the number of haplotypes and haplotype diversity) in the giant freshwater prawn, differ from marine fishes which generally have haplotype numbers and diversity levels ranging from 6-17 and 0.6-0.9, respectively (Nugroho, 2001). However the variability levels are comparable to those of freshwater fishes like tilapia (Nugroho *et al.*, 2002). Relatively low genetic variation in giant freshwater prawn stocks could be due to their relatively limited capability to migrate compared to marine fishes. Low genetic variability of giant freshwater prawns indicate mainly natural selection or genetic drift in these populations.

Some stocks share similar major haplotypes. Sukabumi-Java and GI Macro possess haplotype #1; Sukabumi-Jawa, Pangkalanbun-Kalimantan, and Jambi-Sumatera, haplotype #3; GI Macro, Sukabumi-Jawa, Jambi-Sumatera and Pangkalanbun-Kalimantan, haplotype #5; while giant freshwater prawns from Makassar-Sulawesi have haplotypes #11 and #12 unique to them. Major haplotypes found common in Sukabumi-Java and GI Macro as well as Jambi-Sumatera and GI Macro can be attributed to the fact that they share common ancestries. The Sukabumi-Java stock is comprised of the first GI Macro stock that was developed from three giant freshwater prawn stocks namely, Citanduy, Citarum (West Java), and Musi (Sumatera). There is a high probability that this aforementioned GI Macro stock has interbred with the existing local population in Sukabumi-Java. Giant freshwater prawns from Pangkalanbun-Kalimantan share similar composite haplotypes with Sukabumi-Java, Jambi-Sumatera and GI Macro, however, the Kalimantan stock still possess haplotypes (#8 and #9) unique only to them. This indicates that there are ecological barriers in the migration of this stock.



Table 2. Composite haplotype frequencies of the mtDNA CO-I region in Indonesian stocks of the giant freshwater prawn. The haplotypes resulted from the use of endonucleases Hae III, Rsa I, Mbo I, and Taq I

No	Type of Composite Haplotype	Stock				
		Sukabumi-Java N=5	GI Macro N=5	Pangkalanbun-Kalimantan N=6	Jambi-Sumatera N=5	Makassar-Sulawesi N=6
1	BAAA	0.200	0.400	-	-	-
2	CAAA	0.200	-	-	-	-
3	ABAA	0.200	-	0.333	0.400	-
4	BBAA	0.200	-	-	-	-
5	AAAA	0.200	0.200	0.167	0.400	-
6	BCAA	-	0.200	-	-	-
7	CCAA	-	0.200	-	-	-
8	ACAA	-	-	0.167	-	-
9	DBAA	-	-	0.333	-	-
10	EBAA	-	-	-	0.200	-
11	FBAA	-	-	-	-	0.667
12	FAAA	-	-	-	-	0.333
	No. of Haplotypes	5	4	4	3	2
	Haplotype Diversity (h)	0.280	0.260	0.236	0.200	0.111

Statistically significant genetic differences were noted among the five giant freshwater stocks ($P < 0.05$) based on an AMOVA (Analysis of Molecular Variance). Fst pairwise comparison tests showed that the difference was observed between the giant freshwater prawns from Makassar-Sulawesi and the other four stocks. A significant difference was also detected between giant freshwater prawns from Pangkalanbun-Kalimantan and the GI Macro (Table 3).

Table 3. Pairwise comparison of Fst

Population	Sukabumi-Jawa	GI Macro	Banjarmasin-Kalimantan	Jambi - Sumatera	Makassar-Sulawesi
Sukabumi-Jawa	-				
GI Macro	0.528	-			
Pangkalanbun-Kalimantan	0.2376	0.0272*	-		
Jambi-Sumatera	0.6480	0.0787	0.8161	-	
Makassar-Sulawesi	0.0112*	0.0013**	0.0093**	0.0214*	-

** - significant at level, $P < 0.0$

The observed differences are due to the fact that the giant freshwater prawns from Makassar-Sulawesi have different major composite haplotypes compared to the other populations. This could indicate the possibility that the stock from Makassar-Sulawesi is composed of a different freshwater prawn subspecies due to ecological barriers. De Bruyn *et al.* (2004), confirmed a biographical barrier in giant freshwater prawn populations from Tanah Genting Kra. Differences in the flora and fauna found in this area are explained in terms of the presence of the biogeographic barrier known as the Wallace line. The Wallace line theory may explain why there are differences in the stock structure of the Makassar-Sulawesi prawns (Eastern side of the Wallace line) as compared to the others (Western side).

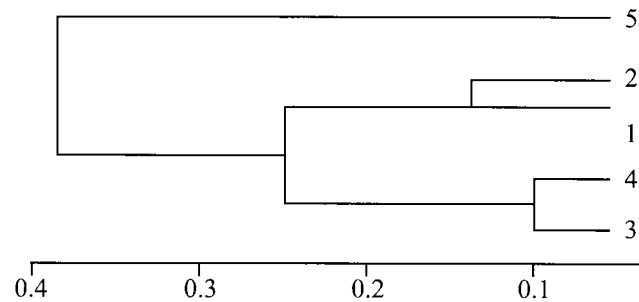
Genetic distance (Nei and Tajima, 1981) based on restriction sites from four endonucleases is listed in Table 4. The average genetic distance among the stocks is estimated at 0.1690. The longest genetic distance was observed between Makassar-Sulawesi and GI Macro stocks, while the shortest distance is between Sukabumi-Jawa and GI Macro stocks.



Table 4. Genetic distance among giant freshwater prawn stocks collected in Indonesia

Population	Sukabumi-Jawa	GI Macro	Banjarmasin-Kalimantan	Jambi - Sumatera	Makassar-Sulawesi
Sukabumi-Java	-				
GI Macro	0.0289	-			
Pangkalanbun-Kalimantan	0.0828	0.2340	-		
Jambi-Sumatera	0.0447	0.2147	0.0131	-	
Makassar-Sulawesi	0.2325	0.3628	0.2565	0.2655	-

A dendrogram based on the genetic distance data showed three clusters founding the giant freshwater prawn stocks examined. The first group is composed of giant freshwater prawns from Sukabumi-Java and the GI Macro. Pangkalanbun-Kalimantan and Jambi-Sumatera stocks form the second cluster while the giant freshwater prawns from Makassar-Sulawesi are uniquely separated from the other groups (Figure 2). The close relationship between GI Macro and Sukabumi-Java may indicate the possibility of the occurrence of gene flow between the two stocks as the GI Macro was disseminated to the farmers in Sukabumi-Java in 2001. On the other hand, the Jambi-Sumatera and Pangkalanbun-Kalimantan stocks lumping in one cluster may be due to a correlation between biological variability and earth history (de Bruyn *et al.*, 2004).



Note: 1 = Sukabumi-Java
2 = GI Macro
3 = Pangkalanbun-Kalimantan
4 = Jambi-Sumatera
5 = Makassar-Sulawesi

Figure 2. UPGMA dendrogram showing the relationship between the five giant freshwater prawn stocks from Indonesia

The Makassar-Sulawesi giant freshwater prawns are phenotypically better than the other stocks as they are relatively bigger than the prawns from the other stocks. It has been noted that the average body weight of Makassar-Sulawesi prawns is about 83g (Ali *et al.*, unpublished). Taking into consideration the level of genetic variability of the Makassar-Sulawesi stock based on the composite haplotype, this stock is an inbred line. In spite of the fact that the Makassar-Sulawesi is inbred, considering that the stock is phenotypically larger than the others, it can be used for outcrossing. Hybridization of the Makassar-Sulawesi with the other stocks may be an effective alternative breeding program in improving the quality of giant freshwater prawn seedstocks in Indonesia.

Conclusion

Significant genetic differences among giant freshwater prawn from Makassar-Sulawesi and the other stocks were observed. The average genetic variability based on composite haplotypes is 0.217, with an average genetic distance of 0.169. Giant freshwater prawns from Makassar-Sulawesi can be used for outcrossing during the next phase of the breeding program for giant freshwater prawns in Indonesia.



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