APPLICATION OF MICROSATELLITE DNA MARKERS FOR GENETIC DIVERSITY ANALYSIS IN WILD AND DOMESTICATED STOCK OF *Macrobrachium rosenbergii*

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DISSERTATION SUBMITTED IN FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF MASTER OF BIOTECHNOLOGY

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Abstract

Ten microsatellite loci (*Mbr*-1, *Mbr*-3, *Mbr*-4, *Mbr*-5, *Mbr*-7, *Mbr*-8, *Mbr*-10a, *Mbr*-10b, *UVC*-807 and *UVC*-817) were used to assess the genetic diversity and makeup of different populations of *Macrobrachium rosenbergii* in Malaysia. A total of 120 individuals from four hatcheries and 116 individuals from four wild populations were studied. The samples were Sarawak River (SRWK), Terengganu River (TRGN), Timun River (NSBL), Kedah River (KDH), Thailand (THAI), National Prawn Fry Production and Research Centre (NAPFRE), Mun's Aquaculture (HatA) and Wong's Aquaculture (HatB). Allele sizes of all samples were scored using the ABI 3100 Genetic Analyzer. A total of 161 alleles were detected at 10 loci across the eight populations. All domesticated and wild populations demonstrated relatively high genetic variation with an average of 9.00 to 13.10 alleles per locus (*A*) and observed heterozygosities (H_o) ranging from 0.5833 to 0.8000. All the studied populations deviated from Hardy–Weinberg equilibrium proportions at a number of loci. Genetic distance computed by Nei (1978) denotes that the shortest genetic distance was between KDH and NSBL (*D*=0.2087) while the greatest was between HatA and NAPFRE (*D*=0.9333). Analysis of molecular variance (AMOVA) revealed significant differentiations in all populations with moderate F_{ST} values of between 0.0311 and 0.1438. Phylogenetic analysis depicted a close genetic relationship of *M. rosenbergii* between HatB and NSBL while samples from HatA diverged and was not clustered with any populations though the three populations originated from the same area. Microsatellites analysis revealed high levels of genetic variation and genetic differentiation among all the populations tested. Regular monitoring on genetic diversity and structure of *M. rosenbergii* is needed for genetic improvement and management programmes.

Abstrak

Sepuluh lokus mikrosatelit (*Mbr*-1, *Mbr*-3, *Mbr*-4, *Mbr*-5, *Mbr*-7, *Mbr*-8, *Mbr*-10a, *Mbr*-10b, *UVC*-807 and *UVC*-817) telah digunakan untuk menilai kepelbagaian dan susunan genetik *M. rosenbergii* di Malaysia. Sejumlah 120 individu dari empat pusat pembenihan dan 116 individu dari populasi liar telah dikaji. Sampel-sampel ini adalah SRWK, TRGN, NSBL, KDH, THAI, NAPFRE, HatA dan HatB. Saiz alel bagi semua sampel ditentukan dengan menggunakan ABI 3100 Genetic Analyzer. Sebanyak 161 alel telah dikenalpasti pada sepuluh lokus tersebut menerusi semua populasi. Kesemua populasi liar dan domestikasi menunjukkan tahap variasi genetik yang tinggi dengan purata alel per lokus (*A*) adalah dari 9.00 hingga 13.10 manakala nilai heterozigositi cerapan (H_o) adalah dari 0.5833 hingga 0.8000. Kesemua populasi didapati menyimpang dari HWE pada beberapa lokus. Tahap jarak genetik tertinggi dicatatkan di antara populasi domestikasi iaitu di antara sampel HatA dan NAPFRE (*D*=0.9333) manakala nilai renggang genetik terendah direkod di antara sampel KDH dan NSBL (*D*=0.2087). Perbezaan genetik yang signifikan telah dikesan dalam semua populasi melalui analisis molekular varians (AMOVA) dengan nilai F_{ST} diantara 0.0311 dan 0.1438. Kedua-dua sampel ini adalah dari populasi liar. Analisis filogenetik menunjukkan hubungan genetik yang sangat rapat di antara HatB dan NSBL manakala sampel HatA mencapah dan tidak membentuk kluster dengan mana-mana populasi walaupun ketiga-tiga populasi ini adalah dari kawasan yang sama. Analisis mikrosatelit menunjukkan tahap variasi dan perbezaan genetik yang tinggi di kalangan semua populasi yang dikaji. Pemantauan secara berterusan terhadap diversiti dan struktur genetik *M. rosenebrgii* adalah perlu untuk program penambahbaikan genetik dan pemeliharaan.

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LIST OF ABBREVIATIONS

A	allele number
AFLP	amplified fragment length polymorphism
AMOVA	analysis of molecular variance
bp	base pair
dATP	2'-deoxyadenosine 5'-triphosphate
dCTP	2'-deoxycytidine 5'-triphosphate
dGTP	2'-deoxyguanosine 5'-triphosphate
dNTP	deoxyribonucleotide
dTTP	2'-deoxythymidine 5'-triphosphate
ddH_2O	deionized distilled water
DNA	deoxyribonucleic acid
D	genetic distance
FAO	Food and Agriculture Organisation
F_{is}	absolute value of inbreeding coefficent
F_{ST}	pairwise genetic differentiation
GDA	Genetic Distance Analysis
g	gram
g	force of gravity
H_e	expected heterozygosity
H_o	observed heterozygosity
HWE	Hardy-Weinberg equilibrium
LD	linkage disequilibrium
$MgCl_2$	magnesium chloride
min	minutes
mtDNA	mitochondrial DNA
N_e	effective number of alleles
PHYLIP	Phylogeny Inference Package
PCR	polymerase chain reaction
RAPD	random amplified polymorphic DNA
RFLP	restriction fragment length polymorphism
s	seconds
T_A	annealing temperature
TBE	Tris-borate-EDTA acid
UPGMA	unweighted pair group method with arithmetic averaging