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Research Article

Population genetics of *Penaeus semisulcatus* from Persian Gulf and Oman Sea using newly developed DNA microsatellite markers

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Abstract

A population genetics study on *Penaeus semisulcatus* from Persian Gulf was performed to assist in the selection of suitable broodstocks for future breeding programs. Eight novel microsatellite loci were developed to study population genetics structure of *P. semisulcatus* in three population sites, Persian Gulf and Oman Sea (Jask, Hormoz and Kuhestak). There were incidences of heterozygosity deficiency and significant deviations from Hardy Weinberg equilibrium (HWE) at most loci (p<0.001). However, four loci (loci E2 and B9 – Hormoz; loci C6 – Kuhestak; and loci H9 – Jask) found to be in HWE. Micro-Checker analysis revealed null alleles in the three microsatellite loci (B5, C6 and C9). Pairwise F_{st} comparison based on allelic and genotypic frequencies indicated that the three populations were significantly differentiated from each other (p<0.05). High levels of pairwise F_{st} (0.106) and low levels of Nm (2.103) observed between Hormoz and Jask populations indicated restricted gene flow between the two populations. On the other hand, low levels of pairwise F_{st} (0.016) and high levels of Nm (15.876) observed between Hormoz and Kuhestak populations indicated high gene flow between these populations. In this study, the assignment test was examined in order to find gene flow connectivity between the three populations. Overall, results revealed high gene flow between Hormoz and Kuhestak and restricted genetic flow between Jask and both Hormoz and Kuhestak populations, providing new input for selection of genetically-suitable broodstocks.

Keywords: *Penaeus semisulcatus*, Genetics, Molecular marker, Polymorphism, Microsatellite

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Introduction

Aquaculture is a strategic and rapidly developing industry. Worldwide, it is growing at an average compound annual rate of 9.2% since 1970 (Barnabe 2018; Gozari *et al.* 2016). Since 1969 utilization of prawns in Iranian waters began in Persian Gulf (Pourmozaffar *et al.* 2019a). *Penaeus semisulcatus* is one major commercial shrimp species in Persian Gulf (~95%) (Niamaimandi *et al.* 2008; Pourmozaffar *et al.* 2019b). The green tiger prawn is widely distributed from south and east Africa to India and Sri Lanka, including Red Sea, Persian Gulf and western Madagascar (Jahromi and Othman 2011). This species lives in estuarine and marine areas from 2 to 30m depth (Alam *et al.* 2017). Preferential range of salinity and temperature for *P. semisulcatus* was reported to be between 38-41 ppt and 28-32°C, respectively (Pourmozaffar *et al.* 2019c; Türkmen 2007). The global capture production in this species reached 12,300 tons in 2014 (FAO, 2017). Stock assessment of *P. semisulcatus* recently reported to be around 873 tons per annum in Persian Gulf. However, heavy exploitation of shrimps in natural habitats may reduce wild populations to an unsustainable level (Tamadoni Jahromi *et al.* 2020a; You *et al.* 2008). Taxonomic status of morphotypes of *P. semisulcatus* has been in question in order to select the best line for brood stocking and related aquaculture programs. Therefore, knowledge of genetic patterns of stocks is considered to be an important tool to understand natural resources in order to ensure sustainability (Heras *et al.* 2016). The genetic compositions of populations may differ in growth rates, disease resistance or other important characteristics (Mandal *et al.* 2012).

Microsatellites have been utilized as genetic markers for studying geographical distribution of populations (Akib *et al.* 2015). Microsatellite enrichment approaches have also been used to amplify the quantity of colonies in a given library containing microsatellite motif of interest (Nunome *et al.* 2006). Microsatellites have been used to identify genetic diversity in several marine organisms such as bivalves (Scott *et al.* 2016), fish (Mojekwu and Anumudu 2013), mollusks (Bierne *et al.* 1998), squid (Shaw and Boyle 1997) and have also been characterized in lobsters (Tam and Kornfield 1996) and shrimps (Heras *et al.* 2016; Li *et al.* 2007; Maggioni *et al.* 2003; Zhou *et al.* 2009). In aquaculture research, microsatellite markers are especially powerful for parentage determination (Taris *et al.* 2005). They also are very useful for assessment of genetic diversity in hatchery populations (Hara and Sekino 2003). The genetic variation of *P. monodon* populations was analyzed by Mandal *et al.* (Mandal *et al.* 2012) suggesting that microsatellites are suitable for shrimp genome study and brood stock management. Factors such as geographic location, marine currents, life cycle and ecological characteristics

can influence genetic distance and differentiation between populations (Sere *et al.* 2017). In addition checking of genetic difference with molecular markers exhibits changes in variability caused by genetic drift, inbreeding or selection in breeding projects or changes in biosynthetic genes (Cruz *et al.* 2004; Tamadoni Jahromi *et al.* 2020b).

To date little information is available for analyzing population structure of *P. semisulcatus* in Persian Gulf and Oman Sea. Therefore, the objective of our study was to explore genetic diversity of natural populations of *P. semisulcatus* based on novel developed microsatellite markers for the first time in Persian Gulf and Oman Sea.

Materials and methods

Sample collection

One hundred and twenty samples of *P. semisulcatus* in Iranian waters (banded morphotype) were collected from three major fishing areas (40 individuals from each location) in Hormozgan Province namely Hormoz Island 57°06'N and 56° 29'E, Kuhestak 26°47'N and 56°49'E and Bandar-e Jask 25°33'N and 57°44'E (Fig. 1). Then, the sample was kept in -18 °C (Nazemi *et al.* 2017; Nazemi *et al.* 2014) These samples were subsequently analyzed to evaluate polymorphism of the newly developed microsatellite markers.

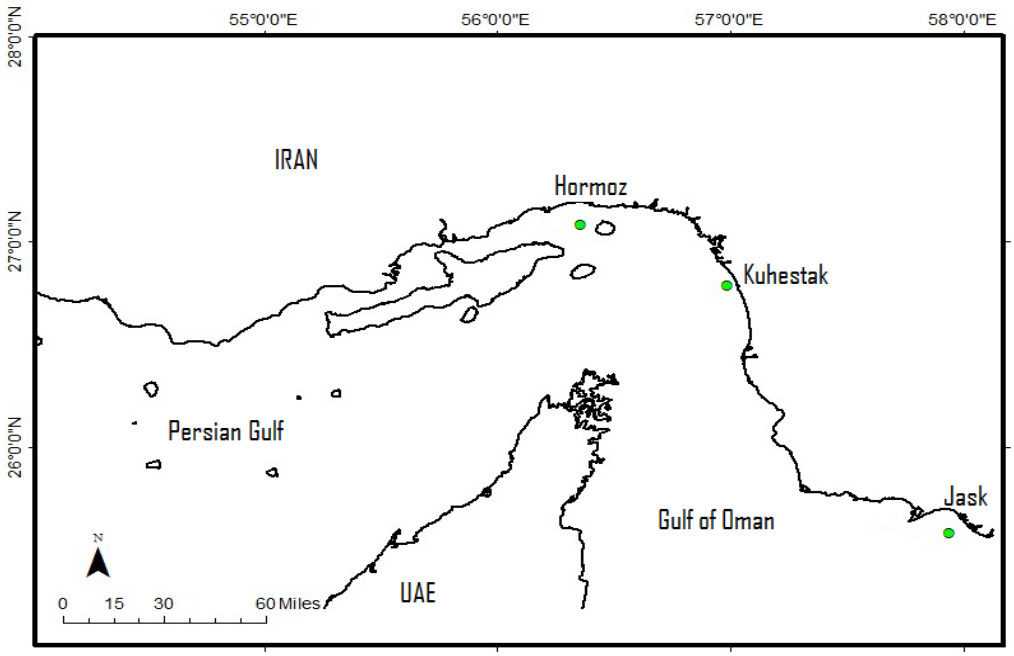

Figure 1: Sampling areas in Persian Gulf and Oman Sea of microsatellite population study

DNA extraction

DNA extraction was performed using Phenol chloroform method employed as described by (Gozari *et al.* 2019a; Gozari *et al.* 2019b; Pirian *et al.* 2016; Tamadoni Jahromi *et al.* 2019) RNAse was used to denature the RNA by

performing "optional" treatment during DNA extraction (Gozari *et al.* 2019c).

Genomic DNA quality and quantity

Agarose gels (1% weight/volume) were used to fractionate high molecular weight DNA and for observation of the quality of extracted DNA. DNA quantity was estimated by spectrophotometer using a Biophotometer (Eppendorf) (Gozari *et al.* 2018; Tamadoni Jahromi *et al.* 2016).

Development of microsatellite libraries

Genomic DNA libraries enriched for microsatellites were constructed based on Edwards *et al.* (1996) with modifications as per Nguyen *et al.* (2007). The plate containing clones with insert was sent to the service provider, 1st BASE Laboratories, Malaysia for direct sequencing in both directions using BigDyeTM terminator cycle sequencing conditions. From the sequences obtained, microsatellite motifs were identified. Once identified, primer pairs were designed for the flanking regions of each microsatellite loci using Primer3 plus software program (http://frodo.wi.mit.edu/primer3/input.htm). Of 96 clones which had insert DNA, 34 clones were chosen for primer design and were used for evaluation of microsatellite polymorphisms in *P. semisulcatus*. The rest were eliminated because they either contained inserts that were too small (<10 repeats) or very short flanking sequence between the repeat sequences. The criteria used when designing primers included primer length (20-28 bp), Tm of between 55-65ºC and G+C content of between 45-65%. Each primer was fluorescently labeled. Primers were synthesized by 1st BASE Laboratories, Malaysia. Only eight of 34 designed primers produced good amplified PCR products with fixed annealing temperature consisting of seven dinucleotide and one trinucleotide repeat. The rest of the primers were either not easily amplified or produced nonspecific bands. Primer pairs were tested on 40 *P. semisulcatus* from Hormoz to test for usefulness. Also, the primer pairs were used amplifying 120 *P. semisulcatus* individuals belonging to Hormoz, Kuhestak and Jask areas in this population genetic study.

Amplification of microsatellite loci

In amplification of microsatellite loci, approximately 100ng template DNA was used in each 25µL reaction, containing 0.6µM of forward and reverse primers, 25mM of $MgCl_2$, 10mM of dNTPs (Promega), 5X PCR buffer (Promega) and 5U of *Taq* polymerase (Promega). Amplification products were verified electrophoretically on a 2% agarose gel, stained with Ethedium Bromide (EtBr) and visualized under an UV transilluminator. The cycling profile was as follows: initial denaturation at 94ºC for 4.30 min, followed by 40 cycles for denaturation at 94ºC for 30s, annealing for 45s at specific

temperature for all the primers (Table 1), and extension at 72ºC for 1 min and 30s, with a final extension of 72ºC for 5 min and soaked at 10ºC.

Data analysis

Fragment analyses were performed using an ABI3730, GeneScan-500 Liz with size standard. Peak Scanner fragment analysis software version 1.0 (Applied Biosystems) was used to score microsatellite data. The presence of null alleles, scoring error due to stuttering or large allele dropouts were estimated using Micro-Checker 2.2.3 (You *et al.* 2008). Expected and observed heterozygosities were calculated and an exact Hardy–Weinberg probability test was performed using Markov chain method (Mandal *et al.* 2012) with default parameters using GenAlEx software version 6 (Jahromi and Othman 2011).

GenAlEx was used to perform assignment tests based on allelic frequencies distributions and also estimation of F_{st} for describing the level of heterozigosity in populations (Peakall and Smouse 2006). Individual-based assignment analysis, which assigns individuals probabilistically to candidate populations by their multilocus genotypes, is extensively used to measure rates of gene flow and dispersal (Waser 1998), and have become typical tools in molecular ecology (Berry *et al.* 2004).

Assignment tests involved using an individual DNA to find out where that individual was hatched and elucidating rates of gene flow and migration. The concept behind assignment tests is to use individual genotypes to assign individuals to populations or clusters. This information can be used to investigate whether the individual was hatched in the same place or it moved in from elsewhere during its lifetime.

Results

Number of repeats in 34 chosen for primer design ranged from 11 to 43. (AG)n dinucleotide repeats were most abundant, whereas trinucleotide repeats with only one clone for (TGT)n, one for (GAT)n, one for (CAA)n and one for (ATT)n detected were in minority.

Only eight of the 34 designed primers produced good amplified PCR products with fixed annealing temperature consisting of seven dinucleotide and one trinucleotide repeats. The rest of the primers were either not easily amplified or produced nonspecific bands. The eight primer pairs were then used to amplify 40 *P. semisulcatus* individuals belonging to Hormoz area to test for its usefulness in population genetic study. A total of 59 alleles were recorded from 40 screened individuals with eight microsatellite loci. Number of alleles ranged from 5-12 per locus. Allele sizes ranged from 150 to 422bp across eight microsatellite loci. Observed heterozygosity ranged from 0.100 to 0.800, while expected heterozygosity ranged from 0.534 to 0.869 (Table 1).

Frequency of each allele at a given locus is shown in Tables 2 and 3. Each

population had an array of unique alleles which was present only once in a population but absent in other populations. A total of 18 unique alleles were found in the three populations, six in the first (Hormoz), eight in the second (Kuhestak) and four in the third population (Jask). Maximum and minimum numbers of alleles were observed in locus B5 with 13 alleles and locus F5 with 6 alleles respectively. Maximum allele frequency was recorded in Hormoz population in locus C6 (0.63) for allele number 6. The results showed that observed heterozygosity (H_o) was higher than expected heterozygosity in loci E2 (Hormoz and Kuhestak), H9 (Hormoz) and B9 (Jask), ranging from 0.6 to 0.8.

Table 1: Characterization of eight microsatellite loci of *Penaeus semisulcatus*. Number of individuals examined (*n*), annealing temperature (*Ta*), number of alleles (*Na*), observed heterozygosity (*Ho*) and expected heterozigosity (*He*) are listed for each locus

Locus	N	Size(bp)	Ta(°C)	Na	Ne	Ho	He	*P*	Repeat units	Primer (5'-3')	Accession No.
E2	40	161-183	48	7	2.7	0.700	0.534	0.495	$(GT)_{13}$	F: TGCGTGGAGTAACTGTGAGC R: TGTGGAATTGTGAGCGGATA	HQ877820
H9	40	215-239	48	6	3.0	0.800	0.726	0.039	$(CA)_{11}$	F: CGGGAATTCGATTTAGTCCA R: CTCAAGCTATGCATCCAACG	HQ877823
C9	40	270-316	48	8	3.6	0.100	0.780	<0.001	$(GT)_{17}$	F: ATGCTGTGCTTATGCCATGT R: AGCGCAGGGTCTTTTGATTA	HQ877819
C6	40	400-442	56	7	2.8	0.375	0.556	0.007	$(AG)_{22}$	F: AAGGGAAGATTGGATTGGAGA R: AGTGGGTGTTTCCGATTACG	HQ877818
B5	40	170-220	56	12	7.9	0.375	0.831	<0.001	$(TG)_{13}$	F: ATGCGAGCAGTGTGCTCTT R: CTCAAGCTATGCATCCAACG	HQ877822
F11	40	231-277	58	9	6.1	0.250	0.869	<0.001	$(GAT)_{13}$	F: AAATTGTCCTTCGCAAAAGGT R: CTCAAGCTATGCATCCAACG	HQ877821
B9	40	150-206	56	5	3.4	0.500	0.546	0.204	$(CA)_{11}$	F: TGACAGGCTATCAGGCAGAG R: AGACTGCAGTGAAGGGGTGT	HQ877816
F5	40	124-170	65	5	3.3	0.750	0.689	<0.001	$(TC)_{11}$	F: GGCCGGCAATTATTTAGTCA R: AGGTCGCAGGTCACTGCTAT	HQ877817

Table 2: Allele frequencies (by population) for eight microsatellite loci in *P. semisulcatus*, * denotes unique alleles, A*= the number of each allele

A*	Locus E2			A	Locus H9			A	Locus C9			A	Locus C6			A	Locus B5		
	Hormoz	Kuhestak	Jask		Hormoz	Kuhestak	Jask		Hormoz	Kuhestak	Jask		Hormoz	Kuhestak	Jask		Hormoz	Kuhestak	Jask
1	**0.013***	0.000	0.000	**1**	**0.025***	0.000	0.000	**1**	0.000	**0.013***	0.000	**1**	0.000	**0.013***	0.000	**1**	**0.013***	0.000	0.000
2	0.025	0.013	0.025	**2**	0.000	**0.050***	0.000	**2**	0.275	0.262	0.225	**2**	0.025	0.013	0.000	**2**	0.262	0.050	0.063
3	0.050	0.025	0.000	**3**	0.075	0.013	0.150	**3**	0.000	0.000	**0.025***	**3**	0.025	0.087	0.000	**3**	0.000	**0.025***	0.000
4	**0.038***	0.000	0.000	**4**	0.000	0.075	0.075	**4**	0.038	0.025	0.025	**4**	0.013	0.000	0.075	**4**	0.275	0.237	0.325
5	0.213	0.300	0.100	**5**	0.387	0.525	0.500	**5**	0.025	0.025	0.038	**5**	0.175	0.150	0.038	**5**	0.075	0.125	0.100
6	0.650	0.512	0.813	**6**	0.225	0.125	0.262	**6**	0.138	0.087	0.100	**6**	0.637	0.550	0.025	**6**	0.038	0.050	0.038
7	0.000	**0.050***	0.000	**7**	0.013	0.125	0.013	**7**	0.350	0.425	0.438	**7**	0.112	0.150	0.788	**7**	0.138	0.112	0.075
8	0.013	0.075	0.013	**8**	0.275	0.087	0.000	**8**	0.050	0.100	0.087	**8**	0.000	0.000	**0.063***	**8**	0.063	0.112	0.087
9	0.000	0.025	0.050					**9**	0.075	0.063	0.038	**9**	0.000	0.038	0.013	**9**	0.025	0.112	0.075
								10	0.000	0.000	**0.025***	**10**	**0.013***	0.000	0.000	**10**	0.013	0.087	0.087
								11	**0.050***	0.000	0.000					**11**	0.050	0.050	0.075
																12	0.038	0.013	0.025
																13	0.013	0.025	0.050

Table 3: Allele frequencies (by population) for eight microsatellite loci in *P. semisulcatus*, *denotes unique alleles

	Locus F11				Locus B9				Locus F5		
A	**Hormoz**	**Kuhestak**	**Jask**	**A**	**Hormoz**	**Kuhestak**	**Jask**	**A**	**Hormoz**	**Kuhestak**	**Jask**
1	0.050	0.050	0.000	**1**	0.000	**0.013***	0.000	**1**	0.363	0.325	0.188
2	0.075	0.025	0.000	**2**	0.000	**0.025***	0.000	**2**	0.013	0.063	0.087
3	0.025	0.075	0.000	**3**	0.000	**0.125***	0.000	**3**	0.363	0.287	0.200
4	0.175	0.087	0.213	**4**	0.050	0.013	0.000	**4**	0.237	0.300	0.463
5	0.175	0.213	0.387	**5**	0.125	0.100	0.050	**5**	0.000	0.000	**0.063***
6	0.162	0.275	0.175	**6**	0.138	0.175	0.325	**6**	0.025	0.025	0.000
7	0.050	0.100	0.038	**7**	0.650	0.475	0.412				
8	0.112	0.063	0.125	**8**	0.038	0.075	0.213				
9	0.175	0.112	0.063								

In this study the levels of heterozygosity ranged from 0.1 to 0.8. The minimum value of heterozygosity was observed in loci C9 (0.1) Hormoz, and the maximum value was for loci H9 (0.8), also from Hormoz. Deviation from HWE was significant in most microsatellite loci (p<0.001). However, there were four loci (loci E2 and B9 Hormoz, loci C6 Kuhestak and loci H9 Jask) which were in Hardy-Weinberg equilibrium (Table 4).

Table 4: Summary of Hardy-Weinberg Equilibrium tests for eight microsatellite loci in three separate populations of *P. semisulcatus* generated using GenAlEx software, significant departure from the HWE is denoted as follows: *p<0.05; **p<0.01, ***p<0.001, values in bold (Ns) were not significant

Population	Locus	Degrees of freedom	Chi square (X^2 value)	Probability (*P*) (HWE)
Hormoz	E2	21	11.5	**Ns 0.457**
Kuhestak	E2	21	75.6	***(0.00)
Jask	E2	10	60.2	*(0.020)
Hormoz	H9	15	49.1	*(0.048)
Kuhestak	H9	21	71.3	***(0.001)
Jask	H9	10	6.4	**Ns (0.545)**
Hormoz	C9	28	214.2	***(0.00)
Kuhestak	C9	28	181.2	***(0.00)
Jask	C9	36	205.2	***(0.00)
Hormoz	C6	21	58.7	***(0.001)
Kuhestak	C6	21	32.5	**Ns (0.052)**
Jask	C6	15	55.8	***(0.004)
Hormoz	B5	66	167.4	***(0.00)
Kuhestak	B5	66	215.3	***(0.00)
Jask	B5	55	190.1	***(0.00)
Hormoz	F11	36	198.6	***(0.00)
Kuhestak	F11	36	235	***(0.00)
Jask	F11	15	70.5	***(0.00)

Table 4 (continued):

Population	Locus	Degrees of freedom	Chi square (X^2 value)	Probability (*P*) (HWE)
Hormoz	B9	10	14.8	**Ns (0.236)**
Kuhestak	B9	28	86.4	***(0.00)
Jask	B9	6	17.2	*(0.17)
Hormoz	F5	10	69.5	***(0.00)
Kuhestak	F5	10	48.4	***(0.001)
Jask	F5	10	27.5	***(0.00)

Genetic variation results showed that pairwise F_{st} values were significant among the three populations. Pairwise comparison based on allelic and genotypic frequencies also indicated that the three populations were significantly differentiated from each other (p<0.05). The maximum genetic variation of 0.106 was between Hormoz and Jask populations, and minimum genetic variation of 0.016 was between Hormoz and Kuhestak (Tables 5 and 6).

Table 5: F_{st} values for pairwise comparison based on AMOVA test among different populations of *P. semisulcatus* (below diagonal). Probability values are shown above diagonal

	Hormoz	Kuhestak	Jask
Hormoz	0.000	0.010	0.010
Kuhestak	0.016	0.000	0.010
Jask	0.106	0.082	0.000

Table 6: F_{st} values for pairwise comparison based on allele frequency values among different populations of *P. semisulcatus*

	Hormoz	Kuhestak	Jask
Hormoz	0.000		
Kuhestak	0.014	0.000	
Jask	0.065	0.053	0.000

In this study pairwise F_{st} values based on allele frequency showed moderate genetic divergence between Jask and Hormoz populations (0.065), and low (0.014) genetic divergence between Hormoz and Kuhestak. The later two were geographically close and low variation between them was expected. Similarly, Jask is geographically far apart from both Hormoz and Kuhestak, thus F_{st} values between Jask and Hormoz, and Jask to Kuhestak were higher than that between Hormoz and Kuhestak. Low levels of F_{st} (0.005) and high level of N*m* (46.210) was observed in locus C9 indicated a relatively high gene flow, while observed high levels of F_{st} (0.253) and low level of N*m* (0.737) in locus C6 indicated limited gene flow between these types of loci. Based on AMOVA test results there were high levels of pairwise F_{st} (0.106) and low levels of N*m* (2.103) between Hormoz and Jask populations indicating

restricted gene flow between the two populations. On the other hand, low levels of F_{st} (0.016) and high levels of N*m* (15.876) were observed between Hormoz and Kuhestak populations indicating high gene flow between these populations (Tables 7, 8 and 9).

Table 7: Relationship between pairwise population F_{st} values and estimates of effective number of migrants (N*m*) in three populations based on AMOVA test

Populations	F_{st}	N*m*	Prob
Hormoz via Kuhestak	0.016	15.876	0.02
Hormoz via Jask	0.106	2.103	0.01
Kuhestak via Jask	0.082	2.782	0.01

Table 8: Relationship between pairwise population F_{st} values and estimates of effective number of migrants (N*m*) in three populations based on allele frequency

Population	F_{st}	F_{st}
Hormoz via Kuhestak	0.014	17.564
Hormoz via Jask	0.065	0.065
Kuhestak via Jask	0.053	0.053

Table 9: Relationship between F_{st} values and estimates of effective number of migrants (N*m*) for eight loci. [a] inbreeding coefficient of an individual (I) relative to the subpopulation (S), [b] inbreeding coefficient of an individual (I) relative to the total (T) population, [c] effect of subpopulations (S) compared to the total population (T)

	LocusE2	LocusH9	LocusC9	LocusC6	LocusB5	LocusF11	LocusB9	LocusF5	Mean
H_t	0.522	0.710	0.749	0.696	0.861	0.836	0.670	0.720	
Mean H_e	0.497	0.682	0.744	0.520	0.846	0.816	0.642	0.699	
Mean H_o	0.592	0.650	0.167	0.350	0.383	0.275	0.592	0.717	
$^{a}F_{is}$	-0.190	0.047	0.776	0.327	0.547	0.663	0.079	-0.025	0.278
$^{b}F_{it}$	-0.134	0.085	0.777	0.497	0.555	0.671	0.117	0.004	0.322
$^{c}F_{st}$	0.047	0.039	0.005	0.253	0.018	0.024	0.041	0.029	0.057
N*m*	5.100	6.088	46.210	0.737	13.656	10.232	5.862	8.303	4.128

Gene flow is the transfer of genetic material between populations resulted from movement of individuals or their gametes. Usually gene flow is expressed as a migration rate, *m*, and refers to the number of alleles in a population for each generation that is of migrant origin (Avise, 1994). N*m* is effective number of migrants, where N is effective population size and *m* is migration rate per generation, and measures level of gene flow within populations per generation. The relationship between F_{st} and evaluation of gene flow (*Nm*) among the three populations in each locus was performed using GenAlEx. Based on AMOVA test, the maximum pairwise Fst was obtained between Jask and Hormoz populations (F_{st}=0.106,

p<0.05). These two populations showed minimum number of migrations (N*m*) among the studied populations (N*m*=2.103). Relationship between F_{st} and N*m* based on allele frequency was also confirmed by AMOVA test. These two populations showed maximum pairwise F_{st} (0.065) and minimum N*m* (3.621).

The assignment test showed that Jask population had minimum connectivity (F_{st}=0.065) and minimum gene flow (N*m*=3.621) as compared to both Hormoz and Kuhestak populations. Jask also had maximum distance (400 km) to other populations (e.g., the distance between Hormoz and Jask), and low connectivity between these two areas is expected (Fig. 2).

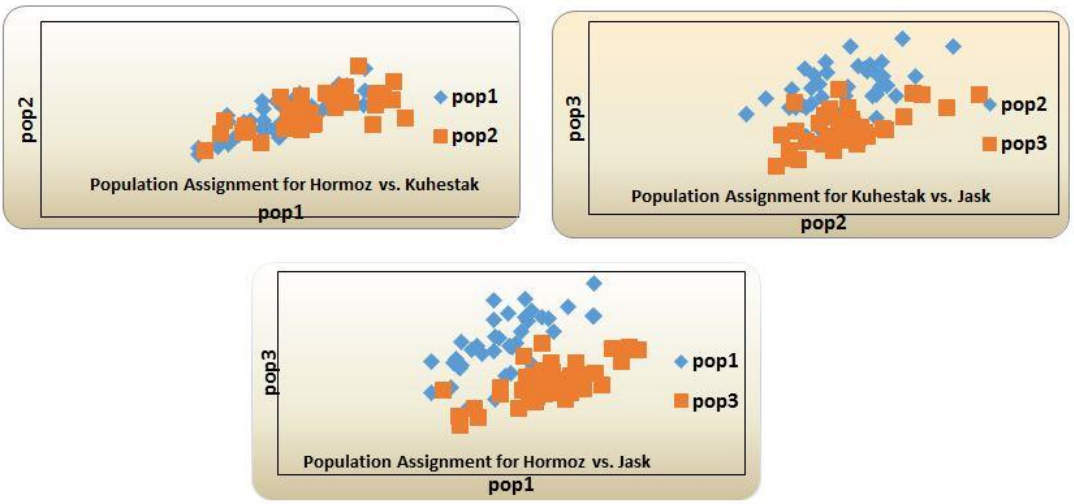

Figure 2: Assignment tests (graphs) based on allele for the three studied populations

Discussion

All eight used loci were highly polymorphic, exhibiting between 5-12 alleles at each locus (Table 1). The high polymorphism (100%) observed in all eight microsatellite loci can be compared to results of (Ball *et al.* 1998) for *P. setiferus* (83%), and Xu *et al.* (1999) and Brooker *et al.* (2000) for *P. monodon* (100%) for six microsatellite loci. Other researchers have also illustrated high numbers of polymorphic alleles per microsatellite locus such as 14-28 alleles which is reported for two loci in *P. monodon* from Thailand (Tassanakajon *et al.* 1998) and also 4-24 loci which was identified for *P. japonicus* (Moore *et al.* 1999). O'Reilly and Wright (1995) mentioned high level of microsatellite loci polymorphism among populations makes them as one of the favorable markers for genome mapping, verifying parentage and also pedigree analyses.

A total of 120 individuals from three locations (Hormoz, Jask and Kuhestak) were investigated for eight microsatellite loci. Number of alleles and allele frequencies for each locus was determined for all eight loci of each

population. Allele frequencies at each locus were calculated based on numbers of alleles generated by GeneAlEx program (Peakall and Smouse 2006).

Population genetics of P. semisulcatus in three Iranian populations

In the past, major investigations of commercial shrimp populations (especially from the Penaeidea family such as *P. monodon*) were performed using allozyme techniques (Benzie *et al.* 1992; Mulley and Latter 1980). By comparison allozyme analysis in penaeid species yielded 14% to 38.6% polymorphic loci. For example, polymorphic allozyme loci accounted for 14% in Australian penaeid prawn *P. latisulcatus* and *M. endeavouri* (Mulley and Latter 1980), 38.6% in *P. vannamei* (Sunden and Davis 1991) and 9.3% in *P. monodon* (Benzie *et al.* 1992). Microsatellite technique opens new doors for investigation of substructure of closely related populations (Estoup *et al.* 1998). In this study, a total of 63 alleles were identified (with the average of eight alleles per locus) in the three populations. Maximum allele frequency was in loci B5 in individuals of *P. semisulcatus* collected from Jask. Jask is, by distance, the furthest from other two study areas, and showed a higher degree of variation from other areas. Slatkin (1985) mentioned that the ability of microsatellite techniques for obtaining unique alleles to discriminate among and between wild shrimp populations is an advantage for these types of markers. Unique alleles could be used as population-specific markers, and good as indicators of gene flow (Bala *et al.* 2017). A total of 18 alleles were found to be unique to the three separate populations. Six unique alleles were found in Hormoz, eight in Kuhestak, and four in Jask (Table 3). These unique alleles may be considered as population-specific markers, and should be useful in breeding studies and broodstocking of *P. semisulcatus* in Persian Gulf and Oman Sea. Although these unique alleles were not abundant, they are potentially important as a specific tool for following pedigrees in breeding programs, and for marker-assisted selection. Such approach has been reported by Wolfus *et al.* (1997), who utilized microsatellite DNA for analyzing genetic diversity in shrimp breeding programs, and by Moore *et al.* (1999) in their study on development and application of genetic markers for the Kuruma prawn, *P. japonicus*. Frequency of allele for each locus was obtained by dividing total number of each allele per population by total number of all alleles at the locus. An allele with a frequency <0.01 was considered as a low-frequency allele. A locus is considered to be polymorphic when frequency of the most common allele (F) is + /< 0.99 (Nei 1987).

Heterozygosity deficit and null alleles

There were many incidences of deficiency/reduction in observed heterozygosity as compared to expected heterozygosity in most loci except for locus E2 for Hormoz and Kuhestak and

locus F5 for Hormoz, Kuhestak and Jask. This deficiency may be due to inbreeding, genetic drift or consequences of illegal overharvesting of *P. semisulcatus* in the studied area. The difference between observed heterozygosity and expected heterozygosity values can indicate important population dynamics. Beardmore *et al.* (1997) mentioned that if the observed heterozygosity is lower than expected, one should seek the cause for the divergence such as inbreeding. They also suggested that if heterozygosity is higher than expected, it means there is high genetic variability and this could be a result of mixing of two populations which were previously isolated. These heterozygosity deficits may also be due to presence of null alleles (Pemberton *et al.* 1995). Population genetics in this study of three populations revealed that almost all loci deviated from HWE, Hardy Weinberg Equilibrium ($p<0.001$). However, three detected loci were in equilibrium (locus E2 in Hormoz, locus C6 in Kuhestak and locus B9 in Hormoz). Micro-Checker (Van Oosterhout *et al.* 2004) was used to find the most possible causes of deviation from HWE. Micro-Checker analysis showed no sign of scoring error owing to stuttering or from large allele dropout. However, Micro-Checker analysis showed signs of null alleles in three microsatellite loci (B5, C6 and C9). The existence of null alleles is common in fish and inheritance of null alleles in microsatellite loci have been reported by Lehmann *et al.* (1996) and van Treuren (Van Treuren 1998). Six cases of heterozygosity deficit were found in population studies of *P. monodon* (Xu *et al.* 2001). They mentioned that the observed deficiency may be due to existence of null alleles (Pemberton *et al.* 1995) which were scored as homozygous instead of heterozygous. Deviation from HWE was tested in that study based on null allele data set. The results showed that there was no heterozygosity deficiency in three large microsatellite loci but the four wild populations investigated in this study were departed from HWE in three small microsatellite loci. Deviation from HWE could be resulted from selection, population mixing or non-random mating (Raymond 1995). In this study, small population size of Hormoz and Kuhestak coupled with migration of broodstocks among the population areas (these two population sites are close) may affect disequilibrium of populations in studied areas. A high level of heterozygosity and therefore genetic variation is important in population studies because it provides a large variety of genotypes for adaptive response to changing conditions (Wang *et al.* 2020). Xu *et al.* (2001) stated that decrease in genetic diversity may enhance susceptibility of organisms to disease and other selective factors, resulting in additional decline in population size. This study suggests that reduction in observed heterozygosity compared to expected

heterozygosity may reduce resistance of studied populations against diseases.

Fixation indices (F_{st}) and effective number of migrants (*Nm*) were estimated in order to understand genetic discrimination between populations. The fixation index (F_{st}) is the standardized measure of population differentiation based on genetic polymorphisms and reveals genetic differences among populations.

Assignment Test

An assignment graph was generated to assign individuals to their original population based on allele frequencies. In this study, the frequency-based assignment test was done using GenAlex software. An individual can be assigned to a source based on the expected frequency of its multilocus genotype in various assumed sources (Paetkau *et al.* 1995). You *et al.* (2008) utilized assignment test in order to distinguish connectivity between various populations of black tiger shrimp (*P. monodon*) in Indo-Pacific region. Results obtained from assignment test illustrated that populations in West Indian Ocean were unique, while other studied populations were partially admixed. This approach can be applied to study migration among populations and thus plays a major role in studies of connectivity of marine populations.

Genetic changes in populations

Values of deviation in heterozygosity due to population subdivision (F_{st}) are regularly expressed as the value of genetic diversity due to allele frequency differences among populations. The values range from 0 to 1. A zero value implies complete panmixis, i.e., the two populations freely interbreed. A value of one would imply the two populations are completely separated and isolated with no gene flow among them (Holsinger and Weir 2009). Results on pairwise F_{st} indicated that the three populations were only slightly different from each other, genetically. Although heterozygosity deficits were observed at most microsatellite loci within the populations, these three populations diverged from each other based on pairwise F_{st} values.

Hartl and Clark (1997) and Wright (1984) classified an F_{st} value between 0.00 to 0.05 to indicate low genetic divergence, a value between 0.05 to 0.15 to indicate moderate genetic divergence, between 0.15 to 0.25 to indicate high genetic divergence, while more than 0.25 indicated a massive genetic divergence between studied populations. Wright (1984) also reasoned that an F_{st} value of less than 0.05 may be considered as an important indication that each population may harbour high but different genetic composition. Furthermore, the amount of F_{st} would not reach 1 most of the time because common polymorphisms due to mutation occur frequently in populations, which reduce F_{st} value (Hedrick 1999; Wright 1984).

Gene Flow

Using F_{st} as an indirect measure of gene flow is suggested by Wright's island model of population subdivision (Slatkin, 1985). Based on (Slatkin, 1985), populations may be treated as replicates that are characterized by just two parameters: population size (N) and migration rate (*m*). The strength of genetic drift is comparative to 1/N, while the strength of gene flow is relative to *m*. In the present study minimum F_{st} 0.014, (p<0.05) and maximum N*m* (17.564) was between Kuhestak and Hormoz. These data showed minimum gene flow between Jask and Hormoz and maximum gene flow between Hormoz and Kuhestak population. Genetic differentiation and gene flow among the three populations may be resulted from many factors such as hydrodynamic structures (water currents) or biological events as shown in other fishery industries (Ruzzante *et al.* 1998). In a marine environment, water currents and also oceanographic characters like eddies can prevent mixing and dispersal of pelagic larvae (Weersing and Toonen 2009). There are several currents generated in Oman Sea (Reynolds 1993), which influence movement of broodstocks or shrimp larvae towards Straits of Hormoz and Persian Gulf as well as affect gene flow especially between Hormoz and Kuhestak populations. These two areas are close (100 km distance) and can exchange larvae. Therefore, gene flow is expectedly high between these two areas as compared to the other population (Jask area). Penaeidae shrimps often live in various habitats during their life cycle. Therefore, they have to migrate between these habitats to complete the life cycle. Four types of migrations are explained by Dall *et al.* (1990): (1) migration of larvae and post larvae from spawning area to nursery ground, (2) migration of juveniles out of the nursery region, (3) migration of adults to the deeper waters offshore, and (4) migration for spawning in various species. Most penaeid species spawn offshore, whereas the juveniles favor estuarine or inshore habitats (second and third types). Vertical migration during pelagic larval phase, attached to transport by water currents, is the mechanism that brings post larvae to their nursery areas (Khorshidian *et al.* 2002). Thus, this movement facilitates gene flow among populations (Chakravarty *et al.* 2018).

Another factor that could increase gene flow between Hormoz and Kuhestak is life cycle behavior and movement pattern of *P. semisulcatus* during spawning season. Jackson *et al.* (2001) described that larvae belonging to Penaeidea family may migrate as much as 100 km between offshore spawning ground and inshore nursery habitats. In conclusion, pairwise comparison based on allelic and genotypic frequencies in three populations of *P. semisulcatus* indicated that the three populations were significantly different from each other (p<0.05). Based on an AMOVA test, high levels of pairwise F_{st} (0.106) and

low levels of N*m* (2.103) observed between Hormoz and Jask populations indicated restricted gene flow between the two populations. By comparison, low levels of F_{st} (0.016) and high levels of N*m* (15.876) observed between Hormoz and Kuhestak populations indicated high gene flow between these populations. The assignment test revealed higher gene flow in Hormoz and Kuhestak and restricted genetic flow between Jask and both Hormoz and Kuhestak populations. High connectivity between Hormoz and Jask may be due to influence of Persian Gulf currents, or life cycle of *P. semisulcatus* in Hormoz Strait. Alternatively, presence of ecological barriers such as mangroves (along Qeshm Island and offshore of Hormoz) may result in restricted genetic flow between Jask and both Hormoz and Kuhestak populations. These findings provide new input for selection of genetically-suitable broodstocks for this important species.

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