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**Research Article**

# Genetic identification of *Penaeus vannamei* broodstocks in Hormozgan and Bushehr Provinces using COI mitochondrial gene

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**Abstract**

Given the role of *Penaeus vannamei* species in shrimp farming of Islamic Republic of Iran's economy (Bushehr and Hormozgan provinces), the proper management of brood stocks in order to reduce the inbreeding coefficient of mentioned species is unavoidable. Sampling has been conducted from two hatcheries in Hormozgan province and one hatchery in Bushehr Province produced post larvae. Total of 18 samples were collected from three regions and used for genetic investigation. Seven haplotypes have been identified from the 502 aligned sequence derived from the COI gene. Phylogeny study showed all samples from three stations showed one clade with two main clusters showed highly Homozygosity of Bushehr and Hormozgan hatcheries. In this study, the maximum amount of fixation ($F_{st}$) statistics was observed between Bushehr and Hormozgan samples with minimal gene flow. Also the minimum amount of Fst was recorded between both hatchery stations from Hormozgan province which had the highest gene flow. Thus, according to the results obtained from this project, any productive application of *vannamei* shrimp brood stocking for post larvae reproducing should be performed by consideration to genetic research and also genetic differentiation between imported brood stocks.

**Keywords**: *Penaeus vannamei*, West white shrimp, Cytochrome oxidase, Persian Gulf

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## Introduction

Shrimp is one of the world's most valuable fishery resources. Shrimp of the genus *Penaeus* constitute a diverse and abundant group of benthic taxa found in the tropical and subtropical waters (Tamadoni Jahromi *et al.* 2019). Consequently this is one of the most important commercial shrimp in the Persian Gulf and Oman Sea (Pourmozaffar *et al.* 2019b).

Marine shrimps of the family Penaeoidea represent approximately one third of the world's commercially important shrimp species and account for over 80% of the wild catch (Tamadoni Jahromi *et al.* 2020a).

Increased susceptibility to diseases and reduced growth may be the significance of low levels of genetic variability (Gozari *et al.* 2016; Pourmozaffar *et al.* 2019c; Wolfus *et al.* 1997). Also Highly pathogenic viruses like WSSV (White Spot Syndrome Virus) are capable of killing over 90% of hatchery-reared in Bushehr and Khuzestan provinces and causes reduction in the main cultured species such as *Penaeus indicus* brood stocks. So the Iranian fisheries organization interests to import a species which is resistant to WSSV for this purpose.

According to FAO (2006), shrimp farming in 2003 yielded a total of 1,278,363 tons globally and 40% of which was the Pacific white shrimp (Patrícia Souza de Lima *et al.* 2010).

*P*. (*Penaeus*) *vannamei*, also known as the Pacific White Shrimp and the White leg Shrimp, is native to the tropical East Pacific from the Gulf of California, Mexico to northern Peru (Tamadoni Jahromi *et al.* 2016). It is the most widely cultured shrimp in the world and is raised in at least 27 countries, with major production operations occurring in the US, Mexico, Central America, tropical South America, China, India, and southeast Asia (Pourmozaffar *et al.* 2019a). Also this species are widely reported from East coasts of USA and Gulf coast waters, Hawaii, South America, and Thailand but this species is not currently known as a remarkable species in North American waters. Wild populations have been reported only from Venezuela. Specific impacts of *P. vannamei* have not been reported.

Genetic definition of stocks has been a major concern towards understanding natural resources in order to ensure sustainability. Different brood stocks or seed stocks often do not show the same growth rate, disease resistance, or other important characteristics (Lester and Pante 1992). Data on such factors and their correlations with certain genotypes are critical in developing a systematic aquaculture programme.

The proper management of brood stocks in order to reduce the inbreeding coefficient of mentioned species is unavoidable. One of the most efficient current methods to determine differentiation between and within species is using mitochondrial DNA (mtDNA) and microsatellites (Akib *et al.* 2015; Tamadoni Jahromi and Othman 2011). Baldwin *et al.* (1998) showed that sequence analyses of mtDNA to be useful in phylogenetic studies between *P. chinensis* populations in four locations. Also Lavery *et al.* (2004) used mitochondrial DNA sequences in to reconstruct the phylogeny of the *P. sensulato* genus of marine shrimp.

In this study COI genes were investigated from the main post larvae producers of *P. vannamei* species in the Bushehr and Hormozgan provinces. This Investigation will help us to have better understanding the taxonomic status and inbreeding coefficient

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relationship in the brood stocks which have been used as broods of *P. vannamei* in Iranian shrimp culture.

## Material and methods

Eighty samples were randomly selected from breeders of three *P. vannamei* hatcheries located in the state of Hormozgan (two hatcheries including Kolahi and Tiab which samples entitled as HOA1-A6 and also HO B1- B6 respectively) and one from state of Bushehr which entitled as BU A1-A6).

Total genomic DNA was extracted from 0.1- 0.2 of frozen muscle tissues (swimming legs) mixed with 600µl DNA extraction buffer (10 mM Tris-HCl, pH 7.5, 100 mM EDTA, 1 µg $ml^{-1}$ proteinase K and 0.05 µg $ml^{-1}$ RNase) and subsequently ground using a mortar and pestle (Gozari *et al.* 2019b). The homogenate was incubated for 12h at 55 ºC. DNA was extracted twice with phenol/chloroform/isoamyl alcohol (25:24:1) followed by extraction with an equal volume of chloroform/isoamyl alcohol (24:1), and precipitated in 100% cold ethanol following Gozari *et al.* (2019a). Extracted genomic DNA was diluted to a working concentration of 50 ng $µl^{-1}$ in deionized water and stored in -20 ºC (Gozari *et al.* 2018). PCR amplification of COI gene was performed using universal primer pair of LCO1490 (5'-GTCAACAAATCATAAAGATATTGG-3') andHCO2198(AAACTTCAGGGTGACCAAAAAATCA-3') (Pirian *et al.* 2016a; Vrijenhoek 1994).

The PCR amplification was carried out in a standard 25 or 50 µl reaction volume with 2 µl of total genomic DNA, 1pmol of each primer, 2.5 mM $MgCl_2$ (optimized from 1.5 mM up to 3.0 mM), 0.2mM dNTPs (Promega), and 1.5 U $µl^{-1}$ *Taq* DNA polymerase (Promega) (Gozari *et al.* 2019c). Amplification was performed in a PTC-200 Peltier Thermal Cycler, (MJ Research, Watertown, MA) with a profile of precycle denaturation at 94ºC for 4.30 min, followed by 30 cycles of 1.30 min at 94 °C, 1 min at 52-56 °C (optimizing annealing temperature), 1 min at 72 °C (extension temperature), and a final extension of 5 min at 72 °C (Tamadoni Jahromi *et al.* 2020b). Amplified DNA from individual shrimp was purified by using a SpinClean Gel Extraction kit (column). Samples were sent to service provider for direct sequencing in both directions. Resulting PCR products were purified using ethanol precipitation and run using Automatic Sequencer 3730xl (Applied Biosystem, Foster City, CA). Statistical analyses including Neighbour-joining, Maximum Liklihood, distance table were carried out through MEGA4 software (Kumar *et al.* 2018).

Individual electropherograms were edited, and sequences aligned using Clustal W (Thompson *et al.* 1994) with adjustments made manually. Sequences were confirmed through BLAST analysis with Genbank sequences .Similarly a Neighbor-Joining (Saitou and Nei 1987) and Maximum Liklihood trees were constructed based on pair-wise genetic distance using the Kimura 2-Parameter algorithm (Kimura 1980). To estimate branch support on the recovered topology, non-parametric bootstrap values were assessed with 1000 bootstrap pseudo-replicates.

DNA sequences (COI genes) from another shrimps including genus, *P. indius*,

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*P*. *monodon* and *P. merguiensis* were used as the out-groups.

## Results

Amplification of the COI gene generated approximately 580 base pairs product for most of the samples have been analysed. After editing, the final consensus sequence of 502 characters were aligned and used for tree construction. Base compositions of the aligned partial COI gene sequences obtained from the 18 samples were adenine (A)= 26.9%, cytosine (C) 17.9 %, guanine (G)=16.3 % and thymine (T)=38.9%. In this study, a total of seven haplotypes were detected for all three stations belonging to Bushehr and Bandar Abass.

The number of individuals was selected based on the number of brood stocks which use in reproductive ponds at different stations (one present of the brood stocks at each station). But these numbers of individuals could detect the level of homozygosity and heterozygosity between different sites which can be extended to the entire population and estimated the genetic discrimination between the stations.

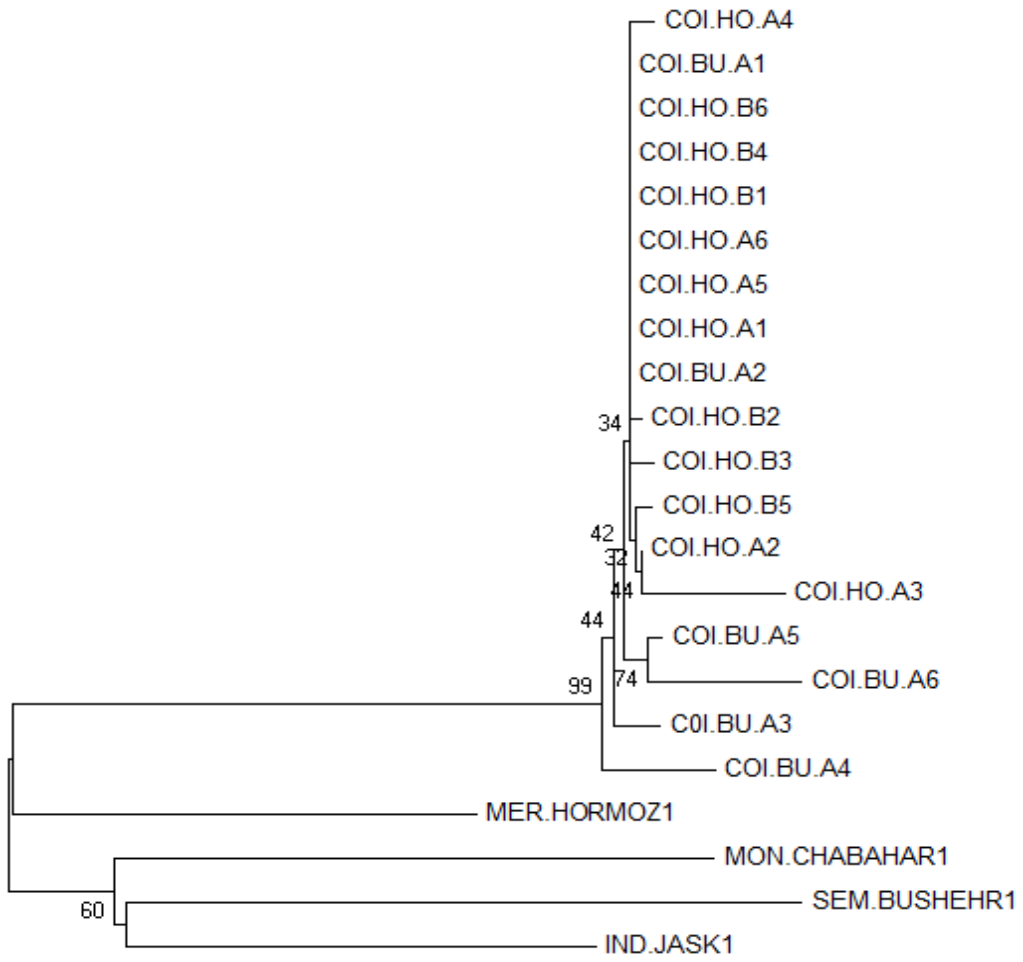



**Figure 1: A Neighbour-joining tree based on genetic distance analysis of COI sequences showing the genetic relationships of *Penaeus vannamei* broodstocks compare to the other species of Penaeidea family. Scale shown refers to genetic distance based on nucleotide substitutions. Numbers at branching points are bootstrap support.**

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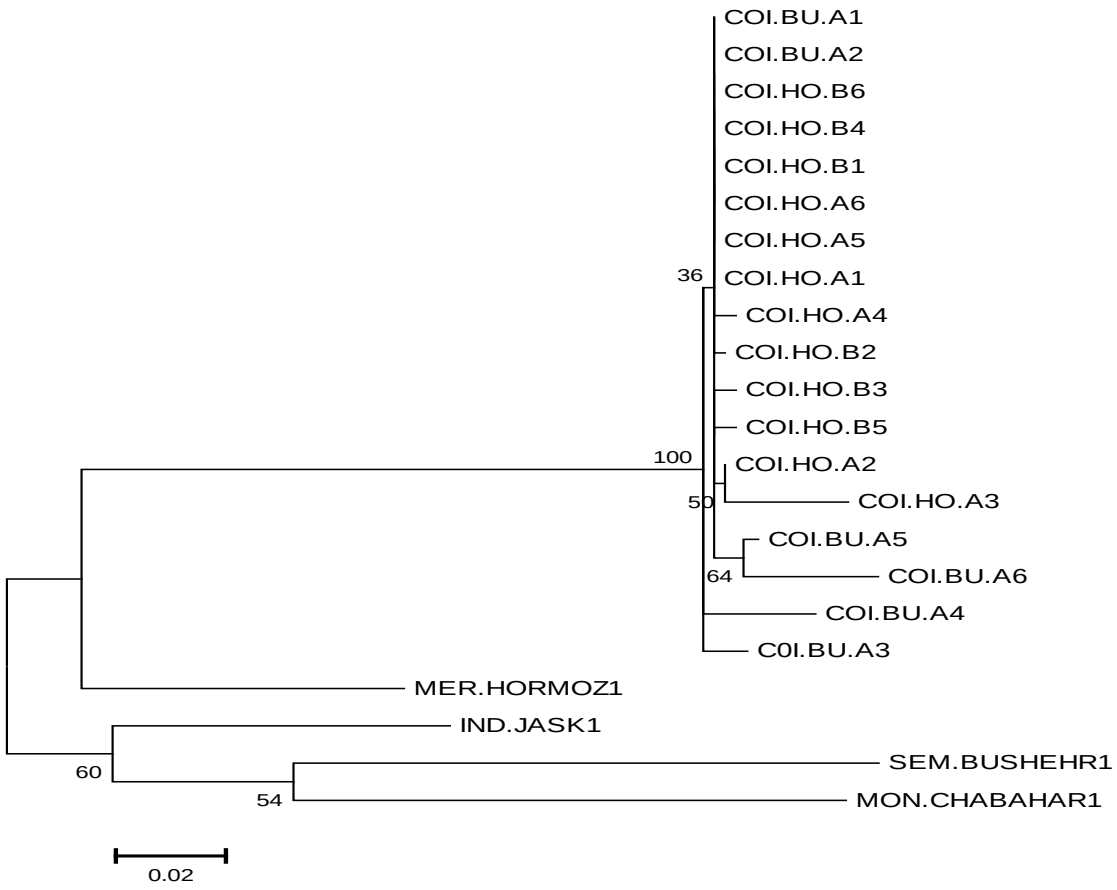



**Figure 2: A Maximum likelihood tree based on genetic distance analysis (based on calculate the Probability the observations which have actually been observed as a function of the model) of COI sequences showing the genetic relationships of *Penaeus vannamei* brood stocks compare to the other species of Penaeidea family. Scale shown refers to genetic distance based on nucleotide substitutions. Numbers at branching points are bootstrap support.**

**Table 1: Pairwise genetic distances between the *Penaeus vannamei* brood stocks compare to the other species of Penaeidea family, revealed from partial COI gene sequences.**

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. COI.HO.A1 | | | | | | | | | | | | | | | | | | | | | | |
| 2. COI.HO.A2 | 0.00 | | | | | | | | | | | | | | | | | | | | | |
| 3. COI.HO.A3 | 0.02 | 0.02 | | | | | | | | | | | | | | | | | | | | |
| 4. COI.HO.A4 | 0.00 | 0.00 | 0.02 | | | | | | | | | | | | | | | | | | | |
| 5. COI.HO.A5 | 0.00 | 0.00 | 0.02 | 0.00 | | | | | | | | | | | | | | | | | | |
| 6. COI.HO.A6 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | | | | | | | | | | | | | | | | | |
| 7. COI.HO.B1 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | | | | | | |
| 8. COI.HO.B2 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | | | | | | | | | | | | | | | |
| 9. COI.HO.B3 | 0.01 | 0.01 | 0.03 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | | | | | | | | | | | | | | |
| 10. COI.HO.B4 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | | | | | | | | | | | | | |
| 11. COI.HO.B5 | 0.01 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | | | | | | | | | | | | |
| 12. COI.HO.B6 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | | | | | | | | | | | |
| 13. COI.BU.A1 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | | | | | | | | | | |
| 14. COI.BU.A2 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | | | | | | | | | |
| 15. COI.BU.A3 | 0.01 | 0.01 | 0.03 | 0.01 | 0.01 | 0.01 | 0.01 | 0.01 | 0.02 | 0.01 | 0.02 | 0.01 | 0.01 | 0.01 | | | | | | | | |
| 16. COI.BU.A4 | 0.04 | 0.04 | 0.06 | 0.04 | 0.04 | 0.04 | 0.04 | 0.04 | 0.06 | 0.04 | 0.05 | 0.04 | 0.04 | 0.04 | 0.06 | | | | | | | |
| 17. COI.BU.A5 | 0.00 | 0.00 | 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.00 | 0.00 | 0.01 | 0.04 | | | | | | |
| 18. COI.BU.A6 | 0.02 | 0.02 | 0.04 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.02 | 0.02 | 0.02 | 0.02 | 0.02 | 0.03 | 0.06 | 0.02 | | | | | |
| 19. SEM.BUSHEHR1 | 0.24 | 0.24 | 0.27 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.24 | 0.25 | 0.24 | 0.24 | 0.24 | 0.25 | 0.26 | 0.24 | 0.24 | | | | |
| 20. IND.JASK1 | 0.18 | 0.18 | 0.21 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.18 | 0.19 | 0.18 | 0.18 | 0.18 | 0.20 | 0.21 | 0.18 | 0.20 | 0.27 | | | |
| 21. MER.HORMOZ1 | 0.16 | 0.16 | 0.18 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.17 | 0.19 | 0.16 | 0.17 | 0.26 | 0.13 | | |
| 22. MON.CHABAHAR1 | 0.23 | 0.23 | 0.25 | 0.23 | 0.23 | 0.23 | 0.23 | 0.23 | 0.24 | 0.23 | 0.24 | 0.23 | 0.23 | 0.23 | 0.25 | 0.27 | 0.23 | 0.25 | 0.22 | 0.24 | 0.25 | |

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**Table 2: Wright's Indices a (Fst) for the Three *Penaeus vannamei* shrimp hatcheries.**

| Bushehr | Hormozgan B | Hormozgan A | |
|---|---|---|---|
| 0.0544 | 0.00323 | 0.000 | HormozganA |
| 0.0592 | 0.000 | 0.00323 | Hormozgan B |
| 0.000 | 0.0592 | 0.0544 | Bushehr |

## Discussion

Neighbour joining analysis based COI sequences further revealed non genetic distinctiveness between the haplotypes. All haplotypes clustered into two separate clades. As it expected, all seven haplotypes of *P. vannamei* including the samples of Bushehr and especially hatchery B from the Hormozgan Province were clustered into one distinct clade, showed significant inbreeding coefficient between the samples from the three studied area. (Figs. 1, 2)

F-Statistic ($F_{st}$) was estimated to understand genetic discrimination between the stations. $F_{st}$ is the standardized measure of population differentiation based on genetic polymorphisms and reveals genetic differences among populations.

The use of $F_{st}$ 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).

Although statistically different from zero, the genetic differences found between the three shrimp hatcheries ($F_{st}$ = 0.00323-0.0592 between Hormozgan hatcheries and Bushehr, respectively) cannot be considered high (Table 2). According to (Wright 1984), values between 0 and 0.05 indicate little genetic differentiation. Luan *et al.* (2006) observed similar values ($F_{st}$ =0.023) in a comparison between wild and cultured populations of *Marsupenaeus japonicus*, whereas (Soto-Hernández and Grijalva-Chon 2005) observed higher values (FST= 0.086) studying allozymes in wild and cultivated *L. vannamei* populations.

Low genetic difference observed in our study indicates that the hatcheries are probable to have organized typically of like genetic structures which agree with Patrícia Souza de Lima *et al.* (2010) who studied on genetics of two marine shrimp hatcheries *L. vannamei* in Brazil. Moreover, the selection of brood stocks in these types of hatchery is usually performed in a random method. Shrimp normally has been provided from different farms to the hatcheries, which can reduce genetic differences.

The differences in $F_{st}$ could be recognized to differences in management and strategy, such as the number of breeding tanks, number of breeders per tack, sex proportion and brood stock selection strategy (Patrícia Souza de Lima *et al.* 2010). The extent to which these variants affect inbreeding remains to be examined.

The southern part of Iran has the highest shrimp farming production in the country (Pirian *et al.* 2016b). In this respect, limited reports about the import the *P. vannamei* brood stocks and estimation the inbreeding coefficient have performed. It is important to conserve and record a high level of genetic variability for breeder which imports *P. vannamei* in Iran. Continued monitoring of these hatcheries could help improving the preservation of this valuable genetic variability.

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In this study, mitochondrial DNA analysis provided a good resolution of genetic divergence among the populations. This type of analysis could be considered as an important tool to be used in brood stocks selection in breeding programs. In this case, different management in brood stocking programs should be performed in order to manage and reduce the inbreeding coefficient in the main shrimp hatcheries and also live shrimp import policy in Iran.

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