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PROOF RESEARCH PAPER

Analysis of Genetic Diversity of White Shrimp (*Metapenaeus Affinis*) from the Northwest of the Persian Gulf Using Microsatellite Markers

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Abstract

We investigated the genetic structure of *Metapenaeus affinis* population from the northern coasts (Khuzestan Province: Bahrakan and Lifeh-Boosiaf region) of the Persian Gulf, using five microsatellite markers. The samples of pleopods tissue of shrimp were taken, DNA extracted and PCR was performed on microsatellite primers. The results revealed that all five microsatellite loci were polymorphic, the number of alleles (*N*a) ranged from 5 to 12 per locus, and the observed and effective number was 7 and 3.67, respectively, also the observed and expected heterozygosis was ranged from 0.1 to 0.77 and 0.1 and 0.83, respectively, which indicated that the two populations of *M. affinis* possessed a rich genetic diversity. The deviation from Hardy–Weinberg equilibrium ($P<0.05$) was observed that due to the heterozygote deficiency in populations. Based on the analysis of molecular variance (AMOVA), the genetic variation among stocks (17%) was much lower than that within stocks (83%), and F_{st}, R_{st}, and N_m were 0.107, 0.372 and 2.092, respectively. The highest genetic distance was 0.571 and the lowest was 0.561. Genetic distance and cluster analysis using UPGMA divided the two stocks into two groups. The present study showed that two different populations of *M. affinis* had a certain genetic diversity.

Keywords: *Metapenaues affinis*, microsatellite, genetic diversity, genetic differentiation, Persian Gulf.

Introduction

The northwest of Persian Gulf supports one of the traditional fishery activities in Iran and Iraq coastal region, where the most important fishery resource depends on the benthic environment. As an economically valuable marine crustacean, the study of genetic diversity of the indigenous species in aquaculture will help to the management the selective breeding programs, because the shrimps are important as the most consumed fishery products due to high demand for them in the world markets (Mehanna *et al.*, 2012).

Penaeus is a genus of prawns, found in tropical and subtropical waters around the world that have aquaculture and economic value in the Persian Gulf and Oman Sea. Jinga shrimp (*Metapenaeus affinis*) is an Indo-West Pacific species of family Penaeidae, which ranges from the Arabian Sea to the Malay Archipelago and Hong Kong (Holthuis, 1980). This white shrimp species that is naturally distributed along the Persian Gulf and Oman Sea water to southern India and Sri Lanka, and in the east is continued in Philippines and Taiwan islands (FAO, 1985). *M. affinis* is introduced as dominant target fishing in the Persian Gulf in the depth 5 to 90 m and restricted to the muddy bottom habitat in the coast, estuary and sea of Khuzestan (native area: the western of Lifeh-Boosif and eastern of Bahrekan), Bushehr and Hormozgan Province of Iran (FAO, 2001), so the conservative management of *M. affinis* resources is required and must include aspects of the genetic diversity of the species to ensure sustainability of fisheries resources. Despite the much information exists about the physiology of this species, there is no information about the genetic relationship between of their population (Baldwin & Bass, 1998).

Climate and geographic changes over many years affected the populations and cause changes in genetic diversity and species extinction. Meanwhile, the identification of wild shrimp stocks is also important to plan to provide a clues to the populations life histories and degree of evolutionary isolation (Okumus and Ciftci, 2003) or a successive generation of wild genetic diversity in domestication and selective breeding programs (Benzie, 2000; Klinbunga *et al.*, 2001; Goyard *et al.*, 2003; Li, Li, Wang, He, & Liu, 2006). Furthermore, use of genetic markers has been greatly ameliorated our knowledge of differentiating between individuals in a population in penaeids (Benzie, 2000).

The most common analysis that used to provide valuable population-level information on variation and the degree of genetic subdivision within populations and between populations of shrimp species is Allozyme (Rosa-Velez *et al.*, 2000; Garcia-Machado *et al.*, 2001). However, molecular markers such as microsatellite (Valles-Jimenez, Cruz, & Perez-Enriquez, 2005; Ball & Chapman, 2003; Zhang, Wang, Li, Zhang, & Kong, 2014) has revealed more variability than allozymes. Microsatellites with characteristics of high reproducibility, polymorphism richness and dominance genome distribution (Song, Li, Liu, Chen, & Gao, 2011), have also been used to estimate the genetic population structure and diversity (Sun, Diaz, Salomon, & Von Bothmer, 2001).

In some penaeids, dispersal capacity and geographic differentiations have been observed during their larval planktonic phases caused by some environmental and biological factors (e.g., oceanographic current and reproductive behavior) (Fe´ral, 2002). Hence, the main objectives of this study were to assess the intra and inter-population genetic variations and genetic differentiation in two populations of *M. affinis* inhabiting the coasts of the Persian Gulf (Bahrakan and Lifeh-Boosif) using the microsatellite DNA markers developed by Fitzsimmons, Moritz, and Moore (1995).

Materials and Methods

Sampled Stocks

A total of 60 samples of wild white shrimp (*M. affinis*) were collected using the bottom trawl net from two different locations along the coast of Khuzestan Province in the North-West of Persian Gulf, Iran (Lifeh-Boosif, n=30 and Bahrakan, n=30) (Figure 1). The studied area lies between latitudes 48°30' to eastern 49°50' and latitude 29°40' to northern 30°10'. The depth of the Bahrakan and Lifeh-Boosif as study area from of 8 to 14 and 2 to 3 meters in depth respectively and the substrate is mainly muddy and sandy in some parts. For all samples, muscle tissue was removed from each specimen and immediately stored in 96% ethanol.

DNA Extraction

Total genomic DNA was extracted from the 40 mg of pleopod muscle of *M. affinis* according to ammonium acetate method as described by Fevolden and Pogson (1997). Genomic DNA was dissolved in 100 µl of TE buffer and stored at -20ºC. The quality of DNA was assessed by agarose gel (1%) electrophoresis and the concentration was adjusted to 50 ng μl^{-1}.

Microsatellite Loci Amplification

Five microsatellite markers with clear and reproducible polymorphic fragments were selected in this study (Table 1). Polymerase chain reaction (PCR) amplification was performed in a total volume of 25µ mixture, including 100 ng of DNA template, 1 µl of forward primer (20 pmol) and 1 µl of reverse primer (20 pmol), 1 µl of 5mM 4dNTP mix, 1 µl of 50mM $MgCl_2$, 2 µl of 10x Taq polymerase buffer, one unit of Taq DNA polymerase (Fermentas, Germany). The temperature profile consisted of initial denaturation for 3 min at 94°C followed by 30 cycles of 30 s at 94°C, 45 s at the respective annealing temperature, and 1 min at 72°C, and a final extension for 7 min at 72°C. The PCR products were evaluated by electrophoresis on 8% denaturing polyacrylamide gels containing 19:1 acrylamide: bis-acrylamide and 5 M urea. Electrophoresis was conducted using a SequiGen sequencing gel electrophoresis system (BIO-RAD Laboratories, Hercules, CA). DNA fragments were visualized by silver nitrate staining (Bassam, Caetano-Anolles, & Gresshoff, 1991). Allele sizes (base pairs) were obtained by comparison to a pBR322 DNA/AluI Marker, 20 (Fermentas, Germany) sequencing ladder. The size of each allele was estimated using the DNAfrag program, version 3.03 (Nash, 1991).

Genetic Diversity Using Microsatellite Markers and Departure from Hardy-Weinberg Equilibrium

A genotypic data matrix was constructed for all loci. The expected heterozygosity (H_o) was calculated directly from observed genotypes and the number of the alleles (A) for each population at each locus was determined using the program MICROCHECKER version 2.2.3 (Van Oosterhout, Hutchinson, Wills, & Shipley, 2004). This program used to identify possible null alleles, large allele dropout, scoring error due to stutter peaks and possible typographic errors, before proceeding with further analyses. Allele and genotype frequencies that were identified as affected by the presence of null alleles were adjusted according to the Brookfield I method (Van Oosterhout *et al.*, 2004). The effective number of alleles (N_e) was calculated using the following formula (Valles-Jimenez *et al.*, 2005):

$$N_e = 1/ \Sigma x^2_{i,}$$

where x_i is the frequency of the i^{th} allele for each locus. Nei's genetic distance (GD) (Nei, 1978) between stocks calculated by POPGENE (Yeh, Yang, & Boyle, 1999). Heterozygous deficiency or excess at each locus was calculated by (Valles-Jimenez *et al.*, 2005):

$$D = (H_o - H_e)/ (H_e).$$

Departures of genotype data from Hardy-Weinberg equilibrium (HWE) at each population were tested, was estimated using the software GENEPOP version 1.31 (Yeh *et al.*, 1999) with 1000

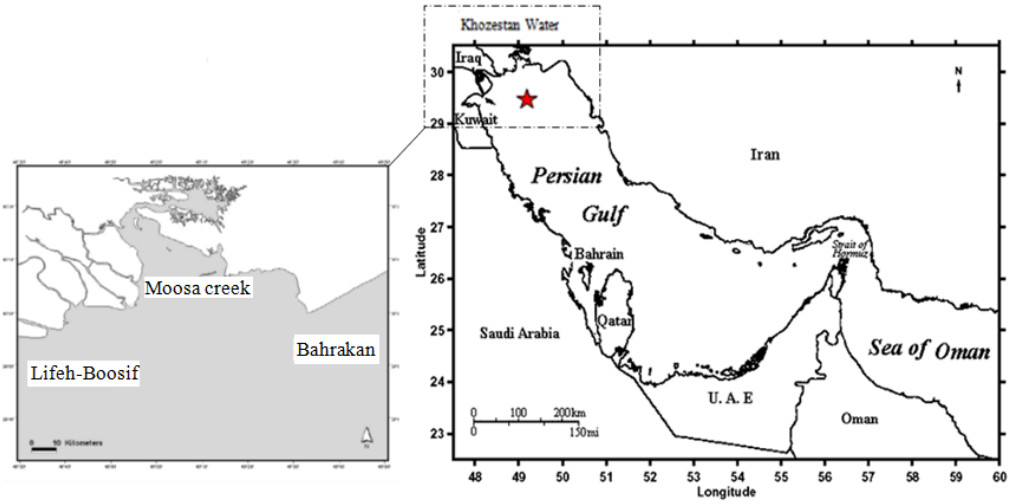

Figure 1. Map of the Persian Gulf and location of the Bahrakan and Lifeh-Boosif white shrimp stocks, along the coast of Khozestan inshore water as study area.

Table1. Detailed characteristics of *M. affinis* amplified microsatellite loci

Locus	Forward & reverse primers(5'→3')	Repeat motifs	Annealing Tm (ºC)	Size (bp)	Na	GenBank
TUZXPm 4.82	F:ATTCATCAGCTAGCCTTG R:CGTTTACTGCATTCACTACC	(CA)6…(A)14(ATA)4	42	120-145	8	AF077594
TUZXPm 2.41	F:AAGGCAGATTTTCTAGCC R:ATCAAGGGAGACATTCAG	(TTTA)14…(CT)3	42	252-284	12	AF077555
TUZXPm 4.9	F:ATCTGACAGGGCACCATAC R:AGTCGAGTCTTGAATAAGCG	(A)23C(AT)21GT(AT)6 (ATTT)2(ATTTATTC)5 (AT)CT(ATTT)8…(AG)3	49	275-284	11	AF077568
TUZXPm 4.45	F:ATCTCTACCAACCTGTCAGC R:TTAGTGAACCCCCTTCGTG	(CA)6(TA)31(TGTA)7(TA)2 (TGTA)4(TA)2TG(TA)3	42	412-434	8	AF077582
TUZXPm 4.85	F:CTTCGGCGGAAATATGTG R:TTGTGTTTGTGCGAGTGC	(CA)4T(AC)G(CA)(CG)3TA (CA)9TA(CA)T(AC)2GC(AC) T (CG)(CA)43T(ACGC)2G (CA)15CTT(AC)6TT(AC)2TC (AC)3(GCTCTC)2(GCACTC) 4	49	268-290	10	AF077596

simulated samples. GENEPOP is a user-friendly Microsoft Window-based computer package for the analysis of genetic variation among and within natural populations using co-dominant and dominant markers and quantitative traits.

To estimate genetic differentiation among individuals in all two stocks, F-statistic (F_{st}) (Wright, 1978) and R_{st} indices (Slatkin, 1995), were calculated using GENEPOP (Raymond & Rousset, 1995). F_{st} estimated for apportioning the variance in allele frequencies among and within stocks and R_{st} was introduced as a counterpart to F_{st} for use with loci which are assumed to follow a generalized the stepwise mutation model (SMM) (Slatkin, 1995). Pair-wise multilocus estimates of the effective number of migrants (N_m) for stocks from Bahrakan and Lifeh-Boosif based on private alleles were computed using GENEPOP version 1.31. The number of alleles per locus and allele frequencies was compared to measure the overall change in genetic diversity of the two introduced stocks, Bahrakan and Lifeh-Boosif. The distribution of genetic variation among and within stocks was analyzed by analysis of molecular variance (AMOVA) with GenAlEx version 6 software package (Peakall, & Smouse, 2006). The GenAlEx version 6 software packages were used for estimating allele frequencies and for applying the homogeneity test between populations. GenAlEx 6 is written in Visual Basic for applications (VBA) within Excel. GenAlEx requires all data to be coded as numbers and formatted within Excel as numeric data.

Differentiation Analyses

The dendrogram was constructed and Cluster analysis was performed to generate a dendrogram based on the unweighed pair group method with arithmetic averages using MEGA software version 4 (Tamura, Dudley, Nei, & Kumar, 2007). The MEGA 4 software includes distance matrix and phylogeny explorers as well as advanced graphical modules for the visual symbol of input data and output.

Results

Genetic Diversity

The genetic diversity of *M. affinis* from two geographic locations in the Persian Gulf was investigated by five microsatellite loci. The number of alleles, size range, and heterozygosity for each microsatellite locus are shown in Table 1. For all stocks of white shrimp, the five microsatellite loci were shown polymorphism and exhibited a high number of alleles per locus. The number of alleles ranged from 5 to 12 per locus, with varied in size from 120 to 434 bp. TUZXPm2.41 had the maximum number of alleles (12) in Bahrakan and the minimum numbers of alleles at TUZXPm4/45 and TUZXPm4/82 were 5 alleles (Table 2). The number of effective alleles per locus displayed a varied degree of polymorphism considerably, from 1.230 at the locus of TUZXm 4.9 to 5.960 at the locus of TUZXm 2.41 in Bahrakan and in all stocks N_e was lower than N_a (Table 2). The population differentiation value between Bahrakan and Lifeh-Boosif was indicating a large differentiation 0.117. The average observed heterozygosity (H_o) values of all stocks in five loci ranged from 0.1 to 0.77. The expected heterozygosity (H_e) varied between 0.1 and 0.83 and was greater than H_o. As shown in Table 3, the Hardy-Weinberg equilibrium, deviations ($P<0.05$) were observed and revealed that populations of Bahrakan and Lifeh-Boosif due to the heterozygote deficiency significant departed from the Hardy-Weinberg law. All of the populations departed from Hardy-Weinberg law at all of the five microsatellite loci ($P>0.05$).

Genetic Differentiation

Altogether, significant F_{st} value of 0.107 indicates substantial genetic differentiation of the *M. affinis* stocks sampled from the Persian Gulf at the five microsatellite loci. The pairwise F_{st} values showed significant genetic differences between all pairs of Bahrakan and Lifeh-Boosif populations (Table 4). Genetic differentiation was displayed for each locus by the fixation indices F_{st} and R_{st} (Table 4). The results of the F-statistic analysis with a mean

Table 2. Allelic frequencies of, *M. affinis* population at two locations and five loci

Population	Parameters[a]	Locus				
		TUZXm 4.82	TUZXm 2.41	TUZXm 4.9	TUZXm 4.45	TUZXm 4.85
Bahrakan	N_a	5	12	5	7	7
	N_e	2.740	5.960	1.230	4.139	2.007
	H_o	0.300	0.367	0.100	0.100	0.233
	H_e	0.635	0.823	0.187	0.793	0.502
Lifeh-Boosif	N_a	8	6	7	7	8
	N_e	3.782	4.826	3.814	2.932	4.627
	H_o	0.767	0.200	0.330	0.300	0.200
	H_e	0.736	0.793	0.738	0.659	0.784

Notes: [a]N_a, observed number of alleles; N_e, effective number of alleles; H_e, expected heterozygosity; H_o, observed heterozygosity

Table 3. Departure from Hardy-Weinberg Equilibrium of, *M. affinis* population at two locations and five loci

Population	Parameters[a]	Pm 4.82	Pm 2.41	Pm 4.9	Pm 4.45	Pm 4.85
	Df	10	45	10	21	21
	χ^2	47.468	124.839	9.41	686.121	58.856
Bahrakan						
	P	0.00***	0.00***	0.00***	0.00***	0.00***
	Df	28	15	21	21	28
Lifeh-Boosif	χ^2	57.940	80.332	117.974	66.892	128.363
	P	0.001***	0.00***	0.00***	0.00***	0.00***

Notes: [a] *Df*, Degrees of freedom. The probability of significant deviation from Hardy-Weinberg equilibrium (*P*) is indicated for each population and locus, *** significant at the 5% level

Table 4. Pairwise F_{st}, R_{st} and N_m estimates among two populations of *M. affinis*

Population 1	Population 2	F_{st}	R_{st}	Nm	*P*
Bahrakan	Lifeh-Boosif	0.111	0.390	2.271	0.01**
Lifeh-Boosif	Bahrakan	0.103	0.354	2.309	0.01**

** significant at $P< 0.01$

of 0.107 indicated that there was moderate differentiation among the stocks. All analyzed of the multilocus F_{st} values showed a significant at $P< 0.01$. According to Wright (1978), F_{st} values ranging from 0 to 0.05, from 0.05 to 0.15, and from 0.15 to 0.25 indicated a low, moderate and high level of genetic differentiation, respectively. The mean of F_{st}, R_{st}, and N_m values were 0.107, 0.372 and 2.90 respectively, between two populations from the Persian Gulf (Table 4). Significant *F* and *R*-statistics values for all *M. affinis* samples were found between Bahrakan and Lifeh-Boosif populations (Table 4). The comparison between the two stocks at the size frequency distribution of the five microsatellite alleles are shown in Figure. 2. A single locus, TUZXPm 2.41, exhibited the same alleles for both stocks of *M. affinis*. The remaining loci shared most of the alleles in both stocks, but also exhibited alleles that were unique to each one, at low frequencies (Figure. 2). Loci TUZXPm 4.9 and TUZXPm 2.41 showed the highest allele size variation, and some unique alleles were present in each stock (Figure. 2). All of the stocks by tests for genetic differentiation per locus showed significant allele frequency differences overall ($P<0.05$). The results of AMOVA (Figure. 3) showed that the two studied stocks of *M. affinis* had high and low genetic diversity within and among populations, respectively. This result, in Pair-wise Population Assignment Graphs using NMS analysis (Figure. 4), indicated that the shrimp in the coast of Khuzestan Province region from the Persian Gulf were genetically distinct.

Genetic Distance and Phylogenetic Reconstruction

To analyze the degree of genetic differentiation among the stocks of *M. affinis* from the Persian Gulf, we used the Nei's original measure of genetic identity (I) and genetic distance (GD) (Table 5). The highest genetic distance and lowest genetic identity were found 0.561, 0.571 respectively. Cluster analysis performed using UPGMA divided the two stocks into two groups (Figure. 5). The Neighbor-joining trees showed the first cluster A included two populations of Bahrakan and Lifeh-Boosif, while the second cluster B belonged to a single population from Bahrakan (Figure. 5).

Discussion

In during of time, a species may carry out micro-evolutionary processes and undergo genetically divergent into sub-populations or stocks, if reproductively and geographically isolated (Carvalho & Hauser, 1994) and have to be treated as separate management units (Moritz, 1994). The occurrence of genetic variation is necessary for adaptation of populations to change in environmental conditions. Therefore, identification of the genetic structure of species is an advantage to manage of aquatic resource (Thai, Pham, & Austin, 2006). To determine the geographical distribution and genetic characteristics of naturally isolated populations of penaeid shrimps, DNAbased markers by high variability and reproducibility showed much hig levels of diversity in natural populations (Benzie, 2000), and is a basic necessity for the principle conservation.

The results showed that based on the number of alleles as the main index of genetic differentiation, the five microsatellite loci were shown polymorphism and exhibited a high number of alleles per locus. The number of alleles for *M. affinis* over the five microsatellite loci ranged from 5 to 12, with varied in size from 120 to 434 bp (Table 2), which was similar to the number (2–13, 5-10, 3-12) reported by Valles-Jimenez *et al*. (2005), Rezaee, Farahmand, and Nematollahi (2016), and Lima, Silva, Oliveira, Maggioni, and Coimbra (2010) for Pacific white shrimp (*Litopenaeus vannamei*) respectively, the number (6-14) that reported by Xu, Primavera, Pena, Pettit, Belak, and Warren (2001) for black tiger shrimp (*Penaeus monodon*), (5-16) by Zhang, Kong, Wang, and Wang (2010) for *P. chinensis*, and (5-15) by Gao *et al*. (2008) for Chinese shrimp *Fenneropenaeus chinensis.* The tests for genetic differentiation showed significant allele frequency differences in all of the stock and suggested a moderate degree of similarity among the Bahrakan and Lifeh-Boosif populations of white shrimp (*M. affinis*) from the Persian Gulf evaluated in the current study. However in all stocks, some allele frequencies were similar but, the exits of private alleles indicated the specific stocks could be formed at certain levels. These results suggested that the stocks of Bahrakan and Lifeh-Boosif before multiple generations through natural selection, might have a common origin and during the selective breeding, new alleles from wild populations were introduced. Analysis of genetic diversity and allele variation among the stocks of white shrimp from the Persian Gulf using the five microsatellite loci has revealed high levels of genetic diversity and source of genetic differentiation among the two different geographic populations of *M. affinis*. These results similar to that reported by, Zhang *et al*. (2014) for seven stocks of *L. vannamei*, which were introduced from Central and South America to China. Heterozygosity (H) values, is the best parameter for the measurement of genetic variation in natural populations that also known as gene diversity (Zhi-min, Li, Fu-liang, & Guo-liang, 2010; Xu *et al.*, 2001). Lima *et al*. (2010) reported that the average observed heterozygosity for the two marine shrimp hatcheries of the Pacific white shrimp *L. vannamei* (Boone, 1931) ranged from 0.143 to 0.841 which was lower than the average expected heterozygosity varied from 0.377 to 0.878. Those data were consistent with our results, which indicated the two studied stocks have rich genetic diversity based on allelic variation. Because in this study, the average observed heterozygosity (H_o) values of all stocks in five loci

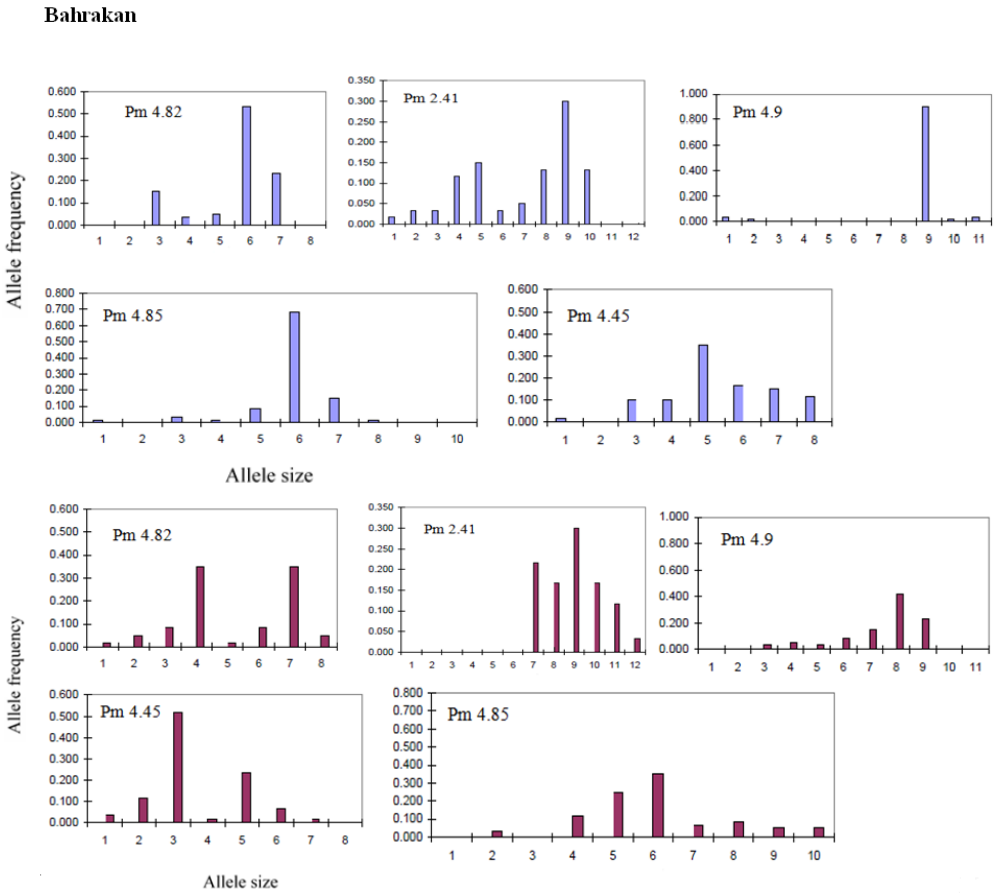

Figure 2. Genetic composition of two stocks at each microsatellite locus of *M. affinis.*

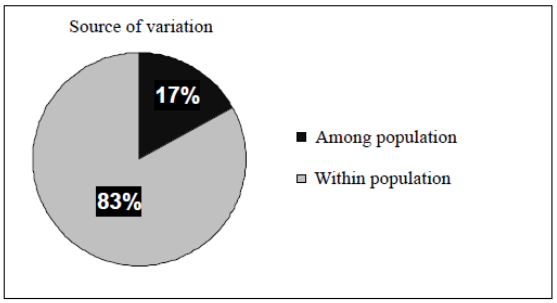

Figure 3. Results of AMOVA, for genetic diversity (Percentage variation (%)) of two stocks of *M. affinis*

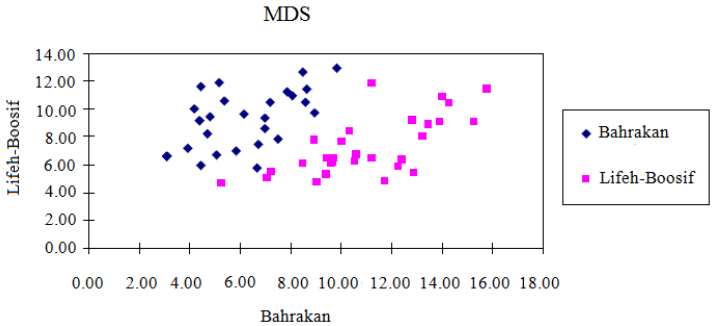

Figure 4. Multidimensional scaling analysis of *M. affinis* based on genome-wide identity-by-state pair-wise distances inferred with complete data at five loci. This graph displays the genetic relationships between Bahrakan and Lifeh-Boosif white shrimp stocks.

Table 5. Nei's original measure of genetic identity and genetic distance among two populations of *M. affinis*

Population[a]	Bahrakan	Lifeh-Boosif
Bahrakan	-	0.571
Lifeh-Boosif	0.561	-

Notes: [a]Data were obtained using GENEPOP (Nei, 1978). Nei's genetic identity (above diagonal) and genetic distance (below diagonal)

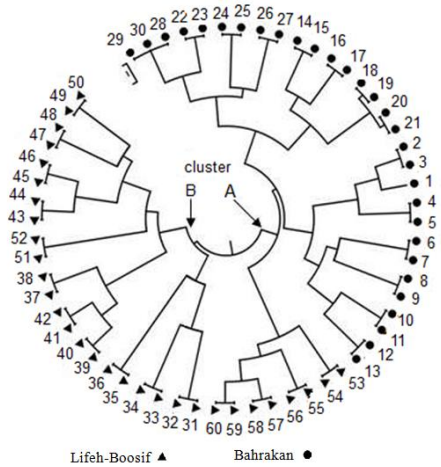

Figure 5. UPGMA dendrogram based on genetic distance showed two major clusters (Bahrakan: samples 1-30; Lifeh-Boosif: samples 30- 60).

ranged from 0.1 to 0.77 reflects the degree of consistency in genetic structure in a two population and the expected heterozygosity (H_e) varied between 0.1 and 0.83 was greater than H_o. According to the Zhang *et al*. (2014), heterozygosity of each stock was higher than 0.5, indicated a high level of genetic diversity. In the current study, the observed heterozygosity for 5 microsatellite loci in all stocks was lower than the expected heterozygosity except at locus of TUZXPm4.82 and indicating heterozygote deficit. As suggested by Zhang *et al*. (2014), one possible reason is that genetic variation changed after several rounds of artificial selection and the stocks had a high heterozygosity within and among populations. The reduction of the observed heterozygosity means less of genetic variation it may result from the forces such as inbreeding, bottleneck, and environment stress or habitat destruction (Supungul *et al.*, 2000). In the present study, all five studied microsatellite loci revealed the varied degree of polymorphism, significant differences in the genetic heterogeneity and allele frequencies. Also, Robainas, Monnerot, Solignac, Dennebouy, and Espinosa (2002) by evaluation the genetic variability from the pink shrimp *Farfantenaeus notialis* (Crustacea, Decapoda) in Cuba reported seven pairs of their primers showed reliable amplification products and five of them was polymorphic. The maximum of heterozygosity was found in white shrimp samples from Lifeh-Boosif populations that indicated, the studied populations have rich genetic diversity based on allelic variation. Lifeh-Boosif area is located at the mouth of the Arvand Rud River that continues toward the Karun river; Persian Gulf, Iran. By entrance the fresh water from the Arvand Rud Rivers to the Persian Gulf, in this region, salinity to somewhat decreased and observed increasing in organic or detritus matter and finally leads to richness. So, for white shrimp, an appropriate environmental condition (nursery grounds) is provided. While in Bahrakan the condition is not so much suitable. The deviation from Hardy–Weinberg equilibrium ($P<0.05$) were observed that due to the heterozygote deficiency in populations of Bahrakan and Lifeh-Boosif. A similar result has been reported by other researcher, including the whiting *Merlangius merlangius* (Rico, Ibrahim, Rico, & Hewitt, 1997), *P. monodon* in Philippine (Xu *et al*., 2001), *P. monodon* in Thailand (Supungul *et al.*, 2002), *P. chinensis* (Meng, Wang, Jang, Liu, & Kong, 2009). Heterozygous deletion can be the result of the defeat to amplify one of the alleles (Machado-Tamayo, 2006), as loss of rare alleles, in addition to null alleles and sample size (Antoro, Na-Nakorn, & Koedprang, 2005). Further, it can be caused by the Wahlund effect that refers to the reduction of heterozygosity due to the subdivision of the local stocks into isolated and differentiated reproductive units (Machado-Tamayo, 2006), or genetic drift and a bottleneck effect, resulting in changes in allele frequency in multi-generational breeding (Zhang *et al.*, 2014). From the comparison of obtained heterozygosity and level of polymorphism variation depended on the locus, we suggest that however the Bahrakan and Lifeh-Boosif stocks had a relatively high level of genetic diversity, there was also to a somewhat degree of genetic

information loss. In tropical species of shrimp, as showed in our results based on genetic differentiation, N_m values higher than 1, is one of the indices of genetic diversity among populations, and is necessary for genetic drift which tends to make populations genetically more heterogeneous (Oliveira, Padua, Zucchi, Vencovsky, & Carneiro Vieira, 2006). An important indicator of the level of genetic differentiation among populations is fixation indices F_{st} and R_{st}. Pairwise genetic differentiation (F_{st}) values range from 0 to 1, with larger numbers correlating to a greater degree of genetic differentiation among groups (Zhang *et al.*, 2014). The mean of pairwise F_{st}, R_{st}, and N_m values were 0.107, 0.372 and 2.90 respectively, between two populations Bahrakan and Lifeh-Boosif of *M. affinis*, indicating that despite high gene flow (N_m) (Oliveira *et al.*, 2006), moderate genetic differentiation exists among populations (Zhi-min *et al.*, 2010). In a similar study, Rezaee *et al*. (2016) determined a F_{st} of 0.133 in Amiri, Gorgeaj, and Gomishan stocks of *L. vannamei*. The results of molecular variance (AMOVA), showed that 17% of the variation occurred among stocks and 83% among individuals within populations that indicating moderate genetic differentiation and remarkable genetic diversity among the two studied stocks of *M. affinis*, consistent with the results of Rezaee *et al*. (2016) and Zhang *et al*. (2014) for *L. vannamei*. A dendrogram based on genetic distance showed two major clusters from two populations. The genetic relationship between two locations (Bahrakan and Lifeh-Boosif), in some clusters, were not discriminated. It may consequence from migration between two different geographic populations. In Conclusions, the data demonstrated that the two studied stocks of *M. affinis*, displayed a rich level of genetic diversity and moderate F_{st} values indicated the importance of constant evaluation of genetic diversity in populations of *M. affinis* shrimp in the coast of Khuzestan Province region from the Persian Gulf, Iran. The heterozygosity values of all stocks in five loci is high (>0.5) that means two populations Bahrakan and Lifeh-Boosif of *M. affinis* have a rich genetic diversity. In order to maintenance genetic diversity of Penaeus shrimp resources for future and design of suitable conservation and management, this data can be applied.

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