Pertanika J. Trop. Agric. Sci. 30(1): 71 - 82 (2007) ISSN: 1511-3701


# A Population Genetics Study on the Malaysian Wild Stocks, *M. rosenbergii* Using Cross Amplified Microsatellite Primers

L.M. SEE [2], S. BHASSU [1], R. HASSAN[1], S.S. SIRAJ[2], S.Y. TAN[2] AND S.G. TAN[2]
[1]*Freshwater Fisheries Research Center, Glami Lemi, 71650 Titi, Jelebu, Negeri Sembilan, Malaysia*
[2]*Department of Biology, Faculty of Science, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia*

**Keywords: Microsatellites, cross amplification, wild stocks, orthologous loci**

## ABSTRACT

*Distantly related species often develop mutations in priming sites of fast evolving gene sequences (e.g. microsatellite loci) over time. Orthologous loci from green-lipped mussel were found to cross amplify the Malaysian giant freshwater prawn,* Macrobrachium rosenbergii *microsatellites, which indicates a high conservation of flanking sequences. This was confirmed by direct sequencing of target loci. Four microsatellite markers were successfully cross-amplified in the 'western' form of* M. rosenbergii *with two containing minor interruptions in their sequences. Number of alleles per locus ranged from 5 to 8 with allele frequencies ranging from 0.0048 to 0.2656. Observed heterozygosities ranged from 0.4369 to 0.6848, but in general, these estimates were lower than expected under Hardy-Weinberg equilibrium. Genetic distance values ranged between 0.0059 and 0.1641 with populations in the UPGMA dendrogram not clustering according to their geographical localities.*

## INTRODUCTION

Prawns of the genus *Macrobrachium* Bate, 1868 (Crustacea: Palaemonidae) are a highly diverse, abundant and widespread group of decapod crustaceans found in circumtropical marine-, estuarine- and freshwaters (de Bruyn *et al.*, 2004). The giant freshwater prawn, *Macrobrachium rosenbergii*, is the largest species in the genus *Macrobrachium* and the most important culture species (Mather and de Bruyn, 2003) It is found in coastal river systems from Pakistan in the west to Vietnam in the east, throughout Southeast Asia and south to northern Australia and Papua New Guinea (de Bruyn *et al.*, 2004).

*M. rosenbergii* as it is currently named taxonomically may be polytypic both regionally and within biogeographical regions (de Bruyn *et al.*, 2004). Two distinct forms of *M. rosenbergii*, an 'eastern' and a 'western' form have been described independently, although the species is currently considered to be monophyletic (Mather and de Bruyn, 2003; de Bruyn *et al.*, 2004; Chand *et al.*, 2005).

Microsatellites are a popular marker of choice in many population studies and have been applied in gene mapping, forensics and behavioural ecological studies (Goldstein and Schlotterer, 1999). However, single locus microsatellite marker development can be tedious, laborious, time consuming, costly and requires specialised facilities and equipments. Conservation of flanking regions surrounding microsatellites had been reported across taxa in many animal species (Schlotterer *et al.*, 1991), which facilitates cross-species amplification in a new target species. Recently, six specific microsatellite primer sets were developed for the 'eastern' form of giant freshwater prawn, *M. rosenbergii*, and the variation characterized (Chand *et al.*, 2005). However, cross amplification of these loci in the related 'western' form of the species was unsuccessful although this form is by

Corresponding author:
Email: *subhabhassu@yahoo.com*

far the most important with respect to worldwide wild fisheries and culture fisheries.

The homologous sequences that existed between two families, *Palaemonidae* and *Mytiloidea*, was very interesting when BLAST analysis performed on RAMS clones of *M. rosenbergii* showed that a number of the sequences were homologous to *Perna viridis* (Bhassu *et al.*, 2005). Thus, microsatellite primers developed from a mollusc, the green-lipped mussel (*Perna viridis*) were evaluated for their potential to cross amplify in Malaysian populations of the 'western' form of giant freshwater prawn. In the gene bank, there are more than 100 microsatellites sequences of *Perna viridis* that can be further tested for its potential for cross amplification in *M. rosenbergii*. Similar studies on marine turtles indicated conservation of flanking sequences spanning approximately 300 million years of divergent evolution (FitzSimmons *et al.*, 1995). Ohno (1970) proposed that without duplicated genes, the creation of metazoans, vertebrates and mammals from unicellular organisms would have been impossible.

It proves that microsatellite loci have great potential for broader applications such as comparative gene mapping and assessing genetic population structures within species. The use of microsatellite loci across species depends on the conservation of priming sites within flanking sequences, which enables amplification and maintenance of repeat arrays long enough to promote polymorphism (Weber, 1990).

The objective of this study was to identify potential microsatellites to be used for cross amplification in *M. rosenbergii* and to aid in finding potential polymorphic microsatellites for use in marker assisted selection programs.

## MATERIALS AND METHODS

### *Samples and DNA Extraction*

*Macrobrachium rosenbergii* were sampled from a total of 11 rivers representing a diverse geographical distribution consisting of 8 populations from east and west Peninsular Malaysia and two populations from the island of Borneo in Sarawak and Sabah (*Fig. 1*). The sample sizes are shown in Table 1. DNA was extracted from 25 mg of tissue following the recommended protocol in QIAamp DNA Mini Kit (Qiagen, Germany).

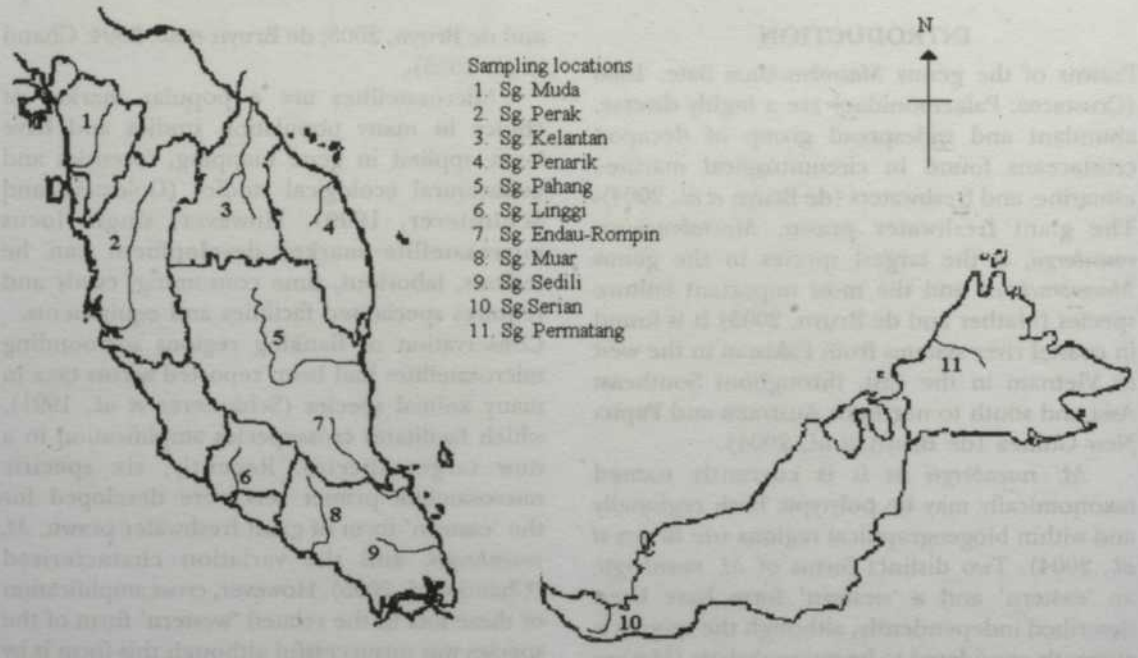


*Fig. 1: Location of sampling sites*

TABLE 1
Sampling sites and sample sizes for 11 populations

| No. | Location | Sample size |
|---|---|---|
| 1 | Sungai Perak, Perak | 20 |
| 2 | Sungai Muda, Kedah | 20 |
| 3 | Sungai Muar, Johor | 20 |
| | Sungai Sedili, Johor | 20 |
| 4 | Sungai Endau, Johor | 20 |
| 5 | Sungai Linggi, N.Sembilan | 30 |
| 6 | Sungai Pahang, Pahang | 30 |
| 7 | Sungai Kelantan,Kelantan | 30 |
| 8 | Sungai Penarik, Terengganu | 30 |
| 9 | Sungai Serian, Sarawak | 20 |
| 10 | Sungai Permatang, Sabah | 20 |

*Microsatellite Screening and Amplification*

Twelve *Perna viridis* microsatellite primers were screened on individuals of *M. rosenbergii* by polymerase chain reaction (PCR). PCR products were electrophoresed and visualized under ultra-violet light. Successfully amplified bands within the expected size range were excised from the gel. Gel excision and purification were performed using the PerfectprepÆGel Cleanup Kit (Eppendorf, Germany).

Excised bands were sequenced in ABI PRISM 377 DNA sequencer using BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, USA) to determine the amplification of microsatellite repeats in the sequence. This process resulted in four primer sets that amplified apparent microsatellites containing fragments in giant freshwater prawn samples.

*Population Characterization*

Based on the screening procedure, four *Perna viridis* microsatellite primer sets that amplified fragments in giant freshwater prawn, OCC26, OCC28, OCC34 and OCC43, were tested in individuals sampled across a large geographical range in Malaysia. The study was carried out by PCR amplification using a Biometra T3 Thermal Cycler. Thermal cycle amplification was performed in 10 $\mu$L total reaction volume, which contained 1x PCR buffer (Promega, USA), 2.5 mM to 3.75 mM of $MgCl_2$ (Promega, USA), 0.2 unit of *Taq* polymerase (Promega, USA), 0.25 mM of each dNTPs (Promega, USA), 50 $\rho$mole of primer pair, deionised water and approximately 20 ng of template genomic DNA.

The general PCR profile consisted of 40 cycles of 94 °C denaturation for 30 s, 30 s at an annealing temperature specific for each primer pair (as shown in Table 2) and 72 °C of elongation for 40 s. The PCR profile was initiated with a 3 min incubation at 95 °C before the cycle began and upon the completion of the cycles, a 5 min incubation at 72 °C was performed. PCR products were electrophoresed on 6% non-denaturing polyacrylamide gels. Sizes of alleles were determined according to M13 sequence ladder.

*Data Analyses*

The number of alleles per locus, number of effective alleles per locus, and observed and expected alleles per locus were calculated. Conformity to Hardy Weinbergy equilibrium was tested using the Markov Chain Method ( dememorization: 1000, batches: 500 and iterations per batches: 1000). All these calculations were performed using the CENEPOP computer package (Raymond and Rousset.1995). The independent t-test comparison was used to check the differentiation of observed heterozygosity among populations (Archie,1985).

The GENEPOP software package was used to calculate F-statistics ($F_{IS}$,$F_{ST}$) test of genetic disequilibrium for each pair of loci, and genetic differentiation between populations (dememorization:100, batches:100 and iteration per batches: 1000). Due to the small number of loci used, the software package did not allow for significant tests for overall $F_{ST}$. The locus-wise $F_{IS}$ for each population was also calculated to detect effects of inbreeding and Wahlund effects.

Genetic distance between populations was based on the Cavalli-Sforza and Edwards (1967) chord distance. The unweighted pair group method with arithmetic averaging (UPGMA) tree including boot-strap values was constructed as dendrograms with the SAHN (sequential, agglomerative, hierarchical and nested clustering) program from NTSYS-pc software version 1.60 (Numerical Taxonomy and Multivariate Analysis System) (Rohlf, 1993). Mantel's test was performed to evaluate correlation between genetic distances and geographical distances (Manly, 1993 suing TFPGA version 1.3 (Miller, 1997)

## RESULTS AND DISCUSSION

Two of the *Perna viridis* microsatellite primer pairs that amplified in *M. rosenbergii* resulted in products, which contained uninterrupted microsatellite sequences, VJ1-1-1- and BP2-49-2 while 2 loci contained minor interruptions of the sequences, VJ1-9-1 and VJ1-11-2. The

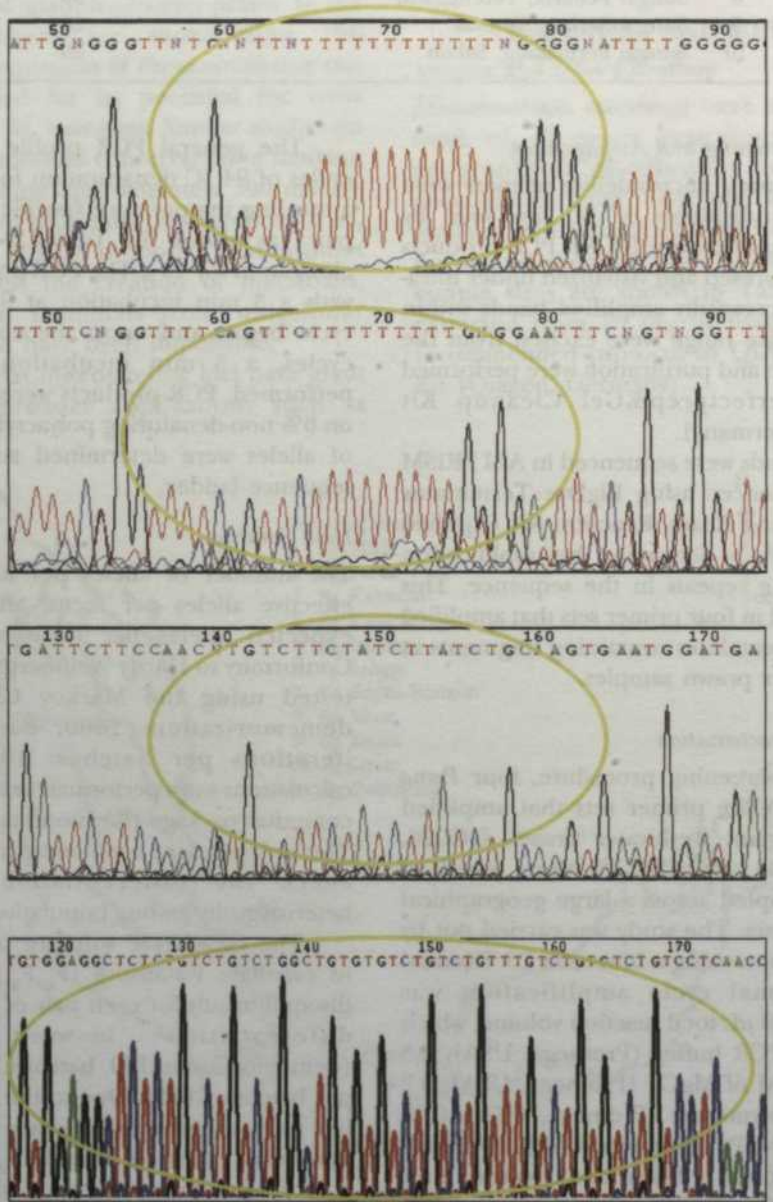

*Fig. 2: Microsatellite regions amplified in M. rosenbergii. (a) VJ1-11-2; (b) BP2-49-2; (c) VJ1-1-1; (d) VJ1-9-1*

amplified flanking sequences are shown in *Fig. 2*. These results suggest cross- amplified microsatellite markers in *M. rosenbergii*.

Microsatellite typing of wild 'western' *M. rosenbergii* populations sampled from Malaysian rivers indicated that all the four loci were polymorphic. Characterization of the four polymorphic loci and a comparison of the repeat sequences between the two species are summarized in Table 2. The number of alleles per locus ranged from 5 to 8 with the overall allele frequency ranging from 0.0042 to 0.2549 (*Fig. 3*). VJ1-1-1 was found to have the highest percentage of effective number of alleles (90.85%), followed by BP2-49-2 (83.45%), VJ1-9-1 (82.62%), VJ1-11-2 (80.69%).

Polymorphisms among the 11 populations was highest for VJ1-11-2, followed by VJ1-1-1, BP2-49-2 and VJ1-9-1. The expected size of the alleles are shown is Table 2. Null alleles were present in few individuals of the Kelantan populations, which could be due to possible mutations at the flanking region of the loci. Studies conducted by Bhassu *et al.*, (2007) indicated that allele at 475 bp of Random Amplified Microsatellite Marker (RAMs) serves as a diagnostic marker in identifying Sg Kelantan samples from the other populations. This could indicate the presence of cryptic species within *Macrobrachium sp.* However, morphology and mt-DNA analysis should be carried out to reconfirm the postulation.

The observed heterozygosity ranged from 0.4421 to 0.7851 (Table 3), which was generally lower than the expected heterozygosity. Such results could be explained by several hypotheses, including methodology bias, null alleles (Jarne and Lagode, 1996) and 'founder effect' during the introduction (Mei *et al.*, 2003).

The $F_{IS}$ values for each population across all loci (Table 3) indicated heterozygote deficiency ($P<0.05$) for all. A comparison of the observed and expected variation indicated that all the loci deviated from Hardy-Weinberg expectations ($P < 0.05$), which might have resulted from the small sample sizes (20 – 30 individuals) analysed per population or the presence of null alleles and high number of alleles. However, three populations, namely Muar, Sedili and Pahang were found not to have deviated from HW equilibrium at locus VJ1-1-1 marker while Kedah did not deviate from HW equilibrium at locus VJ1-11-2. There was no significant linkage disequilibrium between each pair of loci within each of the eleven populations. ($P<0.05$). An independent t-test (Archie, 1985) showed that neither observed nor expected heterozygosity was significantly different between populations.

$F_{ST}$ was 0.062 and significantly different from 0 (Table 4), thus clearly showing that populations of *M.rosenbergii* are divided into subpopulations. The small magnitude of differentiation may be the result of small sample size constraints and large number of alleles, thus increasing the sample size creates high statistical error.

Genetic distance (Cavalli-Sforza and Edwards (1967)) based on the four loci revealed average values between 0.0059 and 0.1641 (Table 5). The dendrogram (*Fig. 4*) showed that the majority of the populations did not cluster according to their geographical location, which may be caused by natural selection and mutation in order to adapt to a new environment. Mantel's test showed no significant correlation between genetic and geographical distances (r =0.1669; Z=1600409.6635; upper tail P= 0.2360; lower tail P =0.8452 )

## CONCLUSIONS

This approach compared to conventional methods is indeed cost effective as it reduces the time taken to isolate microsatellite markers. It utilizes *Perna viridis* microsatellite primers on *M. rosenbergii* stocks for genetic diversity, mutational mechanism, phylogenetic and other studies. Thus more cross-amplified microsatellite primers should be trailed as this study unexpectedly yielded positive results. The dendrogram generated suggested that the clustering of the populations were not exactly in accordance with their geographical locations, which might be due to natural selection in order to adapt to new environments and their breeding systems. The low genetic variation in the eleven populations may have been due to the small sample size and low number of loci used. Therefore there is need to increase the number of loci and sample size. The potential of *Perna viridis* primers will be studied further using large sample populations and at least 20 loci. Distinct genetic differentiation suggested that these populations should be managed separately. Moreover, genetically differentiated populations may show variation of traits important for aquaculture (Kumagai *et al.*, 2004). Therefore economically

TABLE 2
Characteristics of *Perna viridis* microsatellites in *M. rosenbergii*, including GeneBank Accession number (*Perna viridis*), primer sequence, repeat sequence, annealing temperature ($T_A$), expected size (bp) (*Perna viridis*) and size range (bp) (*M. Rosenbergii*)

*Note:* N – Uninterrupted repeat
n – Interrupted repeat

| Primer | GeneBank Accession No. (*Perna viridis*) | Primer Sequence (5' – 3') | Repeat Sequence (*Perna viridis*) | Repeat Sequence (*M. rosenbergii*) | $T_A$ (°C) | Expected size (bp) (*Perna viridis*) | Size range (bp) (*M. rosenbergii*) |
|---|---|---|---|---|---|---|---|
| VJ1-1-1 | DQ010069 | F: CAC CTA GTT CAG GGT CTC TC<br>R: AGC TCT CAT CCA TTC ACT TG | $(TC)_N$ | $(TCT)_n$ | 44 | 174 | 143-276 |
| VJ1-9-1 | DQ010072 | F: TGC GTG TGG AGG CTC TCT<br>R: TCA CCT CTT GGT TGA GGA CA | $(CT)_N$ | $(TCT)_N$ interrupted $(G)_N$ | 40 | 205 | 151-257 |
| VJ1-11-2 | AY850125 | F: ACT CGA TCT CTG TGT TGT TA<br>R: TAG TTT CAG GTT CAC TAT GG | $(TG)_N$ | $(T)_N$ | 44 | 234 | 144-220 |
| BP2-49-2 | AY850129 | F: GTT AAA CAA CCA ACC AAC G<br>R: GTC TTT TTG TCA TTG CAC AC | $(TG)_n$ | $(T)_n$ | 44 | 215 | 122-290 |

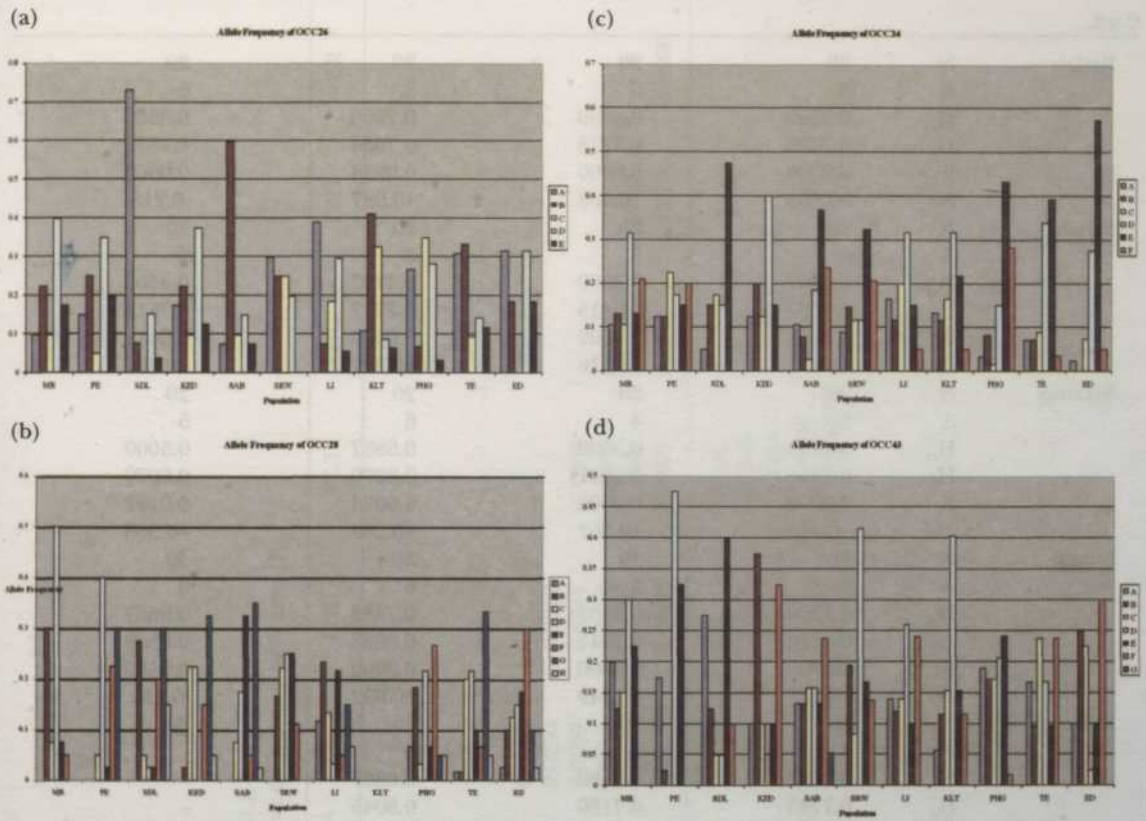


*Fig. 3: Allele frequencies within the 11 populations. (a) VJ1-1-1; (b) VJ1-9-1; (c) VJ1-11-2; (d) BP2-4912*

TABLE 3
Statistical results of *Perna viridis* microsatellite in *M. rosenbergii* populations

| Locus | | VJ1-1-1 | VJ1-9-1 | VJ1-11-2 | BP2-49-2 |
|---|---|---|---|---|---|
| Muar | N | 20 | 20 | 20 | 20 |
| | A | 5 | 5 | 6 | 5 |
| | $H_O$ | 0.7000 | 0.5500 | 0.6316 | 0.7000 |
| | $H_E$ | 0.8013 | 0.7577 | 0.8208 | 0.6628 |
| | P | 0.0195 | 0.0000 | 0.0011 | 0.7000 |
| | *Fis* | +0.129 | +0.279 | +0.235 | -0.058 |
| Perak | N | 20 | 20 | 20 | 20 |
| | A | 4 | 5 | 6 | 5 |
| | $H_O$ | 0.4000 | 0.5000 | 0.6500 | 0.9500 |
| | $H_E$ | 0.6538 | 0.7692 | 0.8462 | 0.7141 |
| | P | 0.0002 | 0.0007 | 0.0220 | 0.0001 |
| | *Fis* | +0.394 | +0.356 | +0.236 | -0.342 |
| Sedili | N | 20 | 20 | 20 | 20 |
| | A | 6 | 4 | 5 | 6 |
| | $H_O$ | 0.8500 | 0.0769 | 0.3500 | 0.1500 |
| | $H_E$ | 0.7526 | 0.4523 | 0.7141 | 0.7885 |
| | P | 0.0566 | 0.0002 | 0.0000 | 0.0000 |
| | *Fis* | -0.133 | +0.836 | +0.516 | +0.814 |

Table 3 *Cont.*

Cont.

| | | | | | |
|---|---|---|---|---|---|
| Kedah | N | 20 | 20 | 20 | 20 |
| | A | 5 | 5 | 5 | 6 |
| | $H_O$ | 0.3500 | 0.4000 | 0.7000 | 0.9500 |
| | $H_E$ | 0.7423 | 0.7718 | 0.7654 | 0.7872 |
| | P | 0.0000 | 0.0000 | 0.0224 | 0.0002 |
| | *Fis* | +0.535 | +0.488 | +0.087 | -0.213 |
| Sabah | N | 20 | 20 | 20 | 20 |
| | A | 6 | 5 | 6 | 6 |
| | $H_O$ | 0.6842 | 0.3000 | 0.4737 | 0.4500 |
| | $H_E$ | 0.8620 | 0.6115 | 0.7767 | 0.7513 |
| | P | 0.0068 | 0.0009 | 0.0009 | 0.0012 |
| | *Fis* | +0.211 | +0.516 | +0.397 | +0.407 |
| Sarawak | N | 20 | 20 | 20 | 20 |
| | A | 5 | 4 | 6 | 5 |
| | $H_O$ | 0.4444 | 0.5000 | 0.5882 | 0.5000 |
| | $H_E$ | 0.7556 | 0.7641 | 0.8200 | 0.8079 |
| | P | 0.0002 | 0.0000 | 0.0094 | 0.0191 |
| | *Fis* | +0.419 | +0.352 | +0.289 | +0.388 |
| Linggi | N | 30 | 30 | 30 | 30 |
| | A | 6 | 5 | 6 | 8 |
| | $H_O$ | 0.5200 | 0.3333 | 0.7333 | 0.9667 |
| | $H_E$ | 0.8278 | 0.7317 | 0.8068 | 0.8508 |
| | P | 0.0000 | 0.0000 | 0.0000 | 0.0409 |
| | *Fis* | +0.377 | +0.549 | +0.092 | -0.139 |
| Kelantan | N | 30 | 30 | 30 | 30 |
| | A | 6 | 5 | 6 | - |
| | $H_O$ | 0.2692 | 0.2609 | 0.6667 | - |
| | $H_E$ | 0.7745 | 0.7150 | 0.8045 | - |
| | P | 0.0000 | 0.0000 | 0.0001 | - |
| | *Fis* | +0.657 | +0.640 | +0.174 | - |
| Pahang | N | 30 | 30 | 30 | 30 |
| | A | 6 | 5 | 6 | 8 |
| | $H_O$ | 0.7241 | 0.4667 | 0.2333 | 1.0000 |
| | $H_E$ | 0.8173 | 0.7328 | 0.7130 | 0.8362 |
| | P | 0.0361 | 0.0000 | 0.0000 | 0.0000 |
| | *Fis* | +0.116 | +0.367 | +0.676 | -0.200 |
| Terengganu | N | 30 | 30 | 30 | 30 |
| | A | 6 | 5 | 6 | 8 |
| | $H_O$ | 0.5238 | 0.5238 | 0.4286 | 0.9667 |
| | $H_E$ | 0.8328 | 0.7677 | 0.7240 | 0.7977 |
| | *P Fis* | 0.0009 | 0.0000 | 0.0000 | 0.0000 |
| | | +0.377 | +0.323 | +0.413 | -0.216 |
| Endau | N | 20 | 20 | 20 | 20 |
| Rompin | A | 6 | 4 | 5 | 8 |
| | $H_O$ | 0.9500 | 0.8947 | 0.1500 | 0.9000 |
| | $H_E$ | 0.7962 | 0.7525 | 0.6000 | 0.8410 |
| | *P* | 0.0068 | 0.0000 | 0.0000 | 0.0000 |
| | *Fis* | -0.199 | -0.195 | +0.755 | -0.072 |

*Note:* N – Number of samples, A – Number of alleles, ( ) – Effective number of alleles, $H_O$ – Observed Heterozygosity, $H_E$ – Expected Heterozygosity, *P* – Probability value estimates regarding deviation from Hardy-Weinberg equilibrium, *Fst* – Population Differentiation, *Fis*:Inbreeding Coefficent. * Significant Deviation at *P*=0.05

TABLE 4
Matrix of population differentiation and structure ($F_{ST}$)

| pop ID | MR | PRK | SDL | KDH | SBH | SRW | LNG | KLT | PHG | TRG | ER |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MR | 0.0000 | | | | | | | | | | |
| PRK | 0.0267 | 0.0000 | | | | | | | | | |
| SDL | 0.1410 | 0.1242 | 0.0000 | | | | | | | | |
| KDH | 0.0710 | 0.0796 | 0.1094 | 0.0000 | | | | | | | |
| SBH | 0.0876 | 0.0759 | 0.1307 | 0.0646 | 0.0000 | | | | | | |
| SRW | 0.0356 | 0.0490 | 0.0989 | 0.0705 | 0.0469 | 0.0000 | | | | | |
| LNG | 0.0579 | 0.0743 | 0.0647 | 0.0418 | 0.0781 | 0.0230 | 0.0000 | | | | |
| KLT | 0.0277 | 0.0384 | 0.1397 | 0.0658 | 0.0267 | 0.0059 | 0.0341 | 0.0000 | | | |
| PHG | 0.0476 | 0.0669 | 0.0703 | 0.0865 | 0.0867 | 0.0212 | 0.0512 | 0.0627 | 0.0000 | | |
| TRG | 0.0705 | 0.0615 | 0.0686 | 0.0222 | 0.0162 | 0.0383 | 0.0336 | 0.0208 | 0.0606 | 0.0000 | |
| ER | 0.0915 | 0.1091 | 0.0705 | 0.0437 | 0.0628 | 0.0540 | 0.0524 | 0.0997 | 0.0478 | 0.0228 | 0.0000 |

MR – Muar
SDL – Sedili
SBH – Sabah
LNG – Linggi
PHG – Pahang
ER – Endau Rompin
PRK – Perak
KDH – Kedah
SRW – Sarawak
KLT – Kelantan
TRG – Terengganu

TABLE 5
Matrix of genetic and geographical distances. Above diagnonal is geographical distance (km), below diagonal is genetic distance according to Cavalli-Sforza and Edwards (1967) chord distance

| pop ID | MR | PRK | SDL | KDH | SBH | SRW | LNG | KLT | PHG | TRG | ER |
|---|---|---|---|---|---|---|---|---|---|---|---|
| MR | 0.0000 | 444 | 197 | 593 | 2389 | 1989 | 139 | 652 | 281 | 544 | 210 |
| PRK | 0.0274 | 0.0000 | 638 | 149 | 2455 | 2025 | 345 | 341 | 401 | 453 | 608 |
| SDL | 0.1641 | 0.1418 | 0.0000 | 787 | 2583 | 2185 | 369 | 660 | 345 | 492 | 92 |
| KDH | 0.0764 | 0.0865 | 0.1228 | 0.0000 | 2604 | 2204 | 494 | 351 | 550 | 463 | 757 |
| SBH | 0.0960 | 0.0821 | 0.1504 | 0.0691 | 0.0000 | 2000 | 2290 | 2674 | 2383 | 2655 | 2553 |
| SRW | 0.0369 | 0.0515 | 0.1098 | 0.0758 | 0.0492 | 0.0000 | 1890 | 2274 | 1983 | 2255 | 2153 |
| LNG | 0.0615 | 0.0803 | 0.0692 | 0.0436 | 0.0847 | 0.0235 | 0.0000 | 564 | 231 | 503 | 321 |
| KLT | 0.0285 | 0.0399 | 0.1624 | 0.0704 | 0.0274 | 0.0059 | 0.0353 | 0.0000 | 440 | 168 | 568 |
| PHG | 0.0500 | 0.0717 | 0.0756 | 0.0947 | 0.0949 | 0.0217 | 0.0540 | 0.0669 | 0.0000 | 272 | 253 |
| TRG | 0.0758 | 0.0655 | 0.0737 | 0.0227 | 0.0165 | 0.0398 | 0.0348 | 0.0212 | 0.0645 | 0.0000 | 401 |
| ER | 0.1008 | 0.1225 | 0.0758 | 0.0457 | 0.0670 | 0.0571 | 0.0553 | 0.1107 | 0.0502 | 0.0233 | 0.0000 |

MR – Muar
PRK – Perak
SDL – Sedili
KDH – Kedah
SBH – Sabah
SRW – Sarawak
LNG – Linggi
KLT – Kelantan
PHG – Pahang
TRG – Terengganu
ER – Endau Rompin

important traits of this population such as growth, disease resistance, and others should be evaluated. Incorporating information on genetic information would enable efficient exploitation of these resources for aquaculture and genetic improvement programs.

## ACKNOWLEDGEMENTS

We would like to thank the Director of Research, Department of Fisheries, Malaysia, Mr. Ismail Awang Kechik for financial support (Grant no: 21-22501-006) and the continued encouragement throughout the research project.

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