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RESEARCH ARTICLE

Colonization and genetic changes of Indo-Pacific immigrant *Saurida undosquamis* (Richardson, 1848) (lizardfish) in the Mediterranean Sea

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Abstract

Genetic pathway of colonization of Lessepsian lizardfish *Saurida undosquamis* populations on the way from the Red Sea to Mediterranean Sea was examined. The lizardfish (*Saurida undosquamis*) samples were collected from the Red Sea (Jeddah) and Mediterranean Sea (Syrian Coast (Lattakia Port), Iskenderun Bay, Mersin Bay, Antalya Bay), and analyzed using mtDNA 16S gene region with mtDNA PCR-RFLP method with 6 restriction enzymes (*Bsur*I *Alu*I, *Hin6*I, *Rsa*I, *Xho*I, *Ehe*I). A total of 16 haplotypes were detected from 150 individuals. AAAAAA haplotype was the most common occurred in all sampling sites present in 40% of individuals. The average haplotype diversity and genetic diversity within populations were 0.4579 and 0.009179, respectively. The average genetic diversity and genetic divergence between populations were calculated 0.025294 and 0.016115, respectively. In Monte Carlo (X^2) pairwise comparisons highly significant differences ($P < 0.001$) between all populations were detected. *S. undosquamis* showed high genetic changes on the pathway of its colonization from south to northward, and there is lack of genetic migration between the Red Sea and Mediterranean.

Keywords: Colonization, *Saurida undosquamis,* Mediterranean, Indo-Pacific, mtDNA.

Introduction

The lizardfish *Saurida undosquamis* (Richardson 1848) is a lessepsian migrant species that penetrated into the Mediterranean Sea from the Indo-Pacific through the Suez Canal (Ben-Tuvia 1966; Gücü and Bingel 1994; Mater *et al*.

1995). *S. undosquamis* invaded the Levant Basin, and established a population of considerable commercial importance (İşmen 2003). First specimens of *S. undosquamis* were collected during 1953 along the coast of Israel (Ben-Yami and Glaser 1974). The first report of *S. undosquamis* in Turkish seas was made by Kosswig (1951). *S. undosquamis* is a demersal species inhabiting sandy and muddy bottoms, at depths generally above 100 m (Golani *et al*. 2002; Gökçe *et al*. 2007). It is reported that the maximum size of *S. undosquamis* is about 50 cm (Bauchot 1987). The distribution range of *S. undosquamis* includes the Indo-West Pacific Ocean (Red Sea, Persian Gulf, Eastern Africa to Japan and Australia) (Froese and Pauly 2006) and Eastern Mediterranean. *S. undosquamis* is one of the most successful colonizers throughout the Levant Basin, which extends as far as the Aegean Sea (Bilecenoğlu *et al*. 2002; Gökçe *et al*. 2007).

In the present study, lessepsian migrant *S. undosquamis* were analysed with mtDNA 16S rDNA region using PCR–RFLP method in order to elucidate genetic changes on the pathway of its colonization from south to northward.

Materials and Methods

Sampling

Samples were collected separately by commercial fishing vessels (trawlers) from five fishing ports in the Red Sea (Jeddah) and Mediterranean Sea (Syrian Coast (Lattakia Port), Iskenderun Bay, Mersin Bay, Antalya Bay) (Figure 1). The sampling size and relevant information for the collected samples as well as abbreviations of sampling areas are given in Table 1.

Mitochondrial DNA Analysis

Total DNA was extracted from muscle using the standard phenol: chloroform: isoamyl alcohol procedure (Sambrook *et al*. 1989). PCR amplification of the mitochondrial ND 5/6 rDNA gene was carried out using the universal primers:

16S-a: 5'- CG (CT) AAG GGA A (ACT) G CTG AAA-3'

16S-b: 5'- CCG GTC TGA ACT CAG ATC ACG TAG -3'

Table 1. Sampling details of *S. undosquamis* used in this study.

Sampling area	Abbreviation	Sample size	MTL*	Collection date
Red Sea	RS	30	32.92 (6.57)	14.12.2008
Syrian Coast (Lattakian Port)	MS1	30	23.37 (2.00)	13.11.2010
Iskenderun Bay	MS2	30	23.29 (1.56)	11.01.2011
Mersin Bay	MS3	30	23.99 (1.45)	25.01.2010
Antalya Bay	MS4	30	20.30 (2.56)	20.01.2011

*MTL:mean total length (mm), standard deviations in brackets

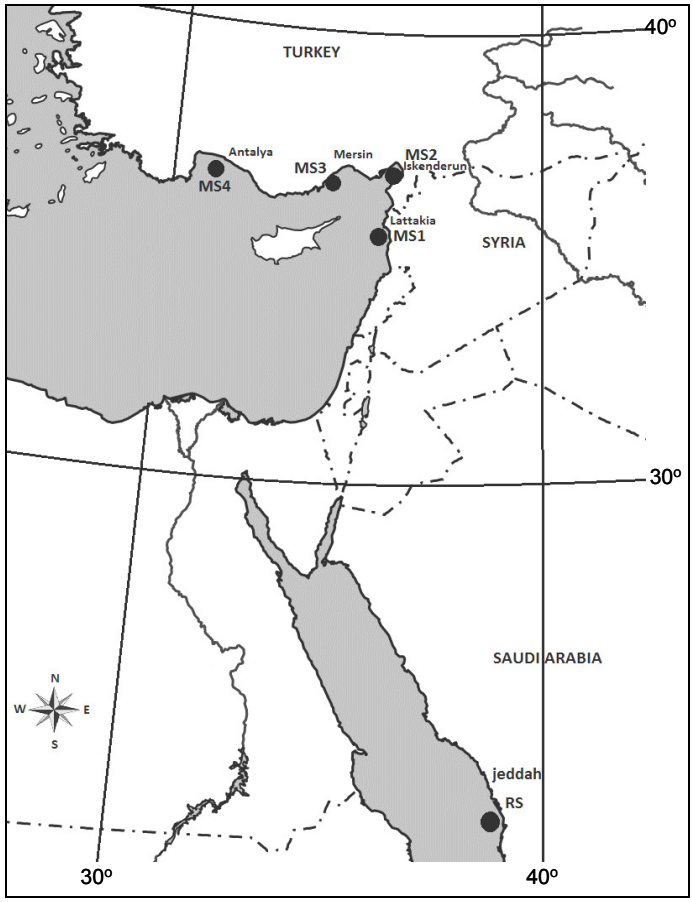

Figure 1. Sampling areas

The amplification was performed with a profile of 94 °C for 4 min, followed by 35 cycles of 94 °C/30s strand denaturation, 52 °C/20s annealing and 72°C/1 min 30 sec primer extensions, and a final 7 min elongation at 72 °C. The ND 5/6 rDNA amplification conditions were: 1.5 µl 10 x polymerase buffer, 0.5 µl dNTP (10 mM), 0.3 µl Taq DNA polymerase (3 U/µl), 0.10 µl primers, 1µl template DNA, and water for a total reaction volume of 25 µl.

The PCR product was restricted with one of 6 endonucleases: *Bsur*I (*Hae*III), *Alu*I, *Hin6*I (*Hha*I), *Rsa*I, *Xho*I, *Ehe*I. The fragments of the restricted DNA samples were separated on 6% polyacrylamide gels, together with a pGem marker (Promega). A modified silver nitrate staining protocol (Tegelstrom 1987) was used to visualize the DNA fragments.

Nucleotide sequence diversities and divergence (Nei and Tajima 1981; Nei 1987) were determined using the REAP computer package programme (McElroy *et al.* 1991). The significance of geographic heterogeneity in haplotype distribution was tested through a Monte Carlo simulation (Roff and Bentzen 1989) with 100 randomisations of the data. A molecular analysis of variance (AMOVA) using F_{ST} was also performed to detect the level of gene flow between populations with Arlequin *v*3 (Excoffier and Schneider 2005). A mismatch analysis was performed using Arlequin *v*3 to compare the occurrence of demographic changes in the populations (Rogers and Harpending 1992). This analysis compares the distribution of the frequency of pairs of individuals who differ by a certain number of nucleotide differences.

The distance matrices of pairwise comparisons among the haplotypes and populations were performed using the unweighted pair-group method with arithmetic averages (UPGMA, Sneath and Sokal 1973), and minimum evolution (ME) methods implemented in the MEGA 3.1 program. The robustness of the internal branches of trees was assessed by bootstrapping (Felsenstein 1985) with 1000 replicates.

PCR-RFLP generated fragment profiles were classified by letters which were then combined to define composite mtDNA haplotype patterns. The size of the restriction fragments were estimated from their mobilities relative to a standard DNA ladder molecular size marker using DNA -FRAG version 3.03.

Results and Discussion

The restriction enzymes (*Bsur*I (*Hae*III), *Alu*I, *Hin*6I (*Hha*I), *Rsa*I, *Xho*I, and *Ehe*I) that showed polymorphic patterns were used to define haplotypes. PCR amplification resulted in a product of approximately 1850 bp, corresponding to about 11% of the mitochondrial genome (Miya *et al.* 2001). A total of 16 composite haplotypes was found from 150 individuals. The composite haplotypes and their occurrence in each population are given in Table 2.

All of the 15 haplotypes, excluding AAAAAA haplotype, were not shared between the populations. This indicates that there is limited gene flow between populations. This high genetic difference between populations may be explained with differential ecological niches. *S. undosquamis* populations inhabiting different habitats in the northeastern Mediterranean are face with different environmental conditions such as topographic structure, predators and types of food available. The number of AAAAAA genotype in overall populations decrease towards the west of Turkish coasts (MS1: 100 %, MS2: 16.6 %, MS3: 6.6 %). This pathway of differentiation may be explained with differential environmental niches in each region which cause the detected genetic differences. This difference can also be due to environmentally induced selection.

Although our study determined 16 haplotypes, 60 out of 150 individuals represented a single haplotype (AAAAAA) and 4 haplotypes were represented with only one individual from population. Shakman (2008) examined genetic structures of 169 individuals from the Mediterranean and Red Sea populations of *Siganus rivulatus*, a lessepsian species of the Mediterranean, with cytochrome b gene region and determined a total of 10 haplotypes. Five out of ten haplotypes were represented with one individual, and also 159 out of 169 individuals represented 1 haplotype.

Haplotype 1 (AAAAAA) was the most common and presented in 40% of individuals. This haplotype is the only haplotype which is unique to the Mediterranean and common in all of the Mediterranean populations (Figure 2). This haplotype may be the reason of remarkable success of *S. undosquamis*'s colonization in the Mediterranean area.

Haplotype and nucleotide diversity values for each population were calculated from the restriction fragment data. Average haplotype diversity (0.4579) and nucleotide diversity (0.009179) within populations was moderate. According to both measures, the lowest level of variation was found in the Iskenderun Bay population (Table 2). The detected zero genetic diversity can be related to a genetic bottleneck caused by overfishing of *S. undosquamis* in Iskenderun Bay. *S. undosquamis* is the target species of trawling. Yoksel (2008) reported that *S. undosquamis* caught in Iskenderun Bay were young individuals and underlined the necessity of taking precautions against overfishing and suggested controlled fishing of *S. undosquamis* in Iskenderun Bay. Similarly, Çiçek (2006) concluded that *S. undosquamis* in Iskenderun Bay has decreased both in terms of biomass and proportion, and this depletion was linked to the pressure of overfishing in this region as supported also by Gücü (2000).

The highest level of genetic variation was determined in the Mersin Bay populations (0.029476). Seven out of 16 haplotypes were observed only in the Mersin population. Due to its topographic structure, Mersin Bay is a shallow and has very large sandy areas. There has been no study reporting the pressure of overfishing in this bay which therefore presented the optimum niche for *S. undosquamis*, and this is not the case for the other regions. Fishing areas for trawling in Mersin Bay are found at waters over 5 miles from the land which is not preferred by trawlers due to economic reasons since in the other regions 1 mile is enough for trawling (personal communication with fishermen). Therefore low fishing pressure, optimum ecological niche and optimum habitation conditions allowed the Mersin population to conserve their genetic diversity since there is no or limited migration from other populations.

Three haplotypes were observed in the Antalya Population. Serpin (2007) concluded that *S. undosquamis* populations in Antalya and Iskenderun are entirely different from each other in terms of morphometric characteristics. In

Table 2. Frequency of 16 composite mtDNA haplotypes from RFLP data within the studied stocks of *S. undosquamis*. Letters reflect individual haplotypes for six restriction enzymes; *Ehe*I, *Bsur*I, *Rsa*I, *Hin6*I, *Xho*I, *Alu*I, (left to right). *H*, haplotype diversity; *N*, nucleotide diversity; *S.E.*, standard error

Haplotypes	Restriction enzymes						Sampling sites					
	***Ehe*I**	***Bsur*I**	***Rsa*I**	***Hin6*I**	***Xho*I**	***Alu*I**	**RS**	**MS1**	**MS2**	**MS3**	**MS4**	**Toplam**
1	A	A	A	A	A	A		23	30	5	2	60
2	B	A	A	A	A	A					16	16
3	C	C	A	A	A	A	14					14
4	D	B	C	B	B	B				14		14
5	C	C	B	A	A	A	13					13
6	B	A	A	A	A	B					12	12
7	A	A	B	A	A	A				4		4
8	C	C	B	A	B	A	3					3
9	A	A	D	B	A	A		3				3
10	D	A	D	B	A	A		3				3
11	D	A	B	C	B	B				2		2
12	D	A	B	B	A	B				2		2
13	D	A	E	B	A	A		1				1
14	A	B	C	B	B	B				1		1
15	D	A	C	A	B	B				1		1
16	D	B	C	B	B	A				1		1
					Total		30	30	30	30	30	150
												Average
					H		0.5944	0.3977	0.0000	0.7367	0.5605	0.4579
					***S.E.* (+/-)**		0.02964	0.07418	0.0000	0.04798	0.03301	0.01601
					N		0.002948	0.010608	0.0000	0.029476	0.002864	0.009179
								334				

the present study, the morphometric differentiation is supported also genetically, and there are specific haplotypes (BAAAAA and BAAAAB) for the Antalya population (Figure 2).

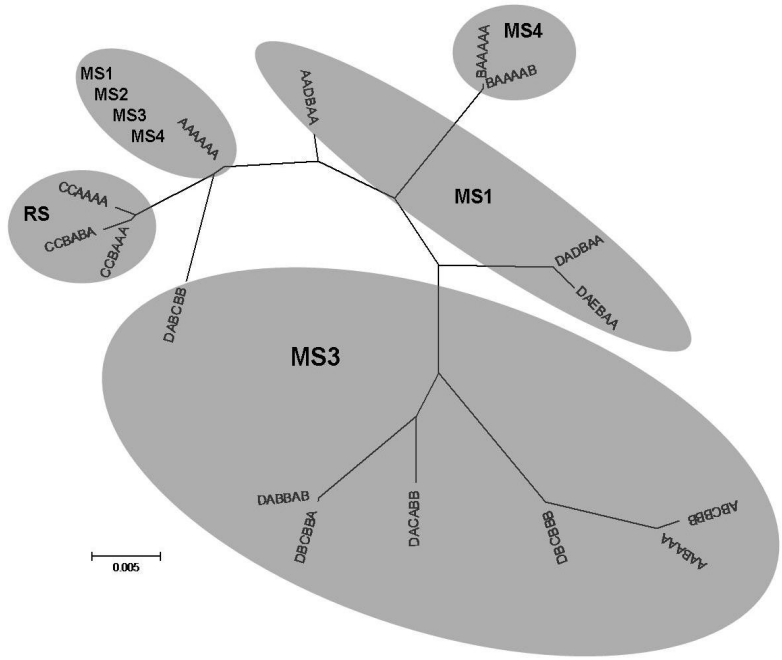

Figure 2. Minimum evolution tree that shows the relationships among the haplotypes

The study revealed that there are three types of haplotypes in the Red Sea population and that population displays a haplotype diversity (0.5944) higher than all populations, apart from the Mersin population. Some studies conducted on lessepsian fishes revealed that the Red Sea migrants in the Mediterranean Sea display a wider range of diversity than the populations in the Red Sea (Azzurro *et al*. 2006; Golani *et al*. 2007). This study confirmed that haplotypes are not shared between the Red Sea and Mediterranean Sea populations. El-Halfawy *et al*. (2007) concluded that female reserves in Suez Bay were depleted due to overfishing. Thus it is considered that the genetic migration between the Mediterranean and the Red Sea was interrupted due to overfishing and other reasons. Therefore there is no any common haplotype between the Mediterranean and Red Sea populations of *S. undosquamis.*

Mean nucleotide divergence among populations of *S. undosquamis* was 0.025294 (+/- 0.0000273). The highest value of pairwise inter-group nucleotide divergence was detected between the Red Sea and Mersin Bay samples

(0.029203), and the lowest (0.001138) between the Mediterranean samples (Syria and Iskenderun Bay) (Table 3).

Table 3. – Pairwise estimates of nucleotide divergence (below diagonal) and FST (above diagonal) values among *S. undosquamis* populations. ***, significance value (P < 0.001)

Stock	RS	MS1	MS2	MS3	MS4
RS	——	0.019885***	0.014387***	0.045415***	0.017762***
MS1	0.013107***	——	0.006442***	0.041854***	0.012685***
MS2	0.012914***	0.001138***	——	0.043732***	0.006487***
MS3	0.029203***	0.021812***	0.028994***	——	0.044296***
MS4	0.014856***	0.005948***	0.005055***	0.028126***	——

The lessepsian migration from the Red Sea to the Mediterranean generally takes place on the Syria-Iskenderun Bay - Mersin Bay route (Ben-Tuvia 1966; Avşar 1999; Mavruk and Avşar 2007). However the first species documented on the Aegean coasts of Turkey and Western Mediterranean coasts of Turkey (Akyol *et al.* 2005; Corsini *et al.* 2005) suggest that *S. undosquamis* population in the Antalya shore do not only come over Mersin Bay. The Red Sea population in this study has as genetic relationship closer to the Antalya population in comparison to the Mersin population.

The genetic relationship between samples is summarized in the form of a UPGMA dendrogram (Figure 3). In the first clad, the Syria and Iskenderun Bay samples clustered as the closest clades, while the Antalya Bay sample was in the neighbouring clad. The Mersin Bay sample clustered as the most divergent (Figure 3). High bootstrapping values were detected for each node on the UPGMA dendrogram. In Monte Carlo pairwise comparisons of haplotype frequencies, there were significant differences between all pairs of populations (P < 0.001) (Table 3).

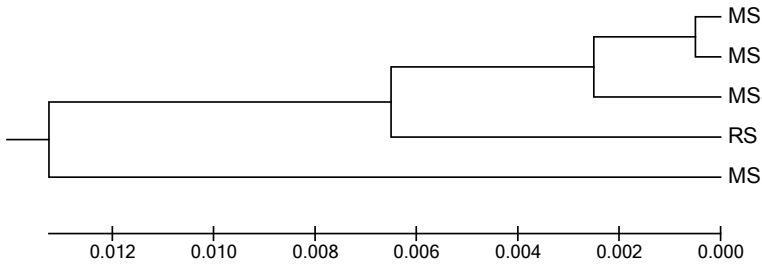

Figure 3. UPGMA phenogram of genetic relationships among populations of *S. undosquamis.* Bootstrap estimates (as a percentage) are indicated above branches. RS, Red Sea; MS1, Syria; MS2, Iskenderun Bay; MS3, Mersin Bay; MS4, Antalya Bay

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İndo-Pasifik göçmeni *Saurida undosquamis* (Richardson, 1848)'in (Iskarmoz balığı) Akdeniz'de kolonizasyonu ve genetik değişimi

Özet

Bu çalışmada Iskarmoz balığı *Saurida undosquamis* (Richardson, 1848) populasyonlarının İndo-Pasifik'ten Akdeniz'e göç yolu üzerinde kolonileşmede gösterdiği genetik değişimin ortaya koyulması amaçlanmıştır. Iskarmoz balığı (*S. undosquamis*)'nın, Kızıldeniz (Cidde) ve Akdeniz'den (Suriye, İskenderun Körfezi, Mersin Körfezi, Antalya Körfezi) örneklenen, populasyonlarının genetik yapısı, mtDNA'nın 16S gen bölgesi için, 6 sınırlama enzimi (*Bsur*I *Alu*I, *Hin6*I, *Rsa*I, *Xho*I, *Ehe*I) kullanılarak tespit edilmiştir. *S. undosquamis* populasyonlarına ait toplam 150 bireyinin incelenmesiyle toplam 16 haplotip saptanmıştır. Örneklerde en fazla gözlenen haplotipin, örneklerin %40'ında gözlenen AAAAAA haplotipi olduğu tespit edilmiştir. Populasyonlar içi ortalama haplotip çeşitliliği 0.4579 (+/- 0.01601), populasyonlar içi ortalama genetik çeşitlilik 0.009179 olarak bulunmuştur. Populasyonlar arası ortalama genetik benzerlik 0.025294, ortalama genetik farklılık ise 0.016115 olarak hesaplanmıştır. Monte-Carlo (X^2) İkili Karşılaştırma Analizi sonucunda, tüm populasyonlar arasında genetik farklılığın önemli olduğu belirlenmiştir ($P < 0.001$). Çalışmada *S. undosquamis*'in mutasyonlar geçirmek suretiyle güneyden kuzeye göçünü sürdürdüğü, Kızıldeniz ve Akdeniz arasında genetik göç mekanizmasının kesildiği tespit edilmiştir.

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