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Research Article

Differentiation among the population of *Argyrops spinifer* in the Iranian waters of the Persian Gulf and Sea of Oman using Cytochrome Oxidase I (COI) gene

Valipour S.[1], Ghavam Mostafavi P.[1*], Shahhosseiny M.H.[2], Kaymaram F.[3]

1Department of Fisheries and Marine Science, Faculty of Natural Resources and Environment, Science and Research Branch, Islamic Azad University, Tehran, Iran
2Department of Microbiology, Islamic Azad University, Shahr-e-Ghods, Iran
3Iranian Fisheries Science Research Institute (IFSRI), Agricultural Research, Education and Extension Organization (AREEO), Tehran, Iran
*Correspondence: mostafavi_pa@srbiau.ac.ir

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Abstract

Argyrops spinifer, commonly known as king soldier bream, is a species from the Sparidae family in the Persian Gulf and Oman Sea. This study was conducted to investigate the genetic diversity and demographic structure of the mentioned species based on the mitochondrial cytochrome c oxidase I gene. DNA was extracted using the ammonium acetate method from the caudal fin of 90 samples. The PCR reaction was performed using a pair of primers of the nucleotide sequences of the mitochondrial COI gene, and its PCR products were sequenced. Based on the results, the mean haplotype diversity was relatively high in Bandar Abbas (1.000), Chabahar (0.93), and moderate in Bushehr (0.533). The mean nucleotide diversity was also 0.194 in Bandar Abbas, 0.022 in Chabahar, and 0.0133 in Bushehr. Tajima's neutrality test and Fu's Fs index between the studied regions were -1.32 and -0.64, respectively ($p \leq 0.05$). The highest FST genetic distance was between the Chabahar and Bushehr populations (0.48). Also, the lowest genetic distance of 0.17 was observed between the Chabahar and Bandar Abbas populations. The results showed the presence of different populations of this genus in the Persian Gulf and Sea of Oman.

Introduction

There are more than 11 species of Sparidae in the Persian Gulf and Oman Sea. *Argyrops spinifer* belongs to the Sparidae family and is known as the king soldier bream in the southern part of Iran. *Argyrops spinifer* is distributed in the Persian Gulf and Oman Sea, as well as from the west to the east of the Indian Ocean and northern Australia (Orrell *et al*., 2002; Froese and Pauly, 2015). This species is a demersal species. The juveniles are found in shallow waters, while adults are found in deeper waters individually (Sommer *et al*., 1996; Randall *et al*., 1997; Al-Mamry *et al*., 2011). The body length reaches 70 cm but is 30 cm on average (Randall, 1995). This species is protogynous (Grandcourt *et al*. 2004) and feeds on benthic invertebrates and mainly mollusks, shrimps, crabs, and nektons (Fouda *et al*. 1998; Ghanbarzadeh *et al*., 2014). Sex reversal of this species is a common reproductive strategy in tropical coral islands (Grandcourt *et al*., 2004; Al Mamry *et al*., 2009). The COI gene in the mitochondrial genome is commonly used in species identification and biodiversity (Bingpeng *et al*., 2018). Geographical distribution, morphological differentiation, and genetic characteristics are the most basic needs for the conservation management of species. It should be noted that morphological indices are not a reliable method for distinguishing between potential populations (Aarbakke *et al*., 2011). Therefore, genetic markers are successfully used to link aquatic populations to the structure of their reserves (Van Herwerden *et al*., 2006). Sequencing the genome of living beings has become easier and error-free with the advancements in sequencing devices and specialized genetic studies software (Hedrick, 1999). Utilizing the genetics of aquatic populations is one of the ways to investigate the intraspecific diversity of aquatic organisms because the study of biodiversity and genetic structure of aquatic populations and also the systematic relationships of these organisms give a clear understanding of the structure of biological communities (Rezvani *et al*., 2006). Fish stocks are a crucial source of livelihood and nutrition, providing 156 million metric tons of food per year, representing some 17% of global animal protein consumed (Action, 2020).

The progressive decline in some species populations highlights the need to develop recovery plans it has long been understood that genetic data is important for planning species conservation (Simberloff, 1988).

Population genetic information plays an important role in conservation biology. Identifying genetic variation within and between populations can provide important information about the extent of interactions between local populations and allow assessment of the contribution of a metapopulation structure to regional persistence (Hanski, 1999).

Today, genetic diversity is used as an index for the ecological status of aquatic ecosystems. One of the ways to investigate the genetic structure of aquatic animals is the genetic study of populations and the identification of intraspecific changes and population structure using mitochondrial DNA molecular markers are two methods for examining the genetic makeup of aquatic animals. This is a crucial step towards the appropriate management and

exploitation of animal food sources (Pourkazemi *et al*., 2012). Population structure and genetic diversity of several fish species were studied using different molecular markers (Ghavam Mostafavi *et al*., 2007; Ghavam Mostavafi *et al*., 2011; Rahimi *et al*., 2016). Mitochondrial DNA (mtDNA) is being used more often to assess changes in genetic diversity compared to other methods (Johnson *et al*., 2018; Zhai *et al*., 2019; Righi *et al*., 2020). Studies on mitochondrial DNA can be used to reveal genetic similarities, phylogenetic classifications, and genetic differences between the populations of a species (Avise, 2004). Significant benefits of the DNA-based methods include their specificity, sensitivity, and speed (Civera, 2003; Rasmussen and Morrissey, 2008; Mazzeo and Siciliano, 2016). Nowadays, the classification and study of diversity in populations are conducted using DNA sequence differences. (COI) is a comparatively conservative gene with a moderate evolution rate. It is one of the most thoroughly studied mitochondrial genes and is suitable as a molecular marker (Sari *et al*., 2015). COI gene sequence variation has been used to study the population diversity and genetic structure of many fish species (Nneji *et al*., 2020; Sarropoulou *et al*., 2022). Molecular genetic studies have demonstrated that the relative paucity of morphological characteristics conceals a high genetic diversity (Shekhovtsov *et al*., 2014). Since *Argyrops spinifer* has an economic value, the study of its population genetics in the studied regions is a prerequisite for ecological and molecular biology activities for this fish (Mosafer *et al*., 2017). *Argyrops spinifer* is one of the most important species known in the waters of southern Iran that has been subjected to overfishing. Despite the economic importance of this species, there is no information about its population structure in the Persian Gulf and the Sea of Oman (Safari *et al*., 2019). Therefore, the present study was conducted to determine possible populations and identify the genetic diversity of *Argyrops spinifer* species in the Persian Gulf and Sea of Oman.

Methods

Sample collection

90 individuals were obtained from different fishing areas in the Persian Gulf and Oman Sea in February 2019 (Bandar Abbas 27° 10' 04"N, 56° 16' 09"E , Chabahar 25° 16' 02"N 60° 44' 34"E, and Bushehr 28° 49' 07"N 50° 54' 28"E). (Fig. 1). Thirty samples were collected simultaneously from each region.

Molecular analyses

The DNA of the samples was extracted by the ammonium acetate method (Saeidi *et al*. 2017). 50-100 mg of the caudal fin was isolated from the soft tissue of the samples and transferred to 1.5 ml tubes. The quantity and quality of DNA extracted was determined by spectrophotometry and 1% agarose gel electrophoresis respectively. PCR was performed from the COI region of the mitochondrial genome using the primers designed by Ward *et al*. (2005).

PCR was performed using 0.5 μL (50 ng) of genomic DNA, 0.3 μL of each forward and reverse primer with a concentration of 10 μL /pm, 1.5 units of *Taq* DNA Polymerase, 12.5 μL Master mix,

to reach a volume of 25 µL at 94°C for 5 min, with 30 cycles.

Figure 1 Sampling stations in the Persian Gulf and Oman Sea (Bandar Abbas, Bushehr and Chabahar). https://vajiramandravi.com/upsc-daily-current-affairs/prelims-pointers/Gulf-of-Oman-upsc/

Each cycle contained 30 seconds of denaturation at 94°C, 60 seconds of annealing at 63.2°C, 30 seconds of extension at 72°C and at the end of the reaction, 5 min of extension at 72°C. After the amplification reaction was completed, 5 µL of the sample was transferred on to 1.5% agarose gel to evaluate the quality of the gene fragments.

The band in question was 650 bp, the accuracy of which was established. PCR products were sent to BIONEER Company in South Korea for sequencing by Dideoxy Chain Termination based on the specific primer. The sequences were submitted to GenBank in NCBI (National Center for Biotechnology Information). All the accession numbers are shown in (Table 1).

Table 1: The sequences submitted in NCBI.

Accession number	OM158728	OM158729	OM158730	OM158731	OM158732	OM158733
Accession number	OM158734	OM158735	OM158736	OM158737	OM158738	OM158739
Accession number	OM158740	OM158741	OM158742	OM158743	OM158744	OM158745

Data analysis

Arlequin software, version 3.5 (Excoffier and Lischer, 2010), have been used for molecular analysis including obtaining the number of haplotypes, bases, and distances between and within the populations, as well as the assessment of the degree of genetic divergence using the F_{ST} index by DNA sp

v.5.0 (Librado and Rozas, 2009). Molecular data was aligned using BLAST (Basic Local Alignments Search Tool) and MEGA 7.0.2 Softwares (Kumar *et al*., 2016). The AMOVA test was used to calculate the F_{ST} by examining the structure of populations at different levels. DnaSP 5.0 software was used to determine haplotypic diversity and nucleotide diversity, to assess the expansion and distribution of the population history, two methods were used and including Tajima's test (D-test of Tajima, 1989) and Fu's Fs test (Fu, 1997). Population history expansion and distribution along with mismatch distribution resulting from the pair differences between the populations were calculated based on factors θ_0, θ_1 (θ before and after population growth) (Excoffier and Lischer, 2010) and τ (unit time of mutation rate) using Arlequin 3.5 and DnaSP 5.10.01 software (Rozas *et al*. 2003). The software Network V5.1 (Rohl, 2004) was used to plot haplotypes network based on the median-joining method (Bandelt *et al*., 1999).

Results

In this study, the quality and quantity of the PCR product were evaluated on 1.5% agarose gel and the resulting bands were observed in the range of 600-650 bp (Fig. 2). The numbers of haplotype in three different regions were 22. The haplotype diversity was relatively high in Bandar Abbas (1.000), Chabahar (0.93), and moderate in Bushehr (0.533). The nucleotide diversity was also 0.037 in Bandar Abbas, 0.005 in Chabahar, and 0.013 in Bushehr. (Table 2).

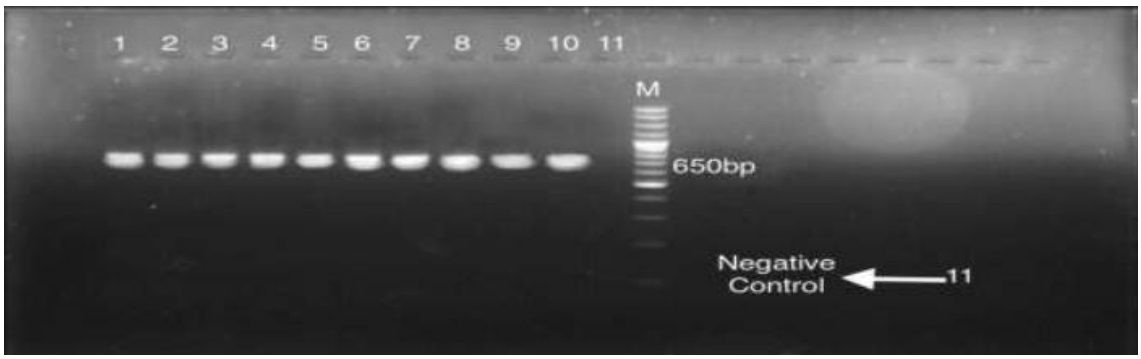

Figure 2: image of the PCR products on 1.5% Agarose gel.

Table 2: DNA polymorphism and haploid and nucleotide diversity of the three regions (Bandar Abbas, Bushehr, and Chabahar).

	Number of sequences	Number of haplotypes	Haplotype diversity	Nucleotide diversity(Pi)	Average number of differences(K)
Bandar Abbas	30	10	1±0.04	0.194±0.01	18.86
Bushehr	30	4	0.53±0.18	0.0133±0.007	8.20
Chabahar	30	8	0.93±0.07	0.022±0.01	2.22

Based on the population history of *Argyrops spinifer* using the mismatch distribution test, which shows pairwise genetic differences between haplotypes. Also based on sudden expansion, population distribution was multimodal in Bandar Abbas, unimodal in Bushehr, and bimodal in Chabahar (Fig. 3).

The result of the median-joining network based on COI gene showed that the *Argyrops spinifer* samples from Bandar Abbas and Chabahar, were located at a short distance from each other with fewer mutations, the samples from Bushehr were located at a further distance with a higher number of mutations in compare to the two other regions. Also, Haplotype number 20 in Bushehr region with a frequency of 22 and haplotype number 11 in Chabahar with a frequency of nine were the most observed haplotypes in the study area. The other haplotypes with a frequency of three showed the least frequency in the study area (Fig. 4).

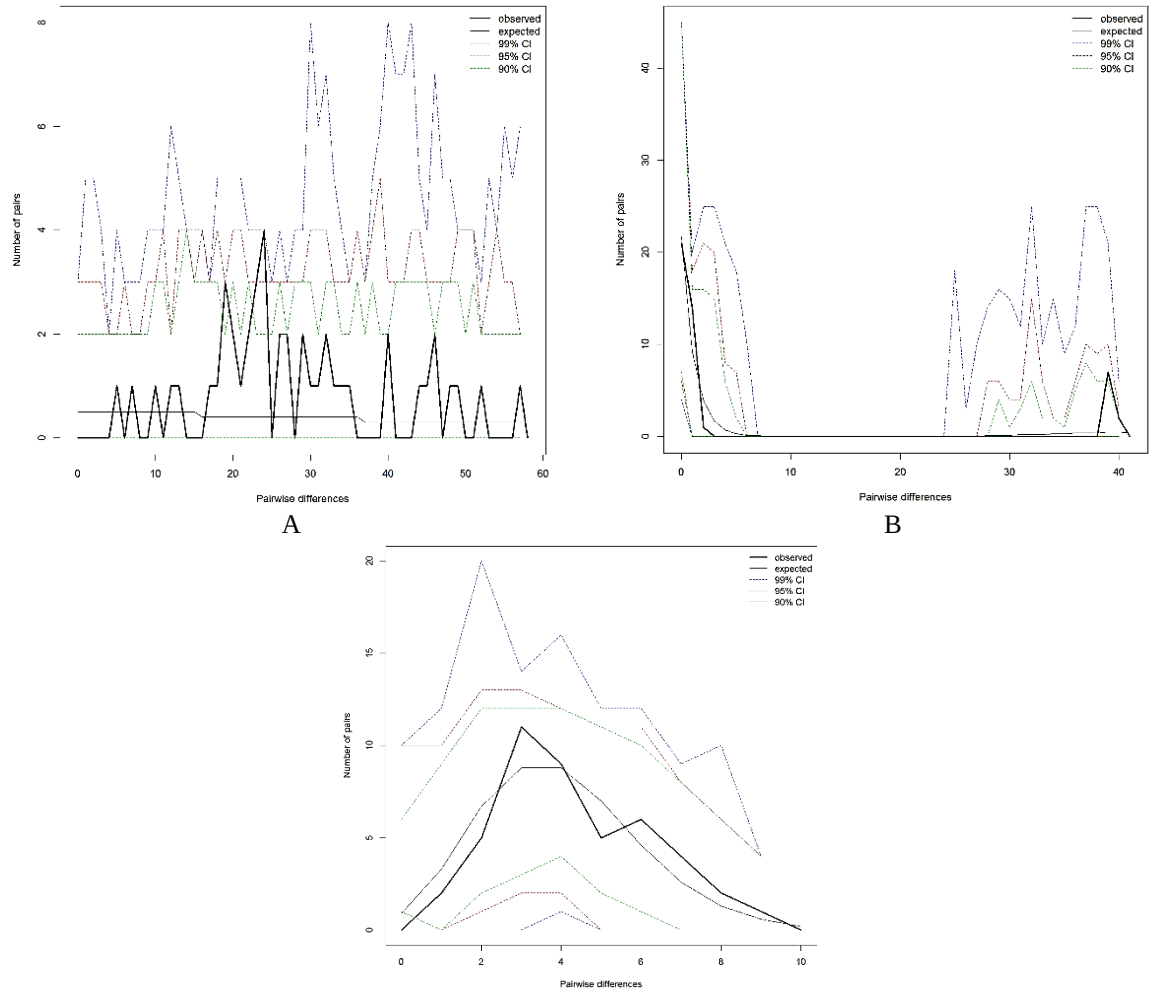

Figure 3: Mismatch distribution of *Argyrops spinifer* populations in studying area (A; Bandar Abbas, B; Bushehr and C:Chabahar).

The calculated value of the (Harpending *et al*., 1993) raggedness index (0.1) confirmed the similarity to the sudden expansion model (Table 3).

Genetic diversity indices and neutrality tests (Fu's FS index and Tajima's D) were -1.32 and -0.64 between the regions, respectively, both of which are negative and not statistically significant ($p \geq 0.05$).

Genetic diversity differences were calculated based on population hierarchy, three populations, and three regions based on the AMOVA test. The highest percentage of differences between the populations was 61.15% and the lowest percentage of genetic differences between members of the species was 38.85% (Table 4).

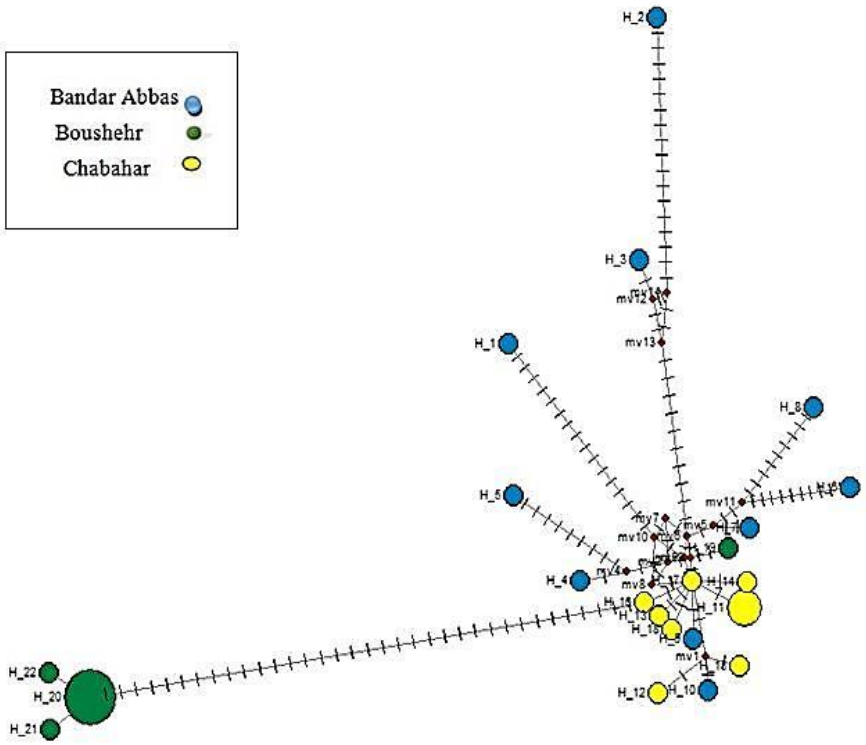

Figure 4: Median-joining haplotype network (Network 10.2.0.0 software) based COI mtDNA of *A.spinifer* sampled from 3 studied regions. Each pie represents a haplotype and its size reflects the frequency of samples. Distances between pies correspond to a number of mutations (*m*) between the haplotypes.

Table 3: COI mismatch distribution test in *Argyrops spinifer*.

Region	Tajima's D		Fu's FS		Mismatch distribution			Goodness-of-fit tests			
	P	D	P	FS	τ	θ_1	θ_0	P	Ragg*	P	SSD
Bandar Abbas	0.3	-0.54	0.09	-1.92	15.46	98.024	4.77	0.46	0.01	0.57	0.02
Bushehr	0.0	-2.10	0.97	4.64	0.62	6837.21	0.035	1.00	0.11	0.00	0.03
Chabahar	0.01	-1.32	0.00	-4.65	2.5	3414.97	0	0.08	0.18	0.07	0.05
Mean	0.11	-1.32	0.35	-0.64	6.19	3450.07	1.60	0.51	0.12	0.21	0.03

*Ragg: Harpending raggedness index, SSD = the sum of the squared deviations. θ_0, θ_1 (θ before and after the population growth), τ (unit time of mutation rate).

Table 4: The AMOVA results for *Argyrops spinifer* populations in the three regions.

Origin of variance	df	SS	Total variance	Percentage of diversity
Among the populations	2	227.03	10.67	61.15
Within the populations	27	183.10	6.78	38.85
Total	29	410.13	17.45	

The F_{ST} index was also used to measure genetic differentiation. The inter-population (intergroup) distance of *Argyrops spinifer* in the three studied regions was measured using DnaSP5.10.01 software. The highest inter-population distance was between Bushehr and Chabahar (0.48) and the lowest inter-population distance was between Chabahar and Bandar Abbas (0.151) (Table 5).

Table 5 Comparing F_{ST} in different *Argyrops spinifer* populations (Kimura 2- Parameter model).

Sampling area	Bandar Abbas	Bushehr	Chabahar
Bandar Abbas	---	0.11	---
Bushehr	---	---	0.48*
Chabahar	0.151*	---	---

*: Significant (p<0.05)

Discussion

The haplotype is a good index of the extent of genetic diversity among populations. The haplotype diversity level can vary from 0 (all population members have similar haplotypes) to 1 (all population members have different haplotypes) (Abiom *et al*., 2005). Bushehr had four haplotypes, while Bandar Abbas had the most haplotypes (10), indicating extremely high genetic diversity in the current study. In Bandar Abbas, 1, 0.53±0.18 in Bushehr, and 0.93±0.07 in Chabahar, the haplotype diversity was measured. In Bandar Abbas, 0.194 in Bushehr, 0.0133 and 0.022 in Chabahar, there existed nucleotide diversity. Additionally, the average number of differences was 2.22 in Chabahar, 8.20 in Bushehr, and 18.86 in Bandar Abbas. There are several reasons for this finding, Bandar Abbas and Chabahar regions are the habitat of different populations of this species, increasing of the indices can indicate high genetic dynamism and a more suitable population structure of this region compared to Bushehr due to more opportunities for natural reproduction, optimal utilization of the fishery resources of the region, and the non-destroyed natural habitat. It is not easy to give biological significance to the mentioned parameters. These findings can indicate the environmental stresses existing in this region. It is worth noting that genetic diversityis greater in species living in unstable and stressful environments compared to the same species living in stable environments; however, during short evolutionary periods (spanning several generations), a species' genetic diversity will be reduced if it is exposed to pollution for a prolonged time, is under fishing pressure within a certain age range, its natural habitat is destroyed, or its spawning

places are restricted. Also, overfishing of adult fish and failure to replace them in several generations causes only a small number to be able to reproduce, as a result of which the fish are born from a few breeders, which again reduces the genetic diversity (Welch *et al*., 2009). Bargelloni *et al*. (2003) examined the phylogeny of five species of Sparidae across the Atlantic-Mediterranean and reported the number of haplotypes for *Pagrus pagrus*, *Lithognathus mormyrus*, *Dentex dentex*, *Pagellus bogaraveo*, and *Spondyliosoma cantharus* as 32, 23, 23 12 and 41, and their haplotypic diversity as 0.92, 0.90, 0.67, 0.56, and 0.98, respectively. Accordingly, the mentioned parameters are consistent with the data obtained from this study.

The most F_{ST} genetic distance was between the Chabahar and Bushehr populations (0.48). Also, the lowest genetic distance of 0.17 was observed between the Chabahar and Bandar Abbas populations. The results showed the presence of different populations of this genus in the Persian Gulf and Sea of Oman.

There are two primary measures of the amount of genetic variation in a population at a locus: heterozygosity and the number of alleles. Allelic richness (number of alleles) is a measure of genetic diversity indicative of a population's long-term potential for adaptability and persistence. It is used less commonly than heterozygosity as a genetic diversity measure, partially because it is more mathematically difficult to take into account the stochastic process of genetic drift for allelic richness (Greenbaum *et al*., 2014). In general, the mean number of haplotype per locus for saltwater fish is 20 (DeWoody and Avise, 2000). In this study, the number of haplotype observed at different loci was 22, which is slightly higher than the number of haplotype in saltwater fish. Varying numbers of haplotype have been reported in the Sparidae family. In general, the population of *Argyrops spinifer* in the Persian Gulf shows a relatively moderate and desirable number of haplotype in different loci compared to other regions and species of the same genus. The number of haplotype in Bushehr was lower, which could be due to the Persian Gulf being an enclosed sea, the low migration of the populations of this species in the Persian Gulf, and the limited gene exchange with other populations. This lower number could also indicate the effects of fishing pressure in these areas (Lind *et al*., 2008).

The MJ-haplotype network showed that the star-like topology was not formed in the study area, suggesting that these populations have not experienced recent population expansion and showing long-term stability (Mila *et al*., 2000; Xue *et al*., 2014). Both neutrality tests (Tajima's D and Fu's Fs) for the studied sequences resulted in -1.32 and -0.64, respectively, indicating the expansion of *Argyrops spinifer* in the three studied regions. The high haplotypic and nucleotide diversity could probably be due to this population expansion (after a period in which the effective population size was low) (Hewitt, 1996; Carstens and Knowles, 2007). This finding is consistent with the results of these tests in this study. In their study of *Dentex dentex* and *Lithognathus mormyrus*, Bargelloni *et al*. (2003), reported Tajima's D test results as -0.72 and -0.99, respectively.

The Fst index describes the differentiation of populations at different levels of genetic structure. An Fst value of 0-0.05 shows low genetic differentiation, 0.05-0.15 shows high differentiation, and >0.25 shows very high differentiation (Balloux *et al*., 2002). Accordingly, the genetic difference between the populations of Chabahar and Bushehr was 0.48, which indicates a high genetic difference, and the lowest value of 0.15 was obtained between the populations of Chabahar and Bandar Abbas, which is quite justifiable due to the lack of gene flow between the studied regions. An Fst>0.05 indicates that gene flow is limited among the populations and does not allow some populations to be subdivided (Hoolihan *et al*., 2006). In a study by Ghasemi *et al*. (2019), Fst levels were 0.01-0.4 among different Sparidae populations, which suggests a high differentiation between the populations. In another study by Ghasemi *et al*. (2019) on *Sparidentex hasta*, the reason for the high differentiation was the low migration of populations of this species between the distribution regions, which is consistent with the biology of this species, which has a slow swimming speed and show vertical migrations. In a study of *Acanthopagrus latus* populations, Syazini *et al*. (2015) identified high genetic differentiation between populations due to extensive migration although only at short distances. In a study of *Sparus aurata* populations in waters around Italy, Franchini *et al.* (2012), also reported geographical distance as the reason for the high genetic differentiation between these populations. Water currents in the Persian Gulf are very slow, and as a result, they have less impact on the movement of fish larvae and juveniles than in other places, such as the waters of western Japan and Korea or monsoon currents in the China Sea and the Taiwan Sea. As a result, it is expected that there be distinct populations of this species in the Persian Gulf, as the populations of the studied regions are highly differentiated. Although sea currents play a major role in Sparidae populations, geographical distance is also a determining factor. Therefore, the results of the AMOVA test in this study based on the Fst index showed high genetic diversity among the populations (61.15) and relatively high genetic differences within the populations (38.85). The test results reported by Morgan *et al*. (2018) for *Chrysophrys auratus* on the east coast of Australia was 76.3.

Conclusions

Based on the present findings, the COI gene is a suitable marker for identifying the species in the Persian Gulf and the Sea of Oman. The higher molecular diversity indices in Bandar Abbas demonstrating the higher genetic dynamics and more appropriate population structure of this region. The most genetic distance was observed between the populations of Chabahar and Bushehr and the lowest genetic distance between the populations of Chabahar and Bandar Abbas which is quite justifiable due to the lack of gene flow between these regions. *Argyrops spinifer* has a favorable and high genetic diversity. There are different populations of this species in the Persian Gulf and the Sea of Oman, and the main factors differentiating the populations include slow swimming, vertical migration, geographical distance,

and the sea currents from the Strait of Hormuz toward the north of the Persian Gulf, which should be considered for the management of the Stock of this economically and ecologically important species.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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