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MOLECULAR GENETIC ANALYSIS FOR WILD POPULATIONS DIVERSITY OF GIANT FRESHWATER PRAWN (*MACROBRACHIUM ROSENBERGII*) IN SOUTH-EAST ASIA USING MICROSATELLIT MARKERS (SSR)

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ABSTRACT

Culture of giant freshwater prawn (*Macrobrachium rosenbergii*) represents one of the important export items of aquaculture industry in South-East Asia. In the present study, genetic diversity of five wild populations of giant freshwater prawn collected from five countries in South-East Asia (Bangladesh, Thailand, Indonesia, Malaysia and Vietnam) were assessed using six microsatellite markers (SSR). A total of 123 alleles were detected over all six microsatelite loci with molecular sizes ranging between 137 bp and 349 bp. All studied populations exhibited relatively high genetic diversity with an average of 10.33 to 15.17 alleles per locus, an average of expected heterozygosity at all loci from 0.747 to 0.883 and fixation index (F_{IS} value) ranged from 0.126 to 0.390 with an average of 0.277. Dendrogram according to Nei's genetic distance separated the studied populations into three clusters. First cluster included Thailand and Malaysia populations while Bangladesh and Vietnam populations were grouped together in the second cluster and Indonesia population was separated in the third cluster. Hence, the results indicate that Indonesia population possessed high level of genetic differentiation than of the other studied populations. The results could be very useful for stock management, selective breeding programs and for the biodiversity conservation of wild resources.

Keywords: *Macrobrachium rosenbergii*, genetic diversity, microsatellite markers, giant freshwater prawn.

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INTRODUCTION

The giant freshwater prawn (*Macrobrachium rosenbergii*) is one of the widely cultured freshwater prawn species all over the world. Production of wild stocks increased from 5246 ton in 1984 to approximately 130,000 ton in 1998 (FAO, 2000) and Asia accounts for more than 98% of global production. Culture of this species has expanded rapidly not only within Asia but also in regions far remote from the natural distribution of the species and is now cultured in at least 43 countries across five continents (FAO, 2000). Freshwater aquaculture has improved rapidly in the Asia-pacific region over the past three decades, both in terms of size of the industry and the diversity of cultivated species (Mather and De-Bruyn, 2003). Three distinct forms of *Macrobrachium rosenbergii* have been recognized; an "eastern", a "western" and an "Australian" form, based on the morphology, allozymes and mitochondrial DNA (De-Bruyn *et al.*, 2004). Due to its commercial importance Macrobrachium species have been extensively studied for their physiology and behavioral patterns. It has been established that Macrobrachium is not monophyletic (Murphy and Austin, 2002) and the genus should be taxonomically subdivided (Jayachandran, 2001).

Information on genetic diversity is a basic requirement for any stock identification, stock enhancement and breeding program for improvement of any aquatic species (Tassanakajon *et. al*, 1997). Though there is a little is known about the level and pattern of genetic diversity of the wild or cultured stocks of *Macrobrachium rosenbergii* (Mather and De-Bruyn, 2003). Molecular analyses are very important to know the population structure of wild and captive stocks so as to gainfully apply the information for genetic improvement programs (Mather and De-Bruyn, 2003). Molecular genetic approaches to resolving systematic questions in Macrobrachium have only been applied recently, when Murphy and Austin (2002) recognized that species and genus level designations did not correspond to traditional morphology based classification schemes.

The application of genetic markers has allowed rapid progress in aquaculture investigations. These studies included parentage

assignments, genetic variability and inbreeding, species and strain identification and the construction of high-resolution linkage maps for aquaculture species (Liu and Cordes, 2004). Molecular markers could provide a method to assist the selection of individuals in prawn-breeding programs, which would facilitate the domestication of the species.

Previous studies of genetic variation among wild populations of *Macrobrachium rosenbergii* based on allozyme markers suggested low genetic diversity and failed to detect population differentiation (Sudsuk and Sodsuk, 1998). In this study, microsatellite markers (SSR) were chosen because they were capable of resolving low genetic differentiation which was un-resoled by allozymes (Liu and Cordes, 2004). Microsatellite markers, also known as simple sequence repeats or SSRs, are clusters of short (usually 2 to 6) tandemly repeated nucleotide bases distributed throughout the genome (Wang *et. al*, 1994). Microsatellite markers distinguish themselves as co-dominant, multiallelic, highly polymorphic genetic markers, requiring small amounts of DNA for straightforward PCR and gel electrophoresis analysis.

Genetic studies on *Macrobrachium rosenbergii* using DNA markers are very rare. A few reports on the development and characterization of a few microsatellite loci from *Macrobrachium rosenbergii* are available (Chareontawee *et al.*, 2006). However no details are available on the genetic diversity of cultured and wild stocks in different regions or countries (Chareontawee *et al.*, 2007). Therefore, the objective of this study was to investigate genetic diversity among five wild populations of *Macrobrachium rosenbergii* using six microsatellite markers (SSR).

MATERIALS AND METHODS

Sample Collections

Freshwater giant prawns; *Macrobrachium rosenbergii* were collected in 2007 from five natural sources from five countries in South-East Asia (Table 1 and Fig. 1). The collection mission was carried out by the team work of molecular population genetic Lab., School of Natural Resource Sciences, Queensland University of Technology (QUT), Brisbane, Queensland, Australia. Tail muscle tissues of the collected samples were removed and stored in 95% ethanol for genetic analyses.

Table 1. Freshwater giant prawn sample collection

Collection sites	Sample name
Meghna River, Bangladesh	Ban
Tapi River, Thailand	Tha
Bengawan River, Java, Indonesia	Ind
Bahand River, Peninsula, Malaysia	Mal
Mekong River, Vietnam	Vie

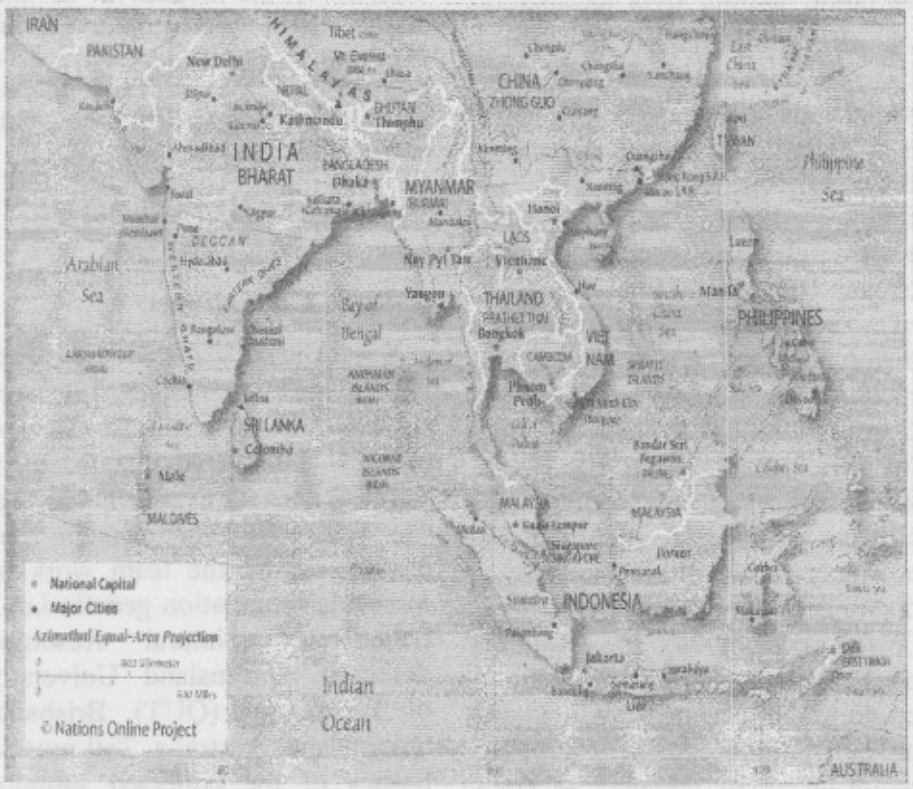

Fig. 1. Map showing sampling sites of five freshwater giant prawn populations

DNA Extraction, PCR Reaction and Screening for Variation

This study was conducted in 2008 during my Postdoctoral fellowship at School of Natural Resource Sciences, Queensland University of Technology (QUT), Brisbane, Queensland, Australia. Total genomic DNA was extracted from 30 individuals of each studied population using a modified salt extraction method (Miller *et al.*, 1988). PCR were carried out with six primers that are specific for six microsatellite loci. The codes, sequences and annealing temperatures of these primers are shown in Table 2. PCR reaction consisted of 2.5µl Roche 10X buffer, 0.5µl 25mM Fisher MgCl2, 1µl Roche deoxy nucleotide triphosphate (dNTP), 0.5µl of each 10mM forward and reverses primers, 0.2µl Roche Taq DNA polymerase, 1µl DNA template (~50ng/µl) to a final volume of 25µl with 16µl ddH_2O. PCR conditions were; 5min at 94◦C for initial denaturation, then 30 cycles of 30s at 95◦C, 30s at relevant annealing temperature, 30s at 72◦C and a final extension step at 72◦C for 8min. Microsatellite polymorphisms were analysed on a Gelscan 2000 System (Corbett Research) in 5% acrylamide gels and run according to the manufacturer's instructions. A 50 –350 bp size standard (Tamra 350) was run at both ends of each gel and in one additional lane to determine allele size. Microsatellite allele sizes were scored using One-D-scan 2.05.

Statistical Analysis

Microsatellite data were checked for presence of null alleles, large allele dropout or errors in scoring due to stutter bands using Micro-checker software version 2.2.3 (Oosterhout *et al.*, 2004). These data were analyzed as diploid data by standard POPGENE (version 1.31), which is a Microsoft windows-based freeware program for population genetic analysis (Yeh, *et al.*, 1999). The estimated parameters were: Allelic frequencies, mean number of alleles per locus (n_a), Chi-square test for Hardy-Weinberg expectations (HWE), Genetic Variation Statistics (Nei, 1987), Heterozygosity Statistics (Nei, 1973), fixation index (F_{is}) as a measure of heterozygote deficiency (Wright, 1978), neutrality test according to Ewens-Watterson test using the algorithm presented by Manly (1985), genetic distance and identity (Nei, *et al.*, 1983). Dendrogram was constructed based on Nei's genetic distances using UPGMA (Unweighted Pair-Group Method of Analysis).

Table 2. The codes, sequences, annealing temperatures and repeat sequence of the used primers

Primer code	Primer sequence	Annealing temperature	Repeat sequence
Mbr-02	F: 5CC CAC CAT CAA TTC TCA CTT ACC R: TCC TTT TCA CAT CGT TTC CCA GTC	62°C	$(GT)_{22}$
Mbr-03	F: 5CA ACT CTA TGT TTC GGC ATT TGG R: GGG GAA TTT TAC CGA TGT TTC TG	60°C	$(AG)_{14}$
Mbr-08	F: 5AA CCA GCC GAC TTA GAC TGT GC R: CGC CAT TTG CGT CTA TCT CTT AC	60°C	$(AGC)_6(AG)_5AA(AG)_4$
Mbr-10	F: 5AT GAC GAT GAT GAG GAA TGA AGC R: TTT CAG GCT ATA TCA AGC AAC AG	62°C	$(ATG)_3A(ATG)_4$
UVC-814	F: 5CA TTT GGC ACA GTG ACG TTC R: CGT CAT TTG CTT AAT GAA TAC TGT TC	57°C	$(GA)_{19}$
UVC-817	F: 5AT GGC CAA GAT GAA AGA TGC R: CTG TCT GTA CCG CAG TCG AA	58°C	$(CT)_{20}$

RESULTS AND DISCUSSION

A total of 123 alleles were detected over all six microsatelite loci, with molecular sizes ranging (R) between 137 bp and 349 bp and average of allele frequencies (F) ranging between 0.038 and 0.100 (Table 3 and Fig. 2). Most of allele frequency values were different among the studied populations and the small values of allele frequencies reflect high level of polymorphism. Allele frequency is a fundamental parameter in the study of evolution because the genetic changes of population are usually described by the changes in allele frequencies (Nei and Kumar, 2000).

Significance of differences between expected and observed allele frequencies were assessed by homogeneity test which depended on performing Chi-square test for the correspondence between observed and expected allele frequencies for each allele per locus and overall populations. All obtained Chi-square values were highly significant with $p < 0.001$ (Table 4), indicating real differences in allele frequencies in the studied populations.

Table 3. Genetic polymorphism at six microsatellite loci in the five populations including sample size (N), observed number of alleles (n_a), allelic size range in bp (R) and average of allele frequency (F)

Population (N)	Locus						Across loci
	Mbr-02	Mbr-03	Mbr-08	Mbr-10	UVC-814	UVC-817	
Ban (30)							
n_a	14	17	5	6	19	20	81
R	303-349	232-270	252-270	152-170	160-204	168-212	152-349
F	0.071	0.059	0.200	0.167	0.053	0.050	
Tia (30)							
n_a	20	20	9	5	16	21	97
R	301-349	224-276	248-268	152-170	160-194	166-212	152-349
F	0.050	0.050	0.111	0.200	0.063	0.048	
Ind (30)							
n_a	14	6	6	7	20	20	73
R	299-341	222-272	252-270	143-170	146-210	168-212	143-341
F	0.071	0.167	0.167	0.143	0.050	0.050	
Mal (30)							
n_a	11	15	5	4	11	16	53
R	297-349	220-274	254-272	137-170	172-194	176-212	137-349
F	0.091	0.067	0.200	0.250	0.091	0.063	
Vie (30)							
n_a	15	22	5	6	15	19	82
R	299-349	222-274	256-274	140-170	152-200	174-214	140-349
F	0.067	0.045	0.200	0.167	0.067	0.053	
All populations (150)							
n_a	24	24	14	10	26	25	137
R	297-349	220-276	248-274	137-170	146-204	166-214	137-349
F	0.042	0.042	0.071	0.100	0.038	0.040	

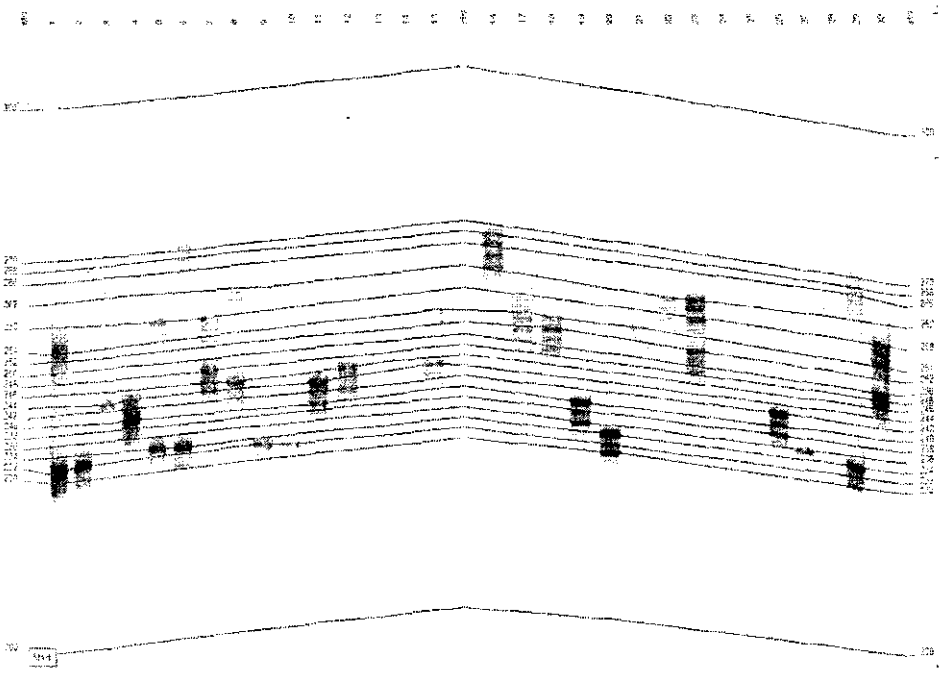

Fig. 2. Results of SSR amplification based on the use of Mbr-03 locus with Bangladesh population using Gelscan 2000 System and One-D-scan 2.05

Table 4. Homogeneity test for correspondence between observed and expected allele frequencies for each allele per locus overall populations

Overall populations-locus	Mbr-02	Mbr-03	Mbr-08	Mbr-10	UVC-814	UVC-817
Chi-square	329.036	158.998	313.714	445.868	233.970	151.718
Degree of freedom	92	92	52	36	100	96
Probability	0.000000	0.000018	0.000000	0.000000	0.000000	0.000252
Homogeneity of allele frequency	Not homogenous	Not homogenous	Not homogenous	Not homogenous	Not homogenous	Not homogenous

Genetic diversity of studied populations was estimated as heterozygosity values (Nei, 1973). The observed heterozygosity (H_o) across all loci ranged from 0.286 (locus Mbr-03 in Ind-population) to 0.929 (locus UVC-817 in Mal and Vie populations). The overall mean H_o (considering the studied populations as one large population) was 0.644±0.132 (Table 5). Nei's expected heterozygosity (genetic diversity: H_e) ranged from 0.413 (locus Mbr-08 in Vie-population) to 0.955 (locus UVC-814 in Ind-population). The mean of overall Nei's H_e (across all loci) was 0.890±0.089 (Table 5).

A precise view over Table 5 with comparing observed heterozygosity (H_o) against expected heterozygosity (Nei's H_e) leads simply to remark a large difference between them either at the population level (mean across all loci) or at the locus level (average across all populations). Where, observed heterozygosity (H_o) values were lower than expected ones (H_e) in most populations except that from Mal-population in locus Mbr-08 and Vie-population in locus Mbr-10. These differences suggest deviation from Hardy-Weinberg equilibrium (H.W.E) proportions and indicate Hardy-Weinberg disequilibrium (H.W.D) where it appears that this deviation could be due to heterozygote deficiency.

Hardy-Weinberg equilibrium (H.W.E) in the studied populations were assessed by testing the degree of correspondence between the observed proportions of genotypic frequencies and that expected from allele frequencies using Chi-square test. Significant departures from Hardy-Weinberg expectations ($p< 0.05$) were observed in 21of 30 (six loci x five populations) single locus exact tests with indicating the absence of H.W.E at each investigated locus in the studied populations (Table 5). The genotypic distribution at all loci deviated significantly from H.W.E in at least one population. Departures from H.W.E at locus UVC-814 were observed in all studied populations.

Fixation index (F_{IS}) as a measure of heterozygote deficiency or excess (Wright 1978) was calculated for all alleles per locus and was found to have positive values at all investigated loci in all studied populations except for Mbr-08 and UVC-817 loci in Mal-population and Mbr-10 in Vie-population (Table 5).

Table 5. Genetic variability at six microsatellite loci in five populations including sample size (N), observed heterozygsity (H_o), expected heterozygsity (H_e), fixation index (F_{is}) and P value for test of HW equilibrium (HW)

Population (N)	Locus						Average across loci
	Mbr-02	Mbr-03	Mbr-08	Mbr-10	UVC-814	UVC-817	
Ban (30)							
H_o	0.724	0.760	0.556	0.464	0.800	0.862	0.694±0.152
H_e	0.883	0.922	0.594	0.560	0.932	0.923	0.819±0.176
F_{is}	0.180	0.176	0.065	0.185	0.142	0.066	0.136
HW	0.106	0.107	0.269	**0.033**	**0.025**	**0.025**	
Tia (30)							
H_o	0.750	0.792	0.667	0.536	0.423	0.700	0.645±0.140
H_e	0.942	0.953	0.791	0.728	0.935	0.948	0.883±0.098
F_{is}	0.190	0.152	0.143	0.251	0.539	0.249	0.254
HW	**0.000**	**0.001**	**0.050**	0.078	**0.000**	**0.000**	
Ind (30)							
H_o	0.551	0.286	0.379	0.367	0.750	0.733	0.511±0.199
H_e	0.895	0.868	0.701	0.670	**0.955**	0.948	0.840±0.124
F_{is}	0.373	0.646	0.450	0.444	0.201	0.213	0.388
HW	**0.000**	**0.000**	**0.000**	**0.027**	**0.005**	**0.001**	
Mal (30)							
H_o	0.655	0.897	0.679	0.448	0.448	**0.929**	0.676±0.208
H_e	0.764	0.920	0.580	0.504	0.783	0.933	0.747±0.175
F_{is}	0.128	0.009	**-0.192**	0.095	0.417	**-0.013**	0.074
HW	**0.031**	**0.043**	**0.000**	0.872	**0.000**	0.383	
Vie (30)							
H_o	0.679	0.714	0.346	0.750	0.407	**0.929**	0.638±0.220
H_e	0.901	0.953	**0.413**	0.615	0.925	0.947	0.792±0.225
F_{is}	0.233	0.237	0.146	**-0.245**	0.551	0.002	0.154
HW	**0.000**	**0.000**	0.713	0.966	**0.000**	0.261	
All populations (150)							
H_o	0.671	0.761	0.529	0.504	0.571	0.828	0.644±0.132
H_e	0.937	0.950	0.736	0.829	0.940	0.950	0.890±0.089
F_{is}	0.281	0.195	0.279	**0.390**	0.390	**0.126**	0.277
HW	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	**0.000**	

Considering the studied populations as one large population (overall populations), the F_{IS} index ranged from 0.126 to 0.390 with an average of 0.277. These positive values indicate high degree of heterozygote deficiency of about 27.7% as an average. The heterozygote deficiency could have resulted from null alleles or small sample size. However, none of our samples showed indication of null alleles at the two loci (Mbr-08 and Mbr-10).

Genetic polymorphism in the studied populations was measured as the mean number of alleles per locus, per population and overall populations (Cavalli-Sforza and Bodmer, 1971) (Table 6). The lowest number of alleles per locus was (n_a= 4 alleles) at locus Mbr-10 in Mal-population and the highest number was (n_a = 20 alleles) at locus Mbr-03 in Vie-population. All studied populations exhibited relatively high genetic variation and were similar with average number of alleles per locus varied from 10.33±4.96 in May-population to 15.17±6.67 in Tia-population. At the overall level (considering the studied populations as one large population), the actual number of alleles (n_a) was higher (ranged from 10 to 26 with mean of 20.50±6.75 across all loci) in comparison with its values at sub-population level reflecting higher number of allelic forms.

Effective number (n_e) of alleles (Kimura and Crow 1964) was also estimated. Table 6 indicated that the lowest (3.73) and the highest (9.38) n_e-values were found at the same loci and populations as their counterparts of n_a-values. Besides, they fluctuated across all loci and populations and overall populations taking the same trend as actual number of alleles (n_a).

Shannon's Information index ($I \geq 0$) as a measure of genetic diversity (Shannon and Weaver, 1949) was different clearly from zero ($I > 0$) and had mean values (averaged across all loci) ranged from 1.78 in Mal-population to 2.35 in Tia-population and 2.61 in overall populations (Table 6). Those values reflect high amounts of genetic diversity. That the n_a, n_e, and Shannon's information index (I) estimates gave values consistent with Nei's H_e values (Nei's genetic diversity) as they show a similar trend in reflecting genetic variability in the studied populations.

The presence of selection in the studied populations was assessed

by Ewens-Watterson neutrality test (Manly, 1985) which depend on the estimation of observed homozygosity (Obs. F) and comparing it with the expected homozygosity under neutrality conditions (neutrality means the absence of selection due to equivalent preferability of all genotypes or by other mean: identical relative fitness of genotypes) or with the confidence limits. The observed homozygosity values per locus per population (Table 7) were all laying between the upper and lower 95% confidence limits indicating that all studied loci were neutral against selection except for locus UVC-814 in Ban, Tia and Ind populations, and locus UVC-817 in Mal and Vie populations. The observed homozygosity values per locus per overall populations (Table 8) were not laying between the upper and lower 95% confidence limits indicating that all studied loci at the level of overall populations were not neutral against selection except for locus Mbr-08. Neutrality that was found at these loci at the level of subpopulations indicates that the "majority of molecular variants that become polymorphic are affected primarily by the interplay of mutations (which generates new variation) and genetic drift leading to the eventual fixation of these variants" (Hedrick, 2000).

Nei's genetic identity was estimated for each population pair (Table 9). The highest value (0.6772) was recorded between Tia-Mal population pair while the lowest value (0.3000) was recorded between Ind-Ban population pair. These small genetic identity values in Ind-Ban, Ind-Mal and Ind-Vie population pairs indicate large differences (dissimilarity) among Ind-population and these populations despite of their being subpopulations of the same species.

Nei's genetic distance among studied populations was calculated depending on genetic identity and will reflect the same discrepancies as with genetic identity. It has the same trend as genetic identity and this will be similarly as just mentioned before. The highest value (1.0000) was recorded in Ind-Ban and Ind-Vie population pairs while the lowest value (0.3899) was recorded between Tia-Mal population pair (Table 9).

The UPGMA-Dendrogram was constructed according to Nei's

genetic distance (Nei, 1978) and separated the studied populations into three clusters (Fig 3). First cluster included Tia and Mal populations while Ban and vie populations were grouped together in the second cluster and Ind-population was separated in the third cluster. Hence, the results indicate that Ind-population show high level of genetic differentiation than of the other studied populations. The results could be very useful for stock management, selective breeding programs and for the biodiversity conservation of wild resources.

Table 6. Observed number of alleles (n_a), effective number of alleles (n_e) and Shannon's Information index (I) at the level of each population and overall populations

Locus	Population															Overall populations		
	Bang			Tlay			Indo			Maly			Viet					
	n_a	n_e	I	n_a	n_e	I	n_a	n_e	I	n_a	n_e	I	n_a	n_e	I	n_a	n_e	I
Mbr-02	14	8.54	2.34	20	13.40	2.78	14	8.29	2.36	11	4.01	1.82	15	8.66	2.42	24	15.14	2.93
Mbr-03	17	12.89	2.70	20	14.96	2.82	6	5.16	1.71	15	10.44	2.48	22	15.52	2.90	24	18.45	3.02
Mbr-08	5	2.46	1.10	9	4.50	1.73	6	3.21	1.39	5	2.32	1.01	5	1.68	0.80	14	3.75	1.76
Mbr-10	6	2.32	1.19	5	3.50	1.35	7	2.93	1.40	4	1.98	0.84	6	2.52	1.16	10	5.76	1.90
UVC-814	19	14.75	2.81	16	12.07	2.61	20	16.16	2.88	11	4.34	1.89	15	10.80	2.51	26	15.72	2.97
UVC-817	20	13.04	2.75	21	14.63	2.86	20	14.63	2.82	16	11.97	2.62	19	14.39	2.78	25	18.72	3.05
Mean	13.50	9.00	2.14	15.17	10.51	2.35	12.17	8.40	2.09	10.33	5.85	1.78	13.67	8.93	2.10	20.50	12.92	2.61
St.D	6.53	5.51	0.79	6.67	5.15	0.65	6.77	5.77	0.68	4.96	4.28	0.73	6.86	5.84	0.89	6.75	6.52	0.60

Table 7. The Ewens-Watterson neutrality test applied to the studied populations at investigated loci

Locus	Population														
	Ban			Tia			Ind			Mal			Vle		
	Obs. F	L95	U95	Obs. F	L95	U95	Obs. F	L95	U95	Obs. F	L95	U95	Obs. F	L95	U95
Mbr-02	0.117	0.103	0.260	0.075	0.070	0.160	0.121	0.105	0.282	0.249	0.128	0.397	0.115	0.095	0.248
Mbr-03	0.081	0.081	0.184	0.067	0.067	0.131	0.194	0.184	0.367	0.096	0.096	0.255	0.064	0.063	0.137
Mbr-08	0.406	0.244	0.764	0.222	0.158	0.484	0.311	0.220	0.697	0.430	0.247	0.800	0.595	0.257	0.786
Mbr-10	0.431	0.219	0.688	0.285	0.248	0.770	0.341	0.201	0.629	0.505	0.303	0.869	0.398	0.220	0.673
UVC-814	0.068	0.075	0.176	0.083	0.087	0.209	0.062	0.070	0.150	0.231	0.129	0.371	0.094	0.094	0.233
UVC-817	0.077	0.070	0.161	0.068	0.067	0.150	0.070	0.070	0.171	0.084	0.090	0.229	0.070	0.074	0.170

Table 8. The overall Ewens-Watterson neutrality test applied to the investigated loci

Locus	Obs. F	L95	U95
Mbr-02	0.0660	0.0827	0.2568
Mbr-03	0.0542	0.0800	0.2281
Mbr-08	**0.2665**	0.1324	0.4800
Mbr-10	0.1737	0.1763	0.6540
UVC-814	0.0636	0.0766	0.2500
UVC-817	0.0534	0.0809	0.2407

Table 9. Nei's Unbiased Measures of Genetic Identity (above diagonal) and Genetic distance (bellow diagonal)

Population	Bang	Tiay	Indo	Maly	Viet
Bang		0.6506	0.3000	0.4569	0.5807
Tiay	0.4289		0.4834	0.6772	0.5375
Indo	1.2039	0.7269		0.3872	0.3277
Maly	0.7833	0.3899	0.9489		0.5645
Viet	0.5436	0.6208	1.1156	0.5719	

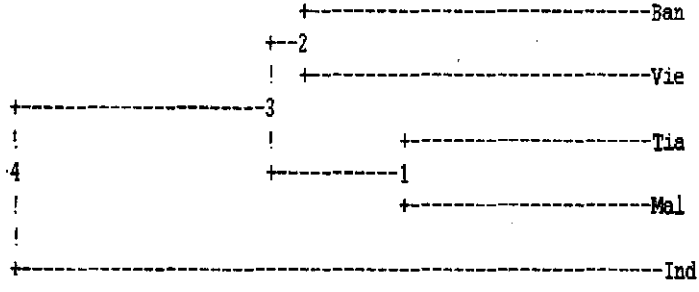

Fig. 3. Dendrogram constructed according to Nei's genetic distance(UPGMA method) shows the genetic relationships among studied populations

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التحليل الوراثى الجزيئى للتباعد بين عشائر برية من جمبرى المياه العذبة العملاق (*Macrobrachium rosenbergii*) فى جنوب شرق اسيا باستخدام واسمات الميكروساتيلايت(SSR)

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يمثل استزراع جمبرى المياه العذبة العملاق واحد من أهم بنود الصادرات فى صناعة تربية الأحياء المائية فى جنوب شرق اسيا. فى هذه الدراسة تم تحليل التباعد الوراثى لخمسة عشائر برية من جمبرى المياه العذبة العملاق تم جمعها من خمسة دول فى جنوب شرق اسيا (بنجلاديش- تايلاند – اندونيسيا- ماليزيا- فيتنام) وذلك باستخدام ستة واسمات من نوع ال SSR. تم الحصول على ١٢٣ أليل من كل الستة واسمات و كانت أطوالها الجزيئية تتراوح ما بين ١٣٧&٣٤٩ زوج من القواعد. أظهرت كل العشائر المدروسة درجة عالية من التباين الوراثى فكان متوسط عدد الأليلات لكل واسم يتراوح ما بين ١٠,٣٣ & ١٥,٢٦ و متوسط المتوقع من الخليط لكل الواسمات ما بين ٠,٧٤٧ & ٠,٨٨٣ وقيمة دليل الثبات الوراثى (F_{ix}) ما بين ٠,١٢٦ & ٠,٣٩٠ بمتوسط قدره ٠,٢٧٧ . وبعمل الدندجرام على أساس التباعد الوراثى تم فصل العشائر الخمسة فى ثلاثة مجموعات المجموعة الأولى احتوت على عشيرتى تايلاند و ماليزيا و المجموعة الثانية ضمت عشيرتى بنجلاديش و فيتنام بينما عشيرة اندونيسيا انفصلت فى المجموعة الثالثة و هذا يؤكد أن عشيرة اندونيسيا أظهرت مستوى عالى من الاختلافات الوراثية عن باقى العشائر. هذه النتائج يمكن أن تكون مفيدة للغاية فى برامج التربية بالانتخاب و فى الحفاظ على التنوع البيولوجى للمصادر البرية.