Genetic diversity of banana prawns *Fenneropenaeus merguensis* in Malaysian waters using microsatellite markers

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Abstract

Aim: To characterize the wild populations of the prawns genetically by using microsatellite markers.

Methodology: Prawns from 11 selected populations were sampled from wild and cultured areas of Peninsular and East Malaysia. Out of thirty primers designed for the species including cross amplified primers, only 22 were successfully polymorphic markers. Throughout populations, observed heterozygosity values was lower than the expected which indicates a probability of a deficit in the heterozygosity. Low heterozygosity suggest that mixture and intermingling probably might have occurred among the population of banana prawns in Malaysia.

Results: The *F* values significantly showed genetic differentiation among the populations. The UPGMA dendrogram grouped the populations according to their geographical origins. Endau Rompin and Sedili were clustered together, while Sarawak and Kelantan populations were sub clustered by themselves. As expected, Kuala Selangor, the cultured population, however, was clustered in a group by itself.

Interpretation: The results of this study would help the farmers in selecting non-inbred and to identify genetically distant or close populations for improvement of brood stock management procedures.

Keywords: *Fenneropenaeus merguensis*, Heterozygosity, Microsatellite markers, Population studies

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Introduction

The fast and unprecedented development of prawns and shrimps industry in aquaculture has captured public's attention and awareness to its possible influence or impact on the natural (wild) population and potential environmental effects (Benzie, 2000). The multinational breeding of *Fenneropenaeus merguensis* has grown like a shot from 1990s to early 2000s. In 2006, the highest aquaculture production was recorded to be 96,633 tonnes (FAO, 2019). In spite of peak achieved, the growth of aquaculture industry has declined tremendously in the past decade with the production of only 24,681 tonnes in 2016, just a decade after its production peak. The decline of global production of this species is alarming. *F. merguensis* contributed less and less to the annual production particularly in Thailand and Indonesia as it was easily substituted by the Pacific white shrimp, *Litopenaeus vannamei* in several farms (FAO, 2010). Due to introduction of alternative species, it is predicted that the production and growth of this species will decline in the immediate future if the situation persist. However, it can be improved later on if studies are improved and development programs are monitored.

To attain the Sustainable Development Goals (SDGs), in particular number 14 which targets and focus on the conservation and sustainable use of ocean and marine resources for development, the genetic basis of wild (natural) brood stock is significant for breeding (culture) this species, in addition to proper management of brood stocks to increase production. Furthermore, important aspects to drive the progress towards achieving the 2030 United Nations Agenda for Sustainable Development depends greatly on a well-timed, responsible, solid and upright data. Depletion of genetic diversity in culture stocks are rather predictable (Kuruma prawn, *Marsupenaeus japonicus*, Luan et al., 2006). This is highly due to new founder population, inbreeding and small effective population size (Rowland, 2013).

Molecular markers are reliable tools to identify genetic variations among populations by comparing the genetic diversities. This approach assist in building a sustainable management program within the species' population (Chauhan and Rajiv, 2010). Microsatellites have been used for an array of applications especially in genome analysis and genetic mapping (Zane et al., 2002), for determining the origin (parents) (Taris et al., 2005) and mainly for evaluating of genetic variations in cultured and natural populations (Al-Barwani et al., 2013).

Microsatellites show polymorphism by having a variable number of tandem repeat motifs of one to six nucleotides long DNA motif resulting in size variation (Kalia et al., 2011). It has gained popularity as molecular markers because it is co-dominantly inherited, simple and fast to assay, a high degree of allelic variation and is relatively abundant. Microsatellite analysis has been adapted by several studies of prawn species, especially

the penaeid; *Penaeus japonicus* (Jerry et al., 2004), *Penaeus monodon* (Aziz et al., 2011; Wuthisuthimethavee et al., 2003) and *Penaeus vannamei* (Wolfus et al., 1997). Although, *F. merguensis* has been highly exploited, relevant molecular information especially on their genetic diversities and status in natural populations are still limited, especially in Malaysia (Lehata et al., 2017). Therefore, the current study was under taken to depict as well as to characterise selected populations of *F. merguensis* in Malaysian waters by using microsatellite markers.

In Malaysia, *Fenneropenaeus merguensis*, is locally called udang kaki merah due to its morphology (Fig. 1). In 1997, a publication by Perez-Farfante and Kensley on prawns of the world, including the penaeoid and sergestoid shrimps has drawn public attention on the taxonomy of this genus. They proposed a revision of the taxonomy by lifting a sub-genera in the genus *Penaeus* to another extensive rank. The new revision has resulted in the substitution of 27 classical penaeid shrimp to almost new set of binomials. The genus of penaeids has been reorganised based on morphological differences, particularly the genital characteristics of these animals. Based on the new taxonomy, Perez-Farfante and Kensley lifted the ranks of the five subgenera (*Farfante penaeus*, *Fennero penaeus*, *Lito penaeus*, *Marsu penaeus* and *Meli certus*) of *Penaeus* to full genera.

F. merguensis is distributed extensively worldwide and it is an important Indo-Western Pacific prawn species that live in shallow waters. Their juveniles are estuarine, while the adults are mostly marine inhabitant. The distribution of this species ranges from the Persian Gulf through the South China Sea to Australia and the Mediterranean Sea (Ozcan et al., 2006).

F. merguensis is highly-priced and of exceptional quality prawn with firm meat and fine flavour. Being favourite among the marine prawn consumers worldwide (us Saher and Noor, 2018), it has potential for aquaculture, especially in net cage culture. Although this is costly to develop some other farms, even small-

Fig. 1 : Banana prawn, '*Fenneropenaeus merguensis*' characteristics: (A) Rostrum; (B) Carapace; (C) Pereiopods; (D) Pleopods; (E) Uropod and (F) Telson.

scale shrimp aquaculture can be profitable. It is a good candidate for aquaculture because of its fast growth, able to adapt to different range of salinities, and they are rather large in size. Although growth performance of *F. merguensis* falls back from other Penaeid prawns, their advantages are short life cycle, their ability to mature and successfully spawn in captivity (Hoang, 2002) ensures production of postlarvae which are consistent and these characteristics boost the opportunity to domesticate this species. Hence, the objectives of this study were to successfully characterise the populations from various sampling sites to exploit the genetic basis and diversity of this species in the Malaysian waters and to also determine the genetic distances between the populations for the goodness of monitoring breeding programs.

Materials and Methods

Collection of samples : The wild and cultured *F. merguensis* samples which consisted of both the males and females were collected from eleven different regions of Malaysia (Table 1) northern, southern, eastern coast of Peninsular Malaysia and East of Malaysia. Fresh prawn samples were taken directly from the fishermen after harvest and were kept at -20°C before examination and analyses.

DNA extraction : Shrimp muscle tissue (body flesh) was used for extracting of the DNA using Promega DNA Extraction Kit. The amount of soft tissue used for extraction was 0.1 – 0.2g. The procedure for DNA extraction included Nuclei Lysis, Lysis and protein precipitation, DNA precipitation and rehydration, and DNA Rehydration Solution. Quantification of DNA samples was performed on 0.8% agarose gel and stained with GelRed, a non-carcinogenic staining solution.

Microsatellite primers, Polymerase chain reaction and Gel electrophoresis : Thirty microsatellite primer pairs developed for *F. merguensis* for polymorphic loci and cross-amplification primers developed for *Penaeus monodon* from Biobasic Canada were tested in this study (Table 2). PCR amplification was

performed with Mastercycler Gradient by Eppendorf. Briefly, the program was set at 94°C for predenaturation, denaturation specific annealing temperature specified for primer pair ranging from 45°C to 55°C , extension at 72°C and finally elongation process at 72°C .

Population study and data analysis : A few analysis of genetic software were carried out to obtain the total number of observed and expected alleles, average allele number per locus, allele frequencies, the observed (H_o) and expected heterozygosity (H_e) across all population. Differences in the population was estimated by using the fixation indices by Wright's (1978) which are the F_{IT} , F_{ST} and F_{IS} in the structure of f , $_$ and F , respectively, for each locus for all populations. This is in accordance to the variations following the Weir and Cockerham (1984) method using the software FSTAT (Version 2.9.3, Goudet, 2002) and POPGEN version 1.32 (Yeh et al., 1999). GENEPOP software (Raymond and Rousset, 1995) was utilised to measure the expansion of inbreeding by reckoning the values of F_{IS} values and the level of significance among each populations, this includes the pair-wise F_{ST} values. Genetic differentiation was described by using F-statistics and calculated according to Wright (1978). These F-statistics were utilised to access the excess and deficiency in heterozygosity as well as differentiation in the population.

Nei's (1978) genetic distance value was calculated by the formula:

$$D = -\ln [G_{xy} / \sqrt{G_x G_y}]$$

Where, G_x is mean of $\sum p_{iz}$, G_y is mean of $\sum q_{iz}$ and G_{xy} is mean of $\sum p_i q_i$, while p_i and q_i are the frequencies of the allele in population X and Y, respectively. The Chi-square tests (goodness of fit) and G log-likelihood ratio test was performed to measure the consistency of genotypic frequencies for each population with the expectations of Hardy-Weinberg. The NTSYS software version 2.1 were used to visualise the relationship across and among the populations by constructing an unweighted pair group method with arithmetic average (UPGMA) dendrogram (Sneath and Sokal, 1973) occupying genetic distance by Nei's (1978).

Table 1 : Sampling sites and sample sizes for *F. merguensis* used in this microsatellite study

No.	Sample sites	Sample size	Species
1	Tanjung Dawai, Kedah	30	<i>F. merguensis</i>
2	Pantai Remis, Perak	30	<i>F. merguensis</i>
3	Batu Maung, Pulau Pinang	30	<i>F. merguensis</i>
4	Langkawi, Kedah	30	<i>F. merguensis</i>
5	Kuala Selangor, Selangor	30	<i>F. merguensis</i> (cultured)
6	Tumpat, Kelantan	30	<i>F. merguensis</i>
7	Bachok, Kelantan	30	<i>F. merguensis</i>
8	Sedeli, Johor	30	<i>F. merguensis</i>
9	Endau Rompin, Johor	30	<i>F. merguensis</i>
10	Bako, Sarawak	30	<i>F. merguensis</i>
11	Daro, Sarawak	30	<i>F. merguensis</i>

Table 2 : List of DNA microsatellites and primers used in genetic variation study

Locus ID	Gene bank Accession No	Primer sequence (5' – 3')	Size (bp)	Annealing temp	Species
GT242	DQ388003	F: CACACGAGTTGATTGTCTGCTR: CACGTTGTGTGTGCAGTGAG	316	48°C	<i>F. merguiensis</i>
GT273	DQ388004	F: CTTCGATAACCGGGATTTGTR: CAACCCAAACACGCATACAC	249	48°C	<i>F. merguiensis</i>
TAA5	DQ388005	F: TGATTTACATGGGTGGCAGAR: TGATGGCATAATGATAACACAA	311	48°C	<i>F. merguiensis</i>
TAA15	DQ388007	F: CAAGGACCCACCAACACAATR: AATTCGAGACATCCCTTTGC	299	48°C	<i>F. merguiensis</i>
TAA16	DQ388008	F: GGCTTTTTCTGACATTGGTR: CACACACTGCACACATGGAA	312	48°C	<i>F. merguiensis</i>
TAA19	DQ388009	F: AAGGACAGGGAGGAGAGAGGR: ACAGGCTGACCAAGGAACAC	193	51°C	<i>F. merguiensis</i>
TAA20	DQ388010	F: TGTCGCTAAAAATCCAAAAGGR: TGCTGTCCATAATCTGCATTG	158	51°C	<i>F. merguiensis</i>
TAA25	DQ388011	F: GCTGTCATCATCATTAACTCCR: CGCGTTTATGCCGATATTTT	252	48°C	<i>F. merguiensis</i>
TAA28	DQ388012	F: AATGAAGAGCAAGGCTGGTCCR: CATCATCATCATCATCTTG	181	51°C	<i>F. merguiensis</i>
TAA30	DQ388013	F: CGGAGGCTGCACCTCTGTR: CCCCCTATAAAGACGGGATT	221	46°C	<i>F. merguiensis</i>
PM580	AY500856	F: AACTGCCTACAGTGTGTGCGR: GAATGGAGCCTGTTGGTTTG	250-340	48°C	<i>P. monodon</i>
PM1091	AY500857	F: TTCACGACCCAGTATGTCAR: CAGGTCGACGGCTCATATTT	232-406	51°C	<i>P. monodon</i>
PM1713	AY500858	F: GTTGGCAGGGGTTGATTCT: TTTATGGCTATGGCTGACAC	261-433	51°C	<i>P. monodon</i>
PM2334	AY500859	F: TAATTTCTCTGCAACGTCCTR: CTGCTCATTATCCAGTCCAT	268-330	51°C	<i>P. monodon</i>
PM2345	AY500860	F: GATATTTCAAGGAATGCTCGR: TAATTCGTGCCTTACCTCAT	143-229	48°C	<i>P. monodon</i>
PM3538	AY500861	F: GAACGTCGGGGGATTACTTR: ATATCACACCGAGGCTTGG	371-441	48°C	<i>P. monodon</i>
PM3852	AY500862	F: TAATGGGCGTAAGTCTTCGGR: TGAAAGGAGTCGGGATATGC	193-303	48°C	<i>P. monodon</i>
PM3945	AY500864	F: TTTGGACTTCACATCGGTGR: CGGCTGAACAGGTCTGAAAT	234-312	48°C	<i>P. monodon</i>
PM4089	AY500866	F: CTTTTTGAAATCGCCCTGTR: CATTATCCCGCTCTTCTGT	243-377	51°C	<i>P. monodon</i>
PM4793	AY500868	F: CATTACACACGTTACCTGR: CCCAGTTCACGTATCGTGTG	227-335	48°C	<i>P. monodon</i>
PM4798	AY500869	F: GCTTGCCTGTGTGCATACTTR: GTTCCCTCGTGTTTACGAA	275-431	51°C	<i>P. monodon</i>
PM5625	AY500875	F: AAAAGCCAGAGGAAACGTGR: ACAGTCACGTACCCACAAA	172-246	51°C	<i>P. monodon</i>
Locus ID	Gene bank Accession No	Primer sequence (5' – 3')	Size (bp)	Amplification	Species
PM528	AY500855	F: GTGTTATTTCCACGGGTGCR: GCTGCAGGAAGTGTGTGCG	245-325	No amplification	<i>P. monodon</i>
PM4018	AY500865	F: CTCCAAGCGACAGACGAGTR: CGAATGCACTGCCTGTATGT	177-255	No amplification	<i>P. monodon</i>
PM4505	AY500867	F: TGCATCTTAAACGCATGCACR: TTGTGCATTCTGCTGAATC	240-307	No amplification	<i>P. monodon</i>
PM4898	AY500870	F: GCCTTGTTACGGTGGAGGTAR: TACACTGCCGTGTGTGTGTG	226-257	No amplification	<i>P. monodon</i>
PM4868	AY500871	F: CGAGTAGGTGGCCAAACAATR: ACTGACAGGTCAAGCGAGGT	293-315	No amplification	<i>P. monodon</i>
PM4927	AY500872	F: CGAATTCGGTGATAGCGTCTR: AATGGCACAAGCAAAAGGAC	332-351	No amplification	<i>P. monodon</i>
PM5213	AY500873	F: TGGACTGAGGTATGCAGCACR: TCCTTGTTTGAACCCCTTTG	190-240	No amplification	<i>P. monodon</i>
PM5271	AY500874	F: AAAACACTCAGGGGAACACGR: TGGCCCCAATGTTCTTTTA	214-380	No amplification	<i>P. monodon</i>

Results and Discussion

Optimization process : The presence of DNA bands on the gel were used to determine the quality of the DNA along with further analyses using a spectrophotometer. Out of 30, only 22 primer pairs (Table 2) were observed to present successful target loci amplification. The primer AY865 was eliminated as it showed very little successful loci amplification. Null alleles existed in all the populations tested, these results was insufficient to analyse the populations due to the level of high degree null alleles obtained. The rest of the eight primers also failed to show any successful product amplification. GT-273 failed to amplify certain populations, however, the primer was still selected for population study owing to its high polymorphism.

Twenty-two primers selected for population analysis were AY-856, 857, 858, 859, 860, 861, 862, 864, 866, 868, 869, 875;

TAA-5, 15, 16, 19, 20, 25, 28, 30; GT242 and 273. Primers were initially subjected to a temperature gradient screening to determine the clearest and sharpest band for population analysis. All the primers produced sharp and clear bands once temperature and reaction mixture optimization was carried out. Microsatellite bands were identified with the reference of a molecular weight extended ladder of 20 base pair (bp) by Promega and by comparing the expected base pair size for each primer. Analysing in general, the prevailing bands for TAA16 primer were at 120 bp, 160 bp and 180 bp. The rest of the bands appeared at 150bp, 180bp, 210bp, 220bp, 230bp, 240bp, 250bp and 260bp, however, these bands were not in large number. Other bands observed during scoring were stutter bands or also known as ghost bands. These bands were not counted or included during scoring as they were not bright and distinct as compared to other bands to avoid confusions and scoring mistakes.

Microsatellite markers analyses

Screening of microsatellite marker : Fig. 2 represents the profiles resulted by the primers used. An important factor during optimisation of PCR is the concentration of DNA template. If low DNA concentration is used in PCR, it will produce unclear bands due to insufficient of DNA for the PCR reaction. However, if the DNA concentration is too high, PCR can not detect and amplify the template due to amplification process.

Heterozygosity : The number of observed alleles per locus ranged from 2.0 to 9.0 with a mean of 5.571. The mean number of observed alleles with a value of 3.417 was highest in the Endau Rompin population whereas the lowest mean number of observed alleles was in the population in Tumpat, Kelantan with a value of 2.000. The number of effective alleles per locus ranged from 1.514 to 4.647 with a mean of 3.193. The mean effective number of alleles was also observed to be the highest in the Endau Rompin population (2.361) while the lowest mean effective number of alleles was observed in the Tumpat population (1.572). Primer AY860, AY861, TAA5, TAA15, TAA16, TAA19, TAA20, TAA25, TAA28, TAA30 and GT242 showed that the number of observed heterozygosity were lower than the number of expected heterozygosity (Table 3) whereas for the rest of the primers, the results showed reverse trend, a higher number of observed heterozygosity as compared to the number of expected heterozygosity.

Across eleven populations, the values of observed heterozygosity were lower as compare to the values of expected heterozygosity in eleven pair of primers showing signs of a deficit heterozygosity. Concurrently, there were also primers which indicated excess heterozygosity. This phenomenon may be

attributed to few explanations such as populations might have mated randomly (Sellars *et al.*, 2009) and no mutation, meaning there were no allele changes in the population. A similar condition was noticed by Chareontawee *et al.* (2007) when they were accessing genetic diversities of *Macrobrachium rosenbergii* in Thailand's hatchery stocks utilising microsatellite markers, where one of the population stocks expressed excess heterozygosity and low allelic diversity.

The deficiency of heterozygosity however, could possibly be caused by various factors. As an example, there are possibilities that the null alleles individuals were homozygotes, which means that there was a high mutation rate in the locus representing too several different alleles which resulted in the mating of individuals in the populations leading to excessive heterozygosity and as a consequence, it did not follow the rules of Hardy Weinberg Equilibrium (HWE) (Vaseeharan *et al.*, 2013). Another possible reason could be due to biased or sampling error and migration (process of immigration of foreign species as a result of self-migratory or translocation) or emigration of the populations. Thai *et al.* (2007) mentioned that the deficiency of heterozygosity can occur in an experimental study due to inbreeding or recent stock mixing at hatchery.

The significant deficiency of heterozygote found in these penaeid populations are possibly due to the following speculations: separation of null or non-amplifying alleles, sub structuring of populations or known as the Wahlund effects, the locus undergo selection such as genetic hitchhiking, biasness during scoring (heterozygotes were scored inaccurately as homozygotes or vice versa) or occurrence of inbred in the population. Zhang *et al.* (2010) observed a similar situation on the study of genetic diversity of *F. chinensis* in the wild and cultured

Fig. 2 : (A) Microsatellite banding profile of *F. merguensis* samples from Pantai Remis using primer pair TAA19; (B) A diagrammatic representation of microsatellite bands in (A). Lane L: Cambrex 20bp extended ladder. Lane 1-15: Individuals from Pantai Remis.

Fig. 3 : Dendrogram constructed based on Nei's (1978) genetic distance values clustered by UPGMA for populations of *F. merguensis* utilizing data from 22 microsatellite loci. (Tjg- Tanjung, K Slgr- Kuala Selangor).

stocks using microsatellite, where they found five cases of observed excess heterozygosity in some of the tested loci, however, as for the whole population, the F_s value showed a significant decrease in the proportion of heterozygous genotype within the populations showing signs of heterozygous deficit in the population.

Genetic variations: The Chi-square test (χ^2) was performed to determine whether the populations obeyed HWE. Significant differences ($P < 0.05$) indicated there was significant deviation from the HWE in twenty primer pairs, while primers AY-859 and GT-273 were in HWE.

F-statistics was calculated to analyse excess or deficient of heterozygosity along with the differences in the population. Table 3 also shows values of F-statistics generated for 11 populations across all the loci utilizing 22 microsatellite loci. The approach was developed to partition the genetic variation in a subdivided population that provided an obvious description of differentiation. This consisted of three different coefficients used to allocate genetic variability to the total population level (T), subdivision (S) and individuals (I). These three fixations are F_{IS} , F_{IT} , and F_{ST} (Weir and Cockerham, 1984). F_{IS} is a measure of the deviations from HWE proportions within subpopulations while F_{ST} is a measure of genetic differentiation over subpopulation and is always positive. For F_{ST} value, primer TAA5 indicated the lowest value (0.3570), while primer AY-859 indicated the highest value (0.9690). A positive value of F_{IS} indicates a deficiency of heterozygosity and a negative value indicated excess heterozygosity. The negative value of F_{IS} can be seen in almost all the primers, except for primers TAA30 and TAA28. The lower genetic variations observed may possibly be

due to increase in the rate of inbreeding in these shrimp populations.

A common reason for HWE deviation was the size or amount of samples. In the present study, the number of samples was different among the populations. Besides, a significant deviation from HWE could be accredited to other factors like loci's high rate of mutations, the population mated non-randomly, population size was limited as well as the overlap of generations in the population and lastly is migration which can eventually lead to population's increase in homozygosity (Lowe *et al.*, 2004; Morin *et al.*, 2009). Ward (2006) while studying excess and deficiency of heterozygotes observed HWE deviation, which probably resulted from either selection or mixing of populations non-randomly.

Distribution of variations is sufficiently different between populations. Across eleven populations, all the twenty-two microsatellite loci showed polymorphic patterns. The highest allele frequency among the populations were found in primer pairs TAA-16, TAA-20, TAA-25, TAA-30, GT-242, TAA-15 and TAA-28. The most common alleles for all primer pairs across the populations were at 100bp and 175bp. The diagnostic alleles were observed at 300bp and only appeared in the Kelantan and Daro populations.

Genetic relationships : The genetic distance was measured and constructed on Nei's standard genetic distances among all population pairs (Nei, 1978) (Table 4). The genetic distance between the Tumpat and the Penang population was highest (0.629) whereas the lowest (0.010) genetic distance was observed between Sedili and Endau.

Table 3 : Heterozygosity and F-statistics for all loci across the populations of *F. merguensis* based on data generated utilizing twenty-two microsatellite loci.

Locus	Obs Het	Exp Het	Nei	Ave Het	FIS	FIT	FST	Nm*
AY-856	1.000	0.579	0.569	0.052	-0.7561	0.9088	0.9480	0.0137
AY-857	1.000	0.707	0.696	0.063	-0.4377	0.9089	0.9366	0.0169
AY-858	0.918	0.710	0.707	0.237	-0.7510	0.5588	0.7480	0.0842
AY-859	0.433	0.345	0.339	0.031	-0.2766	0.9604	0.9690	0.0080
AY-860	0.667	0.775	0.771	0.122	-0.4950	0.8150	0.8763	0.0353
AY-861	0.667	0.668	0.666	0.247	-0.2360	0.6728	0.7352	0.0900
AY-862	1.000	0.702	0.697	0.105	-0.7366	0.8163	0.8942	0.0296
AY-864	1.000	0.509	0.500	0.046	-1.0000	0.9087	0.9544	0.0120
AY-866	0.931	0.587	0.577	0.047	-0.9311	0.9080	0.9523	0.0125
AY-868	0.790	0.711	0.709	0.248	-0.4234	0.6265	0.7376	0.0889
AY-869	0.664	0.651	0.648	0.199	-0.5297	0.6728	0.7861	0.0680
AY-875	0.909	0.728	0.723	0.159	-0.5600	0.7471	0.8379	0.0484
TAA-16	0.346	0.665	0.663	0.282	-0.1207	0.5652	0.6120	0.1585
TAA-19	0.441	0.682	0.681	0.394	-0.0107	0.4644	0.4701	0.2818
TAA-20	0.359	0.664	0.663	0.335	-0.0533	0.5060	0.5310	0.2208
TAA-25	0.565	0.760	0.759	0.402	-0.3906	0.2628	0.4698	0.2821
TAA-30	0.313	0.750	0.749	0.339	0.1309	0.6087	0.5498	0.2048
GT-242	0.598	0.786	0.785	0.362	-0.6927	0.2200	0.5392	0.2137
TAA-5	0.680	0.688	0.686	0.488	-0.1841	0.2387	0.3570	0.4502
TAA-15	0.557	0.668	0.667	0.452	-0.1283	0.3071	0.3859	0.3979
TAA-28	0.170	0.725	0.722	0.112	0.3386	0.9181	0.8761	0.0353
GT-273	0.000	0.000	0.000	0.000	****	****	0.000	****
Mean	5.571	3.193	0.667	0.670	-0.2718	0.6296	0.7088	0.1027
St. Dev	1.886	0.7571	0.260	0.100	0.3523	0.2444	0.2529	0.1332

Effective number of alleles [Kimura and Crow (1964)], Expected heterozygosity as computed using Levene (1949), *significant at 0.05 level; * Nm = Gene flow estimated from $F_{ST} = 0.25(1 - F_{ST})/F_{ST}$.

The UPGMA dendrogram was established following Nei's unbiased genetic distance values (Nei, 1978). It was constructed on 22 microsatellite loci across 11 populations (Fig. 3), which grouped the populations according to their geographical origins. Endau Rompin and Sedili were clustered together while Sarawak and Kelantan populations were sub clustered separately. Kuala Selangor, a cultured population, however, was clustered in a group by itself. Cluster analyses grouped the populations according to their geographical distribution, with the genetic distance observed to be highest between Penang (facing Straits of Malacca) and Tumpat, Kelantan (facing the South China Sea) populations. The lowest genetic distance was observed among Sedili and Endau (these are Johor populations facing the South China Sea) as they were located closely. Gene flow possibly exists between Endau and other populations found in neighbouring regions. However, the genetic distance observed for the Johor populations and the Langkawi population was surprisingly relatively close due to self-migratory and translocation. Similar clustering of populations according to the geographical location was previously observed in the Giant Freshwater Prawn (*Macrobrachium rosenbergii*) using microsatellite analysis in India (Suresh et al., 2015) and in *Penaeus monodon* in the Indonesian natural populations (Imron et al., 2017) as in the present study.

These groupings might be attributed to the fact that different locations construct different selective forces that can affect the heterogeneity of genetic communities between the local populations. Another reason is that variation in habitats give rise to ecological barriers against gene flow which increases the differentiation between local populations genetically. For instance, if migrants travel or migrate from their source population to a new area or environment of different habitats, initially they will be establish rather poorly in the new habitat and it is not likely for them to pass their genes to their offsprings better than the local populations (Cooper, 2000).

Genetic variation that has been documented in wild or natural stocks as of recently does not seem threatened, however, variation within hatchery or cultured stocks is decreasing relative to the wild (natural). This has affected achievement related to growth and disease in some of the farmed populations (Benzie, 2009). Breeding programs that exist in long-term plans aim to achieve a balance and adequate levels of genetic variation in the stocks, but the explanation for the existence of low genetic variations recently might be attributed to the following reasons; random drift deficiency, short life history emerging in a bottleneck situation, natural and artificial transfer of stock or selection. Consequently, breeders and farmers must be sure to maintain a

Table 4: Genetic distance based on data generated utilizing twenty-two microsatellite loci. Nei's (1978).

pop ID	Endau	Bako	Daro	K Slgor	Penang	Remis	Langkawi	Tumpat	Bachok	Tjg Dawai	Sedili
Endau	****										
Bako	0.369	****									
Daro	0.276	0.297	****								
K Slgor	0.136	0.338	0.257	****							
Penang	0.131	0.261	0.238	0.215	****						
Remis	0.073	0.311	0.320	0.228	0.266	****					
Langkawi	0.307	0.567	0.234	0.335	0.230	0.489	****				
Tumpat	0.560	0.243	0.188	0.230	0.629	0.363	0.395	****			
Bachok	0.505	0.255	0.227	0.251	0.505	0.342	0.303	0.464	****		
Tjg Dawai	0.177	0.384	0.391	0.357	0.595	0.320	0.577	0.509	0.375	****	
Sedili	0.010	0.276	0.127	0.198	0.196	0.166	0.044	0.203	0.249	0.265	****

proper population size that is sufficient to decrease the rate of inbreeding and to ensure genetic diversity is adequately achieved in the breeding population.

In general, genetic variation in farmed or hatchery stocks is typically lesser as compared to wild or natural stocks, the extent of decrease mainly depends on the brood stock management (Benzie, 2000; Lorenzen *et al.*, 2010). This can be seen in the Endau population which had the highest genetic variability, while the cultured Kuala Selangor cultured population had the lowest genetic variability. The prawn industry should realise that conservation of this precious populations species should be prioritized. The data from this study supports the intention of proper breeding methods and monitoring genetic breeding programs. This information can be applied for future genetic improvement of breeding, especially in selection of brood stocks and in the construction of proper guidelines for the management of utilising and monitoring these genetic materials.

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