The Study of Population Genetics Structure of *Holothuria parva* in the Persian Gulf Using mtDNASequences

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***Abstract*—In this study in order to identify, determine and compared the genetic diversity of sea cucumber *Holothuria parva* in both regions dayer port and bostaneh port, 16s rRNA gene sequencing was used. A total of 417 nucleotide locus were determined, after consideration of 16s rRNA gene sequences in the NCBI database were consistent with belonging to the species *Holothuria parva* samples was confirmed. In total was 4 haplotype identified in two areas that One haplotype was common in both areas. Haplotype diversity in the dayer port with haplotype 2 and bostaneh port with 3 haplotypes, respectively, 83% and 50% was estimated. Genetic differentiation (F_{st}) Negligible 000.0 and divergences rate (Dxy) 0.0048 and high gene flow (N_m = 1874) between the two regions were estimated. Based on the results of this study are probably samples of Bostaneh port and Dayer port than a similar population, That because high gene flow between the two regions do not have much different and There is also a common haplotype, suggesting a common ancestor of *Holothuria parva* in the region.**

***Index Terms*—*Holothuria parva*, Persian Gulf, genetic diversity, 16s rRNA, Bostaneh, Dayer.**

I. Introduction

Sea cucumbers are group of aquatic animals that are part of marine invertebrates [1]. Holothuroidea are one of the classes of echinoderms, that belonging to the branches of Echinozoa and sub-branches of Echinodermata [2]. This species is distributed in tropical and subtropical regions and mostly have lived in intertidal zone [3]. All echinoderms, have radial symmetry and mostly with calcareous internal skeleton with thorns in the outside [1]. Greatest diversity of them is in tropical shallow and coral reefs [1].

Mitochondrial DNA (mtDNA) analysis is a new approach as a tool for biologists with fisheries purposes are used [4]. In this between mtDNA for various reasons is the key part of the study, in structure and performance in Compared with the nuclear genome is simple and all the individual carry similar mtDNA of their female parent [4]. Of the advantages mtDNA can be no recombination (due to the transfer of the parent female) and high mutation rate noted [5].

16SrRNA gene sequences in many studies for disambiguating of classification and Determining the genetic structure of Different species of aquatics have been used. A unique feature of mtDNA, especially speed of evolution that is 10 times more than nuclear DNA, It has become a useful tool in the analysis of phylogeny [5]. Including studies by this method can be used to study the phylogeny of sea cucumbers (Holothuridae) in Malaysia [6], investigate the phylogenetic sea cucumbers of the coast of Egypt [7], Phylogeny Relationship of commercial Shrimps [5], crab population structure *Portunustrituberculat*us[8], Molecular phylogenetic analysis of shrimp species in Taiwan's economy [9], can be named. Seacucumbers are of Aquatic animal from the Persian Gulf, that ever little research has been done about it. The purpose of this study was to identify possible genetic differences and to determine the genetic diversity among species of sea cucumbers *Holothuria parva* is in these areas.

II. Materials and Methods

After preliminary investigations, accurate identification of the most suitable sites were selected and manually collected samples from the two regions Dayer and Bostaneh was performed. Samples from each region were fixed in 96% ethanol and were transferred to the laboratory for other laboratory works. Initially

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approximately 50 mg from muscle tissue of sea cucumber was isolated and then was placed in the paper napkin in the open air until it is removed alcohol. DNA was extracted by cell disruption, separation of proteins and carbohydrates, of nucleic acids, and finally DNA precipitation with ethanol 70% was performed. To determine the quantity and quality of extracted DNA, of methods agarose gel electrophoresis 1% and spectrophotometry (Bio-photometer) were used. In this study from 1 base primer is used for PCR that is shown in Table I.

TABLE I. PRIMERS USED IN THIS STUDY

Primer	Primer sequence
16sar (F)	5-GGT ATC TTG ACC GTG CAA AG-3
16sbr (R)	5-CTC CGG TTT GAA CTC AGA TC-3

Reaction PCR using template DNA a concentration of 100 ng, $MgCl_2$ at a concentration of 2.5 mM, 2 ml PCR Buffer (10X), 0.5 units of Taq DNA Polymerase, primers 1 at a concentration of 100 micromoles, primer 2 at a concentration of 100 micromoles, dNTP with concentration of 10 micromoles and water rectification to extent that the sample volume reached 25 ml, was performed. In this study was primers for the formation best bands and select the most suitable temperature of the thermal gradient 49-46 were used, which was identified as the optimum temperature 47 ° C for the reaction PCR. In this study primers to form the better bands and select the most suitable temperature of the thermal gradient was 49-46, which was identified as the optimum temperature for the reaction PCR 47 ° C. Thermal cycles for amplification was as follows, Initial denaturing at 95 ° C for 5 min, Then 35 cycle includes 40 seconds at 95 ° C, 35 seconds at 47 ° C and 10 min at 72 ° C and final extension at 72 ° C for 10 min. Ultimately PCR products were transferred to a refrigerator at 4 ° C for further tests, namely quality assessment of PCR products on a 1% agarose gel, be available. Checking and manual editing of sequences was performed using the software Chromas2, Then sequences to ensure the accuracy of the reproduced desired gene in genomic database NCBI, were BLAST. Alignment of sequences obtained by the application of Clustal W was performed in the BioEdite software. Traced the lineage trees of reproductive and genetic distance matrix were performed using the software MEGA.5. Traced phylogenetic trees and genetic distance matrix was performed using the software MEGA.5. Other indices of genetic such as Tajyma tests of types haplotype, And positions of polymorphisms, Haplotype and nucleotide diversity, Genetic divergence test, were calculated using Dnasp ver. 5 software. Excel software was used for drawing.

III. RESULTS

In total were obtained 8 legible sequences. The results of sequencing showed that the amplified district contains 417 bp are after editing sequences. Then sequences by Topical superpositions program (BLAST) were evaluated in the NCBI site. After BLAST samples, was characterized amplified district corresponded with district 16S rRNA. Mentioned sequence contains 99% coverage and identity was with species *Holothuria parva*. By using sequences obtained from a total of 417 nucleotide locus in various software was evaluated and on this basis calculation results showed that of these 417 locus, 406 locus non-variable and And 8 locus are variable.

In total 4 haplotypes was identified, that in the Bostaneh region 3 haplotype (Haplotypes 1, 2 and 3) and in the Dayer region 2 haplotype (haplotype 2 and 4) were observed. Haplotype Number 2 was common in both stations. Also for the desired gene, haplotype diversity at the Bostaneh station 83% and at the Dayer station 50% were estimated. The substitution pattern of nucleotide Based on the substitution of transition type and crossover is calculated (Table II). Also ratio of different nucleotides of total nucleotides were also calculated (Fig. 1). An average ratio of Thymine (26.7%), cytosine (22.7%), adenine (29.7%) and guanine (20.9%) among all the samples studied, with ratio of GC was 43.6 in among all samples, that this ratio between the two region is constant (TableIII).

TABLE II. PATTERN OF NUCLEOTIDE SUBSTITUTION (SUBSTITUTION OF TRANSITIONAL TYPES ARE SHOWN IN BOLD TYPE).

Nucleotide	A	T	C	G
A	-	5.88	4.68	9.57
T	5.88	-	15.96	4.46
C	5.88	20.06	-	4.46
G	12.61	5.88	4.68	-

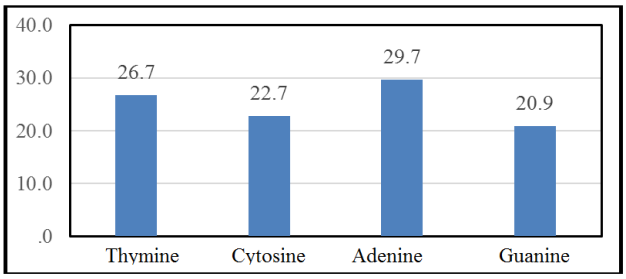

Figure 1. Proportion of different nucleotides sequences obtained from the studied samples

The genetic distance matrix between samples examined, indicating that the genetic distance among the studied samples from 0.000 to 0.015 (Table IV). The results showed that the amount of genetic distinction (F_{st}) between Dayer port and Bostaneh port was very low (0.000), while the amount of gene flow (N_m) 1874.54 Were estimated. The amount of divergence (nucleotide replacement per locus) between the two regions based on divergence test (Dxy) 0.0048 was estimated (standard deviation 0.0034). Also the results of the test Tajima D-Test (-1.70) between the regions was not significant (P>0.10). Phylogenic tree of studied samples to method Neighbor Joining was plotted that examples (B16, D4, D7, B8 and D8) were all in a single branch and Samples B13 and D9 in a sub branch and B5 samples were separated in a sub- branch (Fig. 2).

TABLE III. GENETIC INFORMATION OBTAINED FROM THE STUDIED SAMPLES

Factor	Bostane	Dayer
The number of nucleotide positions	414	415
The number of invariant positions	408	413
The number of polymorphic loci	6	2
Single morphological variable (bivariate)	6	2
The number of haplotype	3	2
Nucleotide diversity Pi	0.007	0.002
Haplotype frequency	83%	50%
Ratio GC	43.6%	43.6%

TABLE IV. MATRIX OF GENETIC DISTANCE BETWEEN SAMPLES EXAMINED.

	B5	B8	B13	B16
B8	0.010			
B13	0.015	0.005		
B16	0.010	0.000	0.005	
D4	0.010	0.000	0.005	0.000
D7	0.010	0.000	0.005	0.000
D9	0.015	0.005	0.010	0.005
D8	0.010	0.000	0.005	0.000

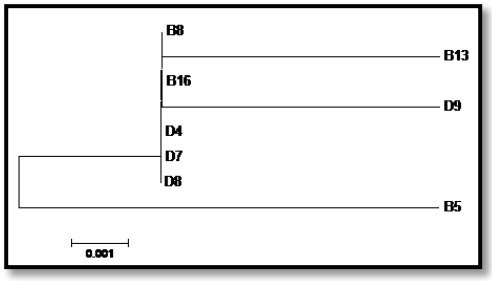

Figure 2. Phylogenetic tree of studied samples to method neighbor joining.

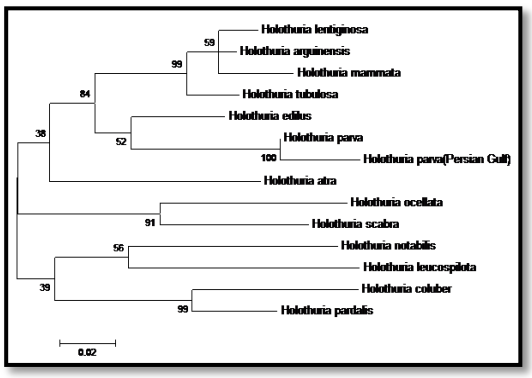

Figure 3. Phylogenetic tree of the sea cucumber *Holothuria parva* Holothuridae Persian Gulf and other species based on 16S rRNA sequences compared to Neighbor Joining.

Based on the results of the amount of divergence between the samples studied with other samples it was found that lowest level of divergence is Between samples of the same species in the region (d= 0.029) And the highest divergence (d= 0.232) between the samples compared with the species *H. coluber* is also phylogenetic trees was drawn to compare Persian Gulf *H. parva* Which Indicative the phylogeny status of Persian Gulf *H. parva* is compared with other species (Fig.3 and 4). This results show samples of the present study and other examples *H. parva* have a common ancestor with *H.edilus*.

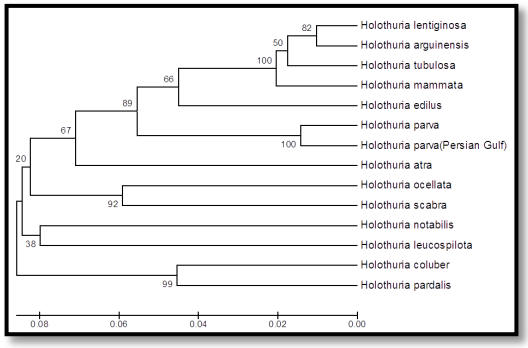

Figure 4. Phylogenetic tree of the sea cucumber *Holothuria parva* Holothuridae Persian Gulf and other species based on 16S rRNA sequence comparison UPGMA method.

IV. DISCUSSION

Sea cucumbers are belonging to the branches of Echinodermata and class Holothuroidae, the sea bed and due to its skeleton the evolved and structure of their ancient respiratory (respiratory tree) are unique creatures [10]. Today these creatures, in addition to nutritional value, due to having effective bioactive compounds in the fields of health and medicine are highly regarded[11]. For this reason we also picked of stocks of sea cucumbers in the country has increased in recent years. Although fisheries data concerning the extent and level of harvesting of these valuable resources, especially in the Persian Gulf coast does not exist, But studies have shown that sea cucumbers Proportion to over-harvesting of them vulnerable [3]and rebuilding rate their reserves is very slow [12], [13].

In the current study, High haplotype diversity and low nucleotide diversity was observed among the samples. Mean haplotype diversity was 0.64, which in Dayer port 0.50 and in Bostaneh port is 0.84. Haplotype existence common between two regions Represents the common ancestor between the two regions is [12]. The overall rate of nucleotide diversity was low in both regions. In other studies, also by using 16s rRNA high haplotype diversity Has been observed in the sea cucumbers [14].The results of this study indicate that genetic distance (By using the index Fst) between samples *H. parva* Bostaneh and Dayer (in terms of gene 16s rRNA) was not significant. Based on the results, probably due to being infinite marine ecosystems and low geographical distance between the two regions Bostaneh and Dayer, high gene flow (Nm= 54.1874) between Dayer port and Bostaneh port samples is established.

In some studies of sea cucumbers also observed significant genetic distance as a result of restrictions limitation on gene flow [13], [15], in this respect Vargara Chen and colleagues in 2010, gained similar results this study. Based on the obtained results can be estimated that the persistence of high gene flow between the two regions, prevents of genetic distance But remain at a high level of genetic diversity, Therefore this species has a relatively

good ability against the environmental changes.Differences in mutation rate 16s rRNA gene (0.5%) relative to cytochrome oxidase (COI) (1.6-3.5%) in sea cucumbers approved [14], But generally 16s rRNA Compared to COI More distance in the same populations has shown [16], [17] Which observed differences to the different mutation rates and gene functional limitations has been attributed [16].

Phylogenetic trees drawn in the current study did not show a separation between the two regions. Based on can be expressed two regions in terms of 16s rRNA gene have no significant genetic distance and genetic isolation was not substantial. Similar studies on sea cucumbers this lack of genetic isolation even in different habitats coastal and lagoons is also seen [14].In general lack of significant genetic differentiation between the two regions and the absence of specific populations and also being a common haplotype and high gene flow, expression of migration and gene exchange between the two regions. The lack of significant Tajima test among pairs of regions can be attributed to the stability of size and population size.

Sequences obtained in the present study were compared with the sequences of other relatives of the same sex, the genetic distances between samples of the present study and other relatives represents genetic distance between 0.029 to 0.232, which indicates 16s rRNA gene an appropriate and effective indicator to identify the species of sea cucumber serves [18], [19].

Persian Gulf samples were in a subdirectory with combined *H. parva* by using Bootstrap 100 index and it was found that these species has a common ancestor With *H.edilus*. Divergence rate investigated also showed that the divergence of the Persian Gulf samples with other species similar from the farthest geographical point, the coast of Mexico, is relatively low (0.029), while the extent of its divergences from other species was relatively high, indicating that the 16s rRNA gene have a High powers in the separation species of sea cucumbers and can be used for encoding sea cucumbers of Persian Gulf.

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