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A Lessepsian invader round herring (*Etrumeus golanii*) with high genetic diversity without bottlenecking in the northeastern Mediterranean Sea

Solmaz Ezgi ÇİFTÇİ[1], Fevzi BARDAKCI[1,2,*]

[1]Department of Biology, Faculty of Arts and Sciences, Aydın Adnan Menderes University, Aydın, Turkey
[2]Department of Biology, College of Science, University of Ha'il, Ha'il, Saudi Arabia

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Abstract: In this study, population genetic structure and genetic diversity of round herring in the Turkish seas were determined using sequence data of mitochondrial DNA control region. Round herring, *Etrumeus golanii* samples were collected from three localities along the Turkish coast of the Mediterranean Sea, (Gulf of İskenderun and Gulf of Antalya) and southern Aegean Sea (Gulf of Marmaris). Genetic variability was high both within and between the populations based on haplotype diversity values. The results have shown that each population has a high genetic diversity with unique mtDNA haplotypes with unique genetic structure. The most important factor in the emergence of this situation is that populations were considered to have migrated from the Red Sea to the Mediterranean Sea by different fish shoals at different time intervals independently of each other. A general acceptance for explanation of high genetic diversity in the Mediterranean Lessepsian species is the lack of bottleneck due to the consecutive invasions.

Key words: mtDNA, control region, Lessepsian species, Mediterranean, Turkey

1. Introduction

The opening of the Suez Canal in 1869 created a connection corridor between the Mediterranean and the Indian Ocean. To date, 666 marine exotic species have been recorded in the Mediterranean Sea and these include more than 90 Lessepsian fish species (Galil et al., 2015; Zenetos and Galanidi, 2020). Round herring, *Etrumeus golanii* is amongst the fish species that expanding its distribution area to the Mediterranean subregions (Zenetos and Galanidi, 2020).

E. golanii is a coastal pelagic species that colonizes the Mediterranean Sea from the Red Sea through the Suez Canal despite the Indian-Pacific distribution (Golani et al., 2006). It is a circumtropical species that its source populations are the Pacific Ocean, Indian Ocean and the western part of the Atlantic Ocean. In the 1990s, it increased its abundance in the coasts of Israel and the area of distribution has expanded to the Gulf of İskenderun and Cyprus (Golani et al., 2006). Between the years of 1994 and1996, six *E. golanii* samples were captured from Karataş (İskenderun Gulf) and it was the first report from the Turkish seas (Basusta et al., 1997). Then, the waters of Cyprus was established in a significant way (Golani, 2000). It was then reported from Rhodes in December 2003 (Corsini et al., 2005), Cyclades Islands in May 2004 (Kallianiotis and Lekkas, 2005), Crete in July 2005, (Kasapidis et al., 2007) and Idra Island in November 2005 (Zenetos et al., 2008). In September 2005, *E. golanii* reached the Island of Lampedusa in the Strait of Sicily (Falautano et al., 2006). Yarmaz et al. (2010) reported a single northern-most example of *E. golanii* on the Dikili coast (Aegean Sea) in February 2009. Recently, *E. golanii* was first reported in Tunisia in 2016 (Gulf of Gabes, Central Mediterranean) (Boudaya et al., 2016). Today, intensive fishing of this species is carried out in the Aegean Sea and Levantine Sea in Turkey.

The present study aims to determine the genetic structure of a Lessepsian species round herring across the northeast of the Mediterranean Sea using sequence analyses of the mtDNA control region. Investigating the genetic structure of the populations of such a Lessepsian species would allow us to delineate the factors shaping up their genetic structure and invasion process. Previous studies on the round herring populations in the northeastern Mediterranean are mainly on their taxonomy and a few are on their biology (Yılmaz and Hoşsucu, 2003; Mehanna and El Gammal, 2005).

2. Materials and methods

The samples examined in the study were obtained from coastal fishers during the fishing season. Sampling localities were Antalya and İskenderun Gulfs in the Mediterra-

* Correspondence: fevzi.bardakci@gmail.com

nean Sea, and Marmaris Gulf in the Aegean Sea (Table 1 and Figure 1). A total of 88 samples of round herring were obtained from the localities studied and stored in 96% ethanol. Total genomic DNA was isolated from approximately 25–50 mg of the caudal muscle of each sample using Purelink Genomic DNA Mini Kit (Invitrogen; Thermo Fisher Scientific,Waltham, MA, USA) according to the protocol given by the manufacturer.

DNA concentrations were checked on Nanodrop1000 (NanoDrop Technologies, Wilmington, DE, USA) at 260–280 wavelength and adjusted to use in PCR reactions.

The primers used for the amplification of 1274 bp the mtDNA control region has been designed using the Primer3 Plus program based on the whole mitogenome of round herring (NC009583) in the GenBank (Forward: Eg-SE-F5'-CACCTCTAACTCCCAAAGCTAGAAT-3' and Reverse: Eg-SE-R 5'-GAAGCATTATGTTTGGAACTT-GCTA-3').

Table 1. Sampling localities, coordinates, sample numbers and sampling date

Populations	Sample numbers	Sampling date	Coordinates
Antalya	34	01.03.2013	36° 30›09.9››N 31°00'05.1"E
İskenderun	26	14.03.2017	36° 46'05.8"N 36°02'05.9"E
Marmaris	28	01.04.2016	36° 47'23.6"N 28°18'41.1"E

The mtDNA control region of the round herring samples, 1× *Taq* polymerase buffer [10× *Taq* buffer; 100 mM Tris-HCl (pH 8.8)], 2.5 mM dNTP mixture (each dATP, dTTP, dCTP, dGTP 0.5 mM; Fermentas, MBI), 2 mM $MgCl_2$ (25 mM; Fermentas, MBI) 0.5 U/µL *Taq* DNA polymerase (5U/µL; Fermentas, MBI), 0.2 pmol/µL of each primer, the final reaction has been completed with sterile distilled water to a volume of 25µL, and has been amplified with 50 ng/µL template DNA. PCR has been performed on the thermal cycler (Bio-Rad T100, Bio-Rad Laboratories, Hercules, CA, USA); temperature loop conditions initial denaturation 94 °C 2 min, denaturation 94 °C 30 s, annealing 55 °C 1 min, extension 72 °C 1 min 35 cycles and 72 °C 5 min final elongation. PCR products were detected on 1.5% agarose gels in TBE buffer (0.5 M Trisma-base, 0.5 M boric acid, 0.01 M EDTA, pH 8,13), stained with 5 µL/100 mL of SafeView (Applied Biological Materials Inc., Richmond, BC, Canada) dye, and visualized under UV light in a gel documentation system (Vilber Lourmat, Collègien, France). PCR products were cleaned using the PCR purifying kits (Invitrogen and PureLink).

Sequencing was carried out single-stranded by Macrogen Europe B.V. (Amsterdam, Netherlands) using the BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA) and an ABI 3730XL capillary sequencer. A length of 997 bp sequence of mtDNA control region were successfully aligned using Clustal W (Thompson et al., 1994) in BioEdit ver 7.1.3.0 (Hall, 1999).

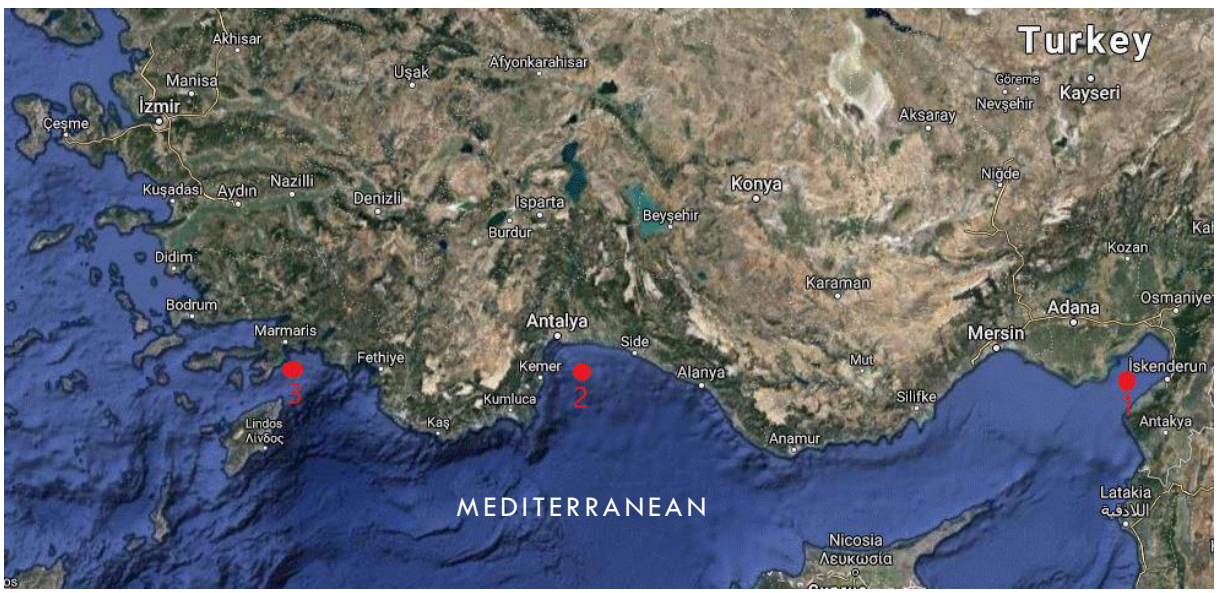

Figure 1. Sampling localities (1. İskenderun, 2. Antalya, 3. Marmaris).

mtDNA control region of round herring specimens was analyzed using DNAsp ver5.10 (Rozas et al., 2003) software to estimate haplotype diversity (*h*), with nucleotide diversity (π) the genetic distance (γ_{ST}, $Gamma_{ST}$) between the population. By using existing data series and genetic distance comparisons (γ_{ST}, $Gamma_{ST}$), neighbor-joining (NJ) trees have been formed through the MEGA 6 (Tamura et al., 2013) program to determine the genetic relationship among the populations. AMOVA (Excoffier et al., 1992) analysis implemented in Arlequin ver.3.5.1.3 (Excoffier and Lischer, 2010) was used to determine the partition of genetic variation within and between the groups and among populations. Groupings were made according to geographical distribution (Group 1: Antalya and İskenderun populations from the Mediterranean/ Marmaris population from the Aegean Sea) and NJ tree clustering of this study (Group 2: Antalya-Marmaris/ İskenderun). Using genetic distance values, *Nm*=0.5 (1/ γST-1) (Takahata and Palumbi, 1985) formula was used to estimate migration rate (*Nm*) among populations. Effective population size (*Ne*) was calculated by using the determined mutation rate ($\mu = 1.29 \times 10^{-5}$) of the *Ameiurus nebulosus* for the mtDNA control region using the formula $\pi = 2Ne.\mu$ (Chen and Herbert, 1999).

3. **Results and discussion**

In this study, 997 bp length mtDNA control region of 88 round herring specimens have been analyzed. Analysis of the sequences of mtDNA control region has revealed 70 haplotypes[1]. The most shared haplotypes were Haplotype 9 and Haplotype 14, which have been found in all localities studied while 68 haplotypes were not shared between any of the localities studied. The mean haplotype diversity and the average nucleotide diversity in all populations were 0.982 and 0.0047, respectively. Although haplotype diversity was the highest in Antalya (0.980) population, values were close to each other among the populations studied. Nucleotide diversity was the lowest in the Marmaris population (0.0038) and the highest in the İskenderun population (0.0048) (Table 2). DiBattista et al. (2014) carried out analyzes of mtDNA *COI* and *cytochrome b* regions to examine the gene flow of *Etrumeus* genus in Australia, along the Western Australian and New South Wales coasts. Haplotype (0.69 ± 0.14) and nucleotide diversity (0.0019 ± 0.0012) in the samples from southern Australia were found to be lower in comparison to other localities. It has been concluded that the time since the last population expansion was newer for New South Wales than for south Australia or western Australia. In this study, haplotype and nucleotide diversity obtained from three sampling localities were found to be higher than *Etrumeus* species distributed in Australian waters. It has been argued that the isolated effect of the throat affects the genetic diversity of the *Etrumeus* genera. One simple explanation for higher genetic diversity in the Mediterranean Sea than Australian waters might be due to the high mutation rate of mtDNA control region used in this study. It is obvious that haplotype diversity of *Etrumeus* species is very high across the cost of Australia although *COI* and *cytochrome b* regions of mtDNA are relatively more conserved than the D-loop region. Our Lessepsian species have also very high haplotype and nucleotide diversity values in the Mediterranean Sea. Such high diversity has also been found in a Lessepsian migrant, *Nemipterus randalli* that has shown a high average haplotype diversity value (0.92 ± 0.04) throughout its native distribution range while relatively lower haplotype diversity value (0.74 ± 0.040) was found in the invasive range of the Mediterranean Sea (Srihari et al, 2021). Similar observations have also been reported for angelfishes (Hobbs et al., 2013). It has been proposed that one possible explanation for such high genetic variation is due to the high fecundity and maturity at early age of fish populations (Romiguier et al., 2014; Martinez et al., 2018). This seems true for the Mediterranean invader round herring since the age at the first sexual maturity of round herring from the Gulf of Suez was reported as early as 1.73 and 1.70 for males and females, respectively (Sanders et al., 1984). In addition, it has also been put forwarded that round herring is a multispawner with high fecundity based on the occurance of different stages of gonad deve-

[1] GenBank Accession Numbers: MW284989–MW285076. Access date 01.10.2021

Table 2. *E. golanii* genetic diversity.

Localities	*N*	N_h	*h*	π	*Ne*
Antalya	34	29	0.980 + 0.017	0.0049 + 0.00053	183
İskenderun	26	22	0.978 + 0.021	0.0048 + 0.00061	185
Marmaris	28	23	0.978 + 0.019	0.0038 + 0.00060	148
Total	88	70	0.982 + 0.008	0.0047 + 0.00034	181

N: number of samples; *Nh:* haplotype number; *h*: haplotype diversity; π: nucleotide diversity; *Ne*: effective population size.

lopment during the spawning period (Osman et.al., 2011). Nucleotide diversity is decreasing towards the localities of İskenderun (0.0048), Antalya (0.0049) and Marmaris (0.0038). This might be an indication of a gradual expansion of the round herring population from the Mediterranean Sea to the Aegean Sea. The records show that round herring expands its geographical distribution area towards the northern latitudes from the Mediterranean to the north of the Aegean Sea, presumably due to the changing hydrological conditions. One possible explanation for the high genetic diversity among the populations with high numbers of unique haplotypes is that they might have gone through different selection pressure but also these populations are more likely colonized these regions by consecutive migrations from the Red Sea to the Mediterranean Sea by different fish shoals at different time intervals independently.

Pairwise comparisons of the γ_{ST} nucleotide diversity and F_{ST} values between the populations based on the haplotype diversity and the genetic distance showed that the Antalya-Marmaris localities pair (γ_{ST}: 0.04285; *Nm*: 11.12) was more close to each other genetically. This is followed by the İskenderun-Marmaris (γ_{ST}: 0.04621; *Nm*: 10.36) and İskenderun-Antalya (γ_{ST}: 0.06578; *Nm*: 7.07) pairs of populations. The F_{ST} comparisons between the Marmaris and İskenderun (F_{ST}: 0.08668) Antalya-İskenderun (F_{ST}: 0.08051) populations was found to be higher than that between Antalya and Marmaris one (F_{ST}: 0.04805) (Table 3). NJ tree of the populations studied was compatible with the results of the genetic differentiation confirms the close genetic relationship between Antalya and Marmaris populations (Figure 2). All these results put forward that the proximity between sampling localities is not effective in the genetic structuring of the populations.

The AMOVA analysis was performed to determine the distribution of genetic variation obtained from the mtDNA control region analysis. AMOVA results have put forward that majority of the genetic variation is due to differences among the populations, while differences between groups were higher in the Group 2 (3.85%) than the Group 1 (–2.77%) (Table 4). The AMOVA analysis indicating that a large portion of the total genetic variation was attributed to the regional divergence that is well in accord with other findings of this study. The migration rate of the sampling localities supports the gene flow (Table 5). Antalya-Marmaris localities have the highest migration rate and this is followed by İskenderun-Marmaris localities.

It is generally assumed that invasion of a new habitat by a species take place by the migration of a small number of individuals from a source population so-called founder effect resulting in a reduction of genetic diversity by genetic drift (Azzure et al., 2006). The absence of genetic differentiation between the Mediterranean and the Red Sea populations of two Lessepsian rabbitfish species (*Siganus rivulatus* and *Siganus luridus*) proposed that the Mediterranean was colonized by a large number of migrants, counteracting any bottleneck event (Hassan et al., 2003).

Unlike other recent Lessepsian invaders (Golani et al., 2007), high genetic diversity have been reported in the invasive Mediterranean *S. luridus* population (*h:* 0.879; π: 0.592) close to the origin Red Sea one (*h*: 0.978, π: 0.958) (Azzure et al., 2006). Bariche and Bernardi (2009) have found a high degree of genetic diversity in the Mediterranean population of the blue-barred parrotfish *Scarus ghobban* (haplotype diversity 1, nucleotide diversity 0.03). A general acceptance for the explanation of high genetic diversity in the Mediterranean Lessepsian species is the lack of bottleneck due to the consecutive invasions (Bucciarelli et al., 2002; Hassan et al., 2003; Hassan and Bonhomme, 2005; Azzurro et al., 2006).

In conclusion, each population belonging to sampling localities contains unique haplotypes, thus each population has a specific genetic structure with high haplotype diversity and haplotype divergence. The most important factor in the emergence of this situation is that the Mediterranean Sea has probably been colonized by the repeated

Table 3. Pairwise comparison of genetic distance values and localities (lower diagonal γST; upper diagonal F_{ST} values).

	Antalya	İskenderun	Marmaris
Antalya		0.08051	0.04805
İskenderun	0.06578		0.08668
Marmaris	0.04285	0.04621	

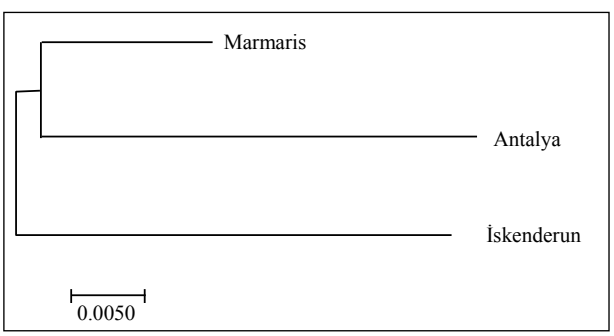

Figure 2. Neighbor-joining tree of *E. golanii* populations.

Table 4. Analysis of molecular variance (AMOVA) based on pair-wise differences among the studied round herring populations.

Variation percentage	Group 1 (İskenderun, Antalya/ Marmaris)	Group 2 (Antalya, Marmaris/ İskenderun)
Va (between groups)	–2.77	3.85
Vb (within groups)	8.95	4.54
Vc (among populations)	93.82	91.61

Table 5. Migration rates among populations (*Nm*)

	Antalya	İskenderun
Antalya		
İskenderun	7.07	
Marmaris	11.12	10.36

migrations of a large flux of immigrants from different source populations without major genetic differentiation. On the other hand, round herring has a wide range of distribution in the northeastern Mediterranean with different trophic and biotic factors from the Red Sea indicates a high adaptive flexibility potential of the species. Findings of this study put forward that the Mediterranean populations might have also been exposed to a high mutational rate and strong selection process that have possibly contributed to genetic structuring of the populations with unique mtDNA haplotypes. Since the high genetic divergence between the studied populations is not related to their geographic proximity and expansion direction, they have probably migrated to İskenderun, Antalya and Marmaris Gulfs in different time intervals using different routes rather than following the Levantine, middle water.

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