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Genetic Structure Four Finger Threadfin (*Eleutheronema tetradactylum*) Population in the Persian Gulf and the Oman Sea Using RAPD Marker

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Abstract: Genetic diversity of *Eleutheronema tetradactylum* was investigated in the Persian Gulf and Oman Sea using 23 pairs RAPD primers. In this study, we collected 158 samples from four locations of the Persian Gulf and Oman Sea; Khoozestan, Bushehr, Sistan va Baluchestan and Hormozgan. The analysis of molecular variance was revealed maximum genetic diversity between Bushehr and Hormozgan populations (0.22). Maximum gene flow was observed from Sistan va Baluchestan region toward the other regions. AMOVA test showed that no significant genetic difference between regions, localities and population individuals (P>0.01). This study was showed dynamic genetic in Khoozestan and Hormozgan populations higher than that of Bushehr population.

Key words: *E. tetradactylum* % RAPD % Persian Gulf % Oman Sea % Genetic Diversity

INTRODUCTION

E. tetradactylum is a species of polynemidae fish distributed from the Persian Gulf of Asia to Papua in New Guinea and North Australia [1, 2]. Many researchers have worked on morphological and other properties of E. *tetradactylum* from the Persian Gulf, but there is no information about the genetic structure and population size of this important commercial fish [3]. *E. tetradactylum* is an important species, valued by commercial and indigenous fishers [4]. However, important commercially fishes make up small amount of the total catch [5]. The gene flow among of marine populations is essential for the management of fisheries and the conservation of marine ecosystems [6-9].

The most of the living things are critically endangered in the Persian Gulf, the main threats include; incidental capture in commercial and recreational fisheries; mortification in marine waste; vessel strikes [10, 11].

Over fishing activity can increase genetics drift chance of can result in changes in the genetic diversity and population structure. In the other hand migration and gene flow, facilitated due to moving of fish eggs and larvae by passive transport or by active adult migration between different locations, first tends to keep populations homogeneous, seconds adds genetic variation to populations. Genetic diversity has established to be the best tool for the study of aqua-organisms for acquiring information about the conservation and management of a species [12].

The goal of this study is to investigate genetic diversity one of the most commercial fish; *E. tetradactylum* in Persian Gulf and Oman Sea by molecular markers, Randomly Amplified Polymorphic DNA (RAPD) [13-16].

MATERIALS AND METHODS

A total of 158 *E. tetradactylum* samples collected from four locations of the Persian Gulf and Oman Sea; Khoozestan, Bushehr, Hormozghan and Sistan va Baluchestan (Figure 1 and Table 1). Samples of muscle

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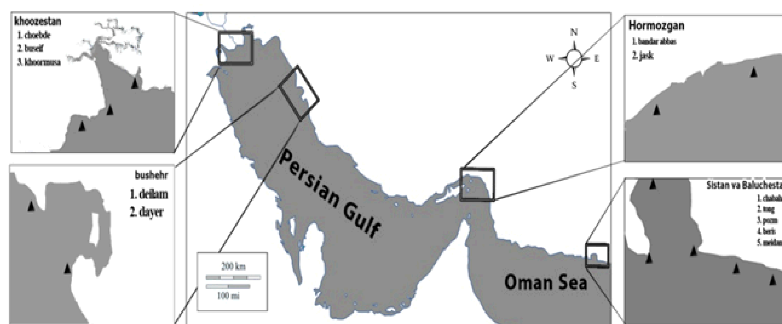

Fig. 1: Map indicating the four sampling locations, of *E. tetradactylum* in the Persian Gulf and Oman sea.

Table 1: Number and distribution of *E. tetradactylum* samples

Region	Station	Samples	Geographical Lat and Long.
Khouzestan	Choebde,Boosaif, Khooremousa	83	E 48.26 29.49 N,
Booshehr	Deylam, Dayer	92	50.79 E, N 28.92
Hormozgan	Jask, Bandar abbas	44	E 56.21, N 27.12
Sistan and baloochestan	Chabahar, Tong, Pozm, Bris, Meydani	15	E 60.29, N 25.32

Table 2: Primers sequences in the RAPD analysis

Primers	Sequences Primer	Annealing°C	GC%
ACS 32	5N-CCC AGC GAT-3N	44	70
AUBC 516	5N-AGC GCC GAC G-3N	44	80
$AOPG_{10}$	5N-GGC TGC ACA A-3N	43	60
$AOPA_{10}$	5N-GTG ATC GCA G-3N	45	60
$AOPA_{2}$	5N-TGC CGA GCT-3N	43	70
$AOPG_{9}$	5N-CGA AGC AGC-3N	45	60
$AOPG_{15}$	5N-AAC TGG ACT G-3N	36	50
$AOPA_{3}$	5N AGT CAG CCA C-3N	45	60
$AOPA_{5}$	5N AGG GGT CTT G-3N	45	60

tissue about 3 to 5 g of soft tissues were preserved in absolute ethanol and stored until DNA extraction [2]. Total genome was isolated from the muscle tissue by proteinase K digestion followed by standard phenol-chloroform extraction, the DNA samples were suspended in ddwater [17].

Nine RAPD primers were used to amplify random fragments (Table 2). PCR amplification was done in a reaction total volume of 25µL, 2.5µL 10X PCR buffer, 0.5µL 10 mm dNTPs, 0.8µL 50mm MgCl2, 1µL of each primer, 0.8µL of Taq DNA polymerase and 1µL diluted DNA reached to final volume 25µL with ddwater. The PCR reaction was performed as follows: an initial incubation at 95°C for 3 min, followed by 35 cycles of PCR (denaturing at 95°C for 45s, annealing at 45°C for 45s and extension at 72 °C for 45s) and a final extension at 72 °C for 3 min. The reactions were performed in a Quanta Biotech thermocycler (England) Excel, PopGene ver 1.31 [18] and GeneALEX ver 6.41 [19] software were used to calculate statistical values, F_{st}, number of polymorphic sites.

RESULTS

RAPD primers were screened on three randomly selected samples. In examination, the effects of Mg^+ concentrations and annealing temperature during PCR ampli?fication, nine primers that formed reproducible fragments on the electrophoresis gel, which were selected for further analysis (Table 2). These nine primers produced 173 bands ranging from 200 to 3000 bp sizes, corresponding to an average of 19.2 bands per primer. RAPD analysis of 158 *E. tetradactylum* samples using 9 primers generated a total of 173 scorable bands (Figure 2). The percentage of polymorphic bands for the four populations; Khoozestan, Bushehr, Hormozghan and Sistan va Baluchestan were 87.86%, 93.6%, 92.49% and 63.01%, respectively (Table, 3).

The number of bands generated by each primer varied, as the highest and lowest number of bands produced by primers AOPA3 and AOPG15, respectively.

Table 3: Number of bands, specific allele, allelic frequency, number and polymorphic locus Percentage

Regions	No. of bands	Specific allele	allelic frequency	No. of polymorphic locus	polymorphic locus%
Khouzestan	161	4	151	152	87.86
Booshehr	152	1	137	161	93.60
Hormozgan	160	4	147	160	92.49
Sistan and baloochestan	110	0	110	109	63.01

Table 4: Hierarchical analysis of molecular variance (AMOVA) result for *E. tetradactylum*

Source of variation	df	F statistic	Variance	% total	p-value
Among regions	1	$0.07 = F_{ct}$	2.36	7	<0.01
Among Population	2	$0.10 = F_{sc}$	3.05	10	>0.01
Within Population	154	$0.17 = F_{st}$	26.25	83	>0.01

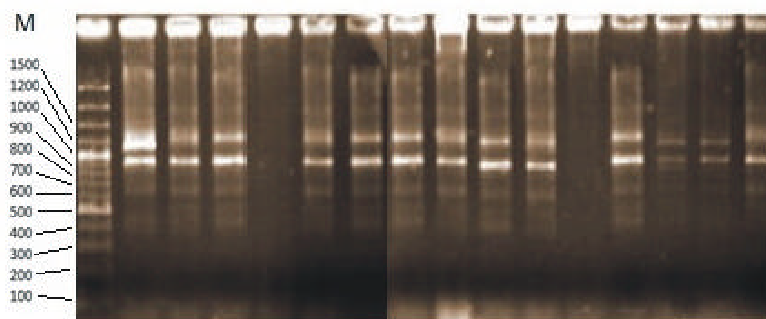

Fig. 2: RAPD patterns of individuals of *E. tetradactylum*) using nine primer.

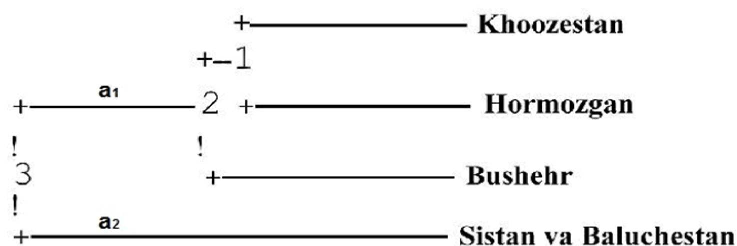

Fig. 3: UPGMA dendrogram of the populations of *E. tetradactylum* based on values of genetic distance in the Persian Gulf and Oman sea (Nei, 1978).

The molecular variance analysis (AMOVA), based upon samples from two groups revealed that maximum genetic distance 4% and minimum genetic distance 1% (Table 4). Genetic variation among the population and within the population was 10% and 88% respectively ($P > 0.01$).

One hundred and seventy three loci were analyzed using AMOVA test. The result was showed the highest gene flow between Khoozestan and Sistan va Baluchestan seas. The polymorphism of *E. tetradactylum* was calculated by PopGene (Table 4).

The UPGMA dendrogram [20], was created on the basis of genetic distance between populations of *E. tetradactylum* from three locations of the Persian Gulf and one location from Oman Sea. It showed two main clusters (a_1 and a_2) that a_1 was divided for three more subclusters (Figure 3).

DISCUSSION

In recent years, increasing the vulnerability of the Persian Gulf and Oman Sea fish due to the uncontrolled entry of various pollutants, etc., necessary to perform basic and applied research, to inform potential population or populations, the protection program like proliferation education, resource management and sustainable fishing and harvesting model is necessary. This is not only important to the exploitation of fisheries resources, but also to ensure the preservation of biodiversity and ecological function of marine ecosystems. Several techniques. Molecular markers successfully determine the relationship between fish stocks and their populations [21]. Genetics Polymorphic DNA is a valuable marker in assessing population structure and conservation of genetic resources of species [22]. Among, molecular

techniques RAPD marker is fast and cheaper which, have been developed for the genetic analysis of fish, [23, 24]. RAPD molecular method has been used effectively for study of genetic variation among fish species, [25-31].

The number of alleles per locus is varied in fishes with different habitant, as on average for marine fish (20.6), freshwater fish (7.5) and for anadromous fish (11.3%), [32]. Genetic variation among populations of marine species was found the low degree than from other habitant species that probably attribute to large effective population sizes, which would because to limit variation due to genetic drift [33]. In this study, average number of alleles was observed low degree for *E. tetradactylum*, which agreement with the finding of [34], who is reported that the larvae of *E. tetradactylum* have lower performance swimmers and poor orientation compared with many other fishes, in terms of speed and stability.

In the current study, the percentage of polymorphic bands in Bushehr, Khoozestan and Hormozghan seas were higher than Sistan va Baluchestan that could be from gene flow among the populations of three areas located in the Persian gulf and in the other hand, gene flow is restricted from Sistan va Baluchestan located in the Oman Sea.

The cluster result indicated that there was a high similarity between the three populations of *E. tetradactylum* from the Persian Gulf, it might be from that frequent gene flow from one population to another. While populations of *E. tetradactylum* from the Oman Sea showed the lowest similarity with populations from the Persian Gulf, because there was a low gene flow between populations of two different regions.

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