UNIVERSITI PUTRA MALAYSIA

Population characterization of Macrobrachium rosenbergii De Man USING EST-SSR MARKERS in WESTERN PENINSULAR MALAYSIA

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POPULATION CHARACTERIZATION of *Macrobrachium rosenbergii* De Man USING EST-SSR MARKERS in WESTERN PENINSULAR MALAYSIA

By

ATIN KHALAJ HEDAYATI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of the Requirements for the Degree of Master of Science

July 2014

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This thesis is dedicated to

my lovely parents

(Mohammad Javad Khalaj Hedayati and Talayeh Etemadzadeh)

for their love, endless support and encouragement.

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

POPULATION CHARACTERIZATION of *Macrobrachium rosenbergii* De Man USING EST-SSR MARKERS in WESTERN PENINSULAR MALAYSIA

By

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July 2014

Chair: Annie Christianus, PhD
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The purpose of current study is to illustrate the utility of EST-derived SSR in characterizing wild populations of *Macrobrachium rosenbergii* in Malaysia's rivers. A novel set of EST-SSR was validated in a full panel of 120 samples from four wild populations through Polymerase Chain Reaction (PCR). Seven EST-SSR loci were identified, characterized, and evaluated on 30 individuals each from the populations namely Sg. Tapah (Perak), Sg. Timun (Negeri Sembilan), Sg. Bernam (Selangor) and Sg. Johor (Johor). The average polymorphic informative content value (PIC) for these seven primers was found to be 0.5355 indicating considerable degree of polymorphism with number of alleles detected ranged from 5 to 17. The observed heterozygosity value count during multi-population analyses ranged from 0.3668 to 0.4554, whilst the expected ranged from 0.5192 to 0.5700. There was linkage disequilibrium (LD) observed between 2 pairs of EST-SSRs loci. All loci have deviation from the Hardy-Weinberg equilibrium (HWE) except EST-Mr-AS-31957, suggesting factors violating the neutral expectation such as selection and non random mating. The F_{IS} index demonstrated indication of inbreeding among individuals of each population. There was evidence that all samples from four sampling sites assessed in this study are drawn from four clusters (k=4). Estimate of fixation index value in pairwise comparisons among the four localities revealed very low magnitude of differentiation (R_{ST} ranged between 0.0000 to the highest of 0.17918).

The findings of this study suggested that intra-specific diversity that occurs between studied populations were not extremely high, as very low variation was detected in pairwise comparisons and genetic structuring analyses. The ongoing gene flow either naturally or via translocations by humans are possible reasons for the low magnitude of genetic differentiation. The overall results suggest that all populations (Tapah, Timun, Bernam, and Johor) were composed of one large possible panmictic population for management purposes at present.

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Also the results indicated that these polymorphic EST-SSR derived from *M. rosenbergii* would be useful for population genetic structure analysis and genetic diversity assessment in prawn populations as part of management policies of natural resources to ensure sustainability of wild broodstock for future development of prawn culture industries.

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Abstrak tesis ini dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Science

PENCIRIAN POPULASI *Macrobrachium rosenbergii* De Man MENGGUNAKAN PENANDA EST-SSR UNTUK DI SEMENANJUNG MALAYSIA

Oleh

ATIN KHALAJ HEDAYATI

Jului 2014

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Kajian ini bertujuan untuk menunjukkan penggunaan EST dari SSR dalam pencirian populasi liar *Macrobrachium rosenbergii* di sungai di Malaysia. Satu set EST-SSR yang novel disahkan dengan 120 sampel panel penuh dari empat populasi liar melalui Reaksi Rantai Polimerase (PCR). Tujuh loci EST-SSR dikenalpasti, dicirikan dan dinilai untuk 30 individu dari setiap populasi iaitu Sg. Tapah (Perak), Sg. Timun (Negeri Sembilan), Sg. Bernam (Selangor) dan Sg. Johor (Johor). Nilai purata kandungan maklumat polimorfik (PIC) untuk tujuh primer ini adalah 0.5355, menunjukkan terdapatnya tahap polimorfisma dengan bilangan alel yang dikesan adalah di antara 5 hingga 17. Pengiraan nilai heterozigositi yang didapati semasa analisi multi populasi adalah dari 0.3668 hingga 0.4554, manakala julat yang dijangka dari 0.5192 hingga 0.5700. Terdapat ketidak-seimbangan hubungan (LD) yang didapati adalah di antara 2 pasang loci EST-SSR. Semua loci tersisih dari keseimbangan Hardy-Weinberg (HWE) kecuali EST-Mr-AS-31957, ini mencadangkan faktor yang bercanggah ke atas jangkaan neutral seperti pemilihan dan pembiakan tidak rawak. Indeks F_{IS} menunjukkan pembiakan sesama sendiri di antara individu di dalam setiap populasi. Bukti menunjukkan bahawa semua sampel dari empat lokasi persampelan yang dinilai dalam kajian ini adalah berasal dari empat kluster (k=4). Anggaran nilai indeks penetapan dalam perbandingan berpasangan di antara empat lokasi menunjukkan jarak pembezaan yang sangat rendah (R_{ST} di antara 0.0000 hingga paling tinggi 0.17918).

Hasil kajian ini mencadangkan bahawa kepelbagaian intra-spesifik di antara populasi yang dikaji adalah tidak terlalu tinggi, kerana di mana variasi yang sangat rendah dikesan dalam perbandingan berpasangan dan analisis penstrukturan genetik. Aliran gen yang ada yang berterusan samada secara semulajadi atau melalui translokasi oleh manusia adalah sebab yang mungkin untuk jarak pembezaan genetic yang rendah. Keputusan keseluruhan mencadangkan bahawa semua populasi (Tapah, Timun, Bernam, dan Johor) adalah terdiri dari satu populasi panmiktik yang besar untuk tujuan pengurusan pada masa sekarang.

Keputusan juga menunjukkan bahawa polimorfik EST-SSR yang didapati dari *M.*

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rosenbergii berguna untuk analisis struktur populasi genetik dan penilaian kepelbagaian genetik dalam populasi udang sebagai sebahagian daripada polisi pengurusan sumber semulajadi untuk memastikan kemampanan induk liar demi perkembangan industri pengkulturan udang di masa akan datang.

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I certify that a Thesis Examination Committee has met on 10 July 2014 to conduct the final examination of Atin Khalajhedayati on her thesis entitled "Population Characterization of *Macrobrachium rosenbergii* De Man using EST-SSR Markers in Western Peninsular Malaysia" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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