RESEARCH ARTICLE

DNA barcoding of Scombrid species in the Turkish marine waters

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Abstract

In order to obtain barcods of nine Scombrid species (*Thunnus alalunga, Thunnus thynnus, Euthynnus alletteratus, Auxis rochei, Katsuwonus pelamis, Sarda sarda, Scomber colias, Scomber scombrus, Scomberomorus commerson*), occurring in the Turkish Seas, mitochondrial DNA Cytochrome Oxidase subunit I (COI) gene was sequenced. COI contained 177 variable and 457 conservative nucleotides of which 175 were parsimony informative over 634 bp. Mean genetic diversity within and between species were 0.002 and 0.117 respectively. The number of detected different haplotypes were 22 out of 35 sequences, and haplotype diversity was 0.96. The highest genetic diversity (0.005) within species were observed for *S. commerson*, and lowest genetic diversity (0.000) was observed for *K. pelamis* and *E. alletteratus*. The highest and lowest nucleotide divergence was observed between *S. commerson* and *S. colias* (0.201) and between *T. alalunga* and *T. thynnus* (0.005) respectively. In Neighbour joining tree, two main phylogenetic nodes were detected; in the first node, *S. scombrus* and *S. colias* grouped together, and in the second main node, three branches were detected on which *S. commerson* was branched first and most divegent from the others and sisterly grouped with *S. sarda*. On the other hand, *A. rochei, E. alletteratus*, *K. pelamis, T. thynnus* and *T. alalunga* were grouped together in third branch in which *T. thynnus* and *T. alalunga* were clustered together.

Key words: DNA Barcoding, mtDNA, COI, biodiversity monitoring, Scombridae

Introduction

Scombrid fishes have a worldwide importance for their economic and ecological value and contain 15 genera and 51 species (Collette *et al. 2001*). This family includes mackerels, bonitos and tunas, showing a worldwide distribution from tropical to subtropical oceans (Collette 2003). Scombrids are epipelagic and generally migratory marine fish, characterized by an elongate and fusiform body although moderately compressed in some genera (Collette *et al. 2001*). Generally, these species have a varied diet with a preference for small pelagics

(e.g. clupeids, mullets, carangids). They feed also on crustaceans, mollusks and cephalopods.

There are ten species of this family (*Scomberomorus commerson* (Lacepède 1800), *Auxis rochei* (Risso 1810), *Euthynnus alletteratus* (Rafinesque 1810), *Katsuwonus pelamis* (Linnaeus 1758), *Sarda sarda* (Bloch 1793), *Scomber colias* Gmelin 1789, *Scomber scombrus* Linnaeus 1758, *Thunnus alalunga* (Bonnaterre 1788), *Thunnus thynnus* (Linnaeus 1758), *Orcynopsis unicolor* (Geoffroy Saint-Hilaire 1817) which are distributed in the Turkish seas (Fricke *et al. 2007*; Turan, *2007*). While *S. commerson* is a Red Sea migrant and the others (*A. rochei, E. alletteratus, K. pelamis, S. sarda, S. colias, S. scombrus, T. alalunga, T. thynnus, O. unicolor)* are origanated from the Atlanto-Mediterranean. On the other hand, *Acanthocybium solandri* (Cuvier 1832) distributed along the Red Sea, Pacific Ocean and Atlantic Ocean, has been reported from the Mediterrenean (Collette and Nauen 1983; Romeo *et al. 2005*), but the way of enterance into the Mediterrenean is still unknown, and there is no record about current population existence. Moreover, *Scomberomorus tritor* (Cuvier 1832) was also reported by Collette and Russo (1979) and Collette and Nauen (1983) from the Mediterranean coastal waters of France and Italy, but no further report exists on the occurence in the Mediterrenean.

Molecular genetic studies on mtDNA have proven useful for examining hypotheses about the phylogeny and phylogeography of marine species (Meyer, 1993; Avise, 1994; Turan *et al.* 2015a). Sequence analysis of mtDNA regions may be a quick tool to reveal phylogenetic relationships of marine species (Avise, 1994; Turan *et al.* 2008; Tabata and Taniguchi, 2000). Since different regions of mtDNA evolve at different rates, specific mtDNA regions have been targeted for inter and intra specific variation (Hauser *et al.* 2001; Mohindra *et al.* 2007; Turan *et al.* 2015b).

DNA barcoding is a global initiative that provides a standardized and efficient genetic marker to catalogue and inventory marine and freshwater biodiversity, with significant conservation applications. The DNA barcoding approach is concentrated on a single part of the mitochondrial genome, chosen because it presents portions conserved across taxa that are appropriate for primer design, while including polymorphism among and within species (Hebert *et al.* 2003; Kress and Erickson, 2008). The first studies for barcoding marine species in Turkish waters was by Kochzius *et al.* (2008) and Kochzius *et al.* (2010) who studied DNA barcoding using three mitochondrial genes 16S rRNA (16S), cytochrome b (cyt b), and cytochrome oxidase subunit I (COI) for the identification of 50 marine fish specieson in European waters, including Turkish seas and found that cyt b and COI are suitable for unambiguous identification of marine fishes. The cytochrome oxidase subunit I (COI) region of the mitochondrial genome is sufficiently diverse so as to allow the specific

identification of a great majority of animal species (Kochzius *et al.* 2008; Kochzius *et al.* 2010).

In addition to simple identification of Scombrids by DNA barcoding, the current level of interspecific and intraspecific genetic variation at Scombrid species which distrubuted in Turkish waters is very important to know. In spite of the wide scientific interest given to this family because of their commerical value, there are rare studies which investigated genetic structure of these species in Turkish waters.

The goal of this study is to evaluate the usefulness of DNA barcoding in the monitoring of the Scombrid species biodiversity distrubuted along the Turkish waters at two levels: by confirming the taxonomic identification, and by determining intraspecific and interspecific variations for nine species commonly found off Turkish marine waters.

Materials and Methods

Specimens of *S. scombrus* were collected from Izmir Bay, *T. thynnus*, *T. alalunga* and *S. sarda* specimens were collected from Fethiye and Antalya, and the others *A. rochei, E. alletteratus, K. pelamis, S. commerson* and *S. colias* were collected from Iskenderun Bay in winter season of 2012-2013. All samples were put in plastic bags individually and frozen at -20 °C till they were transported to the laborator*y*. All tissue samples were stored at -20 °C and 95 % ethanol till the analysis.

Total genomic DNA was extracted from cauadal fins and muscle samples. High salt method described by Asahida *et al.* (1996) was followed for extracting genomic DNA. Each PCR reaction was performed in a total volume of 50 μl containing 0.4 µM of each primer, 0.2 mM of dNTP and 1.25U of *Taq* DNA polymerase (Thermo Scientific) in a PCR buffer that included 20mM of Tris–HCl (pH 8.0), 1,5mM of $MgCl_2$, 15 mM of KCl and 1-2 µl template DNA. Denaturation step at 94°C for 30 s, 50 °C for 30 s, and 72 °C for 45 s for 30 cycles and followed by a final extension for 7 min at 72°C. The set of primers used for PCR amplification described by Ward *et al.* (2005) as as follows:
COIF: 5'-TCAACCAACCACAAAGACATTGGCAC-3'
COIR: 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3'

Visualization of amplified COI gene was done on agarose gel. Quantitation of the PCR product was completed using spectrophotometer. The DNA sequencing was attempted to determine the order of the nucleotides of a gene. The chain termination method by Sanger *et al.* (1977) was applied with Bigdey Cycle Sequencing Kit V3.1 and ABI 3130 XL genetic analyzer. The initial alignments of partial COI sequences were performed with Clustal W program (Thompson *et al.* 1994) and final alignment was completed manually with BioEdit (Hall

1999). After sequence alignment, sequence divergences were calculated using the Kimura two parameter (K2P) distance model (Kimura 1980). The molecular phylogenetic tree was constructed using Mega5 (Tamura *et al.* 2011). A distance-based method as neighbour joining (NJ) (Saitou and Nei 1987) and a cladistic phylogenetic tree as maximum parsimony (MP) criterion were used. The reliability of the inferred phylogenies was evaluated using the bootstrap method (Felsenstein 1985) with 1000 replicates. One species, *Xiphias gladius,* was included as an out group taken from GenBank (HQ024917).

Results

There were 177 variable and 457 conservative nucleotides of which 175 were parsimony informative over 634 bp sequences. The average nucleotide composition was 23.6% A, 29% T, 18.8% G and 28.7% C. Twenty two haplotypes were found out of 35 sequences, and there was no shared haplotypes between species (Table 1).

Kimura 2 paramater method was chosen as a best method for intra- and interspecific variations. Mean genetic diversity between and within species was calculated as 0.117 and 0.002, respectively. The matrix of pairwise distances within species is presented in Table 2. Intraspesific genetic diversity within *K. pelamis* and *E. alletteratus* was observed to be zero while it was highest within *S. commerson* specimens (0.005). The lowest genetic distance is observed between *T. alalunga* and *T. thynnus* (0.005) while the highest one is observed between *S. colias* and Indo-Pacific originated *S. commerson* (0.201).

Table 1. The number of haplotype and its distribution among species

	T.thynnus	K.pelamis	T.alalunga	S.commerson	S.scombrus	S.colias	A.rochei	E.alletteratus	S.sarda
Hap1	2								
Hap2	1								
Hap3	1								
Hap4		2							
Hap5			1						
Hap6			1						
Hap7			1						
Hap8				1					
Hap9				1					
Hap10					1				
Hap11					2				
Hap12					1				
Hap13						3			
Hap14						1			
Hap15						1			
Hap16							1		
Hap17							2		
Hap18							1		
Hap19							1		
Hap20								5	
Hap21									1
Hap22									4

Table 2. The matrix of intraspesific genetic diversity given
in bold (transversal diagonal) and distances between species

|	1	2	3	4	5	6	7	8	9
T. thynnus (1)	**0.002**								
K. pelamis (2)	0.016	**0.000**							
T. alalunga (3)	0.005	0.013	**0.004**						
S. commerson (4)	0.124	0.131	0.128	**0.005**					
S. scombrus (5)	0.159	0.163	0.160	0.190	**0.002**				
S. colias (6)	0.170	0.166	0.169	0.201	0.108	**0.003**			
A. rochei (7)	0.092	0.092	0.092	0.145	0.168	0.142	**0.002**		
E. alletteratus (8)	0.095	0.089	0.094	0.147	0.174	0.165	0.095	**0.000**	
S. sarda (9)	0.108	0.108	0.109	0.138	0.183	0.178	0.109	0.116	**0.001**

Neigbour Joining and Maximum Parsimony phylogenetic approaches resulted in similar tree topologies. In Neighbour joining phylogenetic tree (Figure 1), two phylogenetic nodes were detected; in the first node, *S. scombrus* and *S. colias* grouped together. In the second node two branches were detected; *S. commerson* was in the first branch and *A. rochei, E. alletteratus* and *K. pelamis, T. thynnus* and *T. alalunga* are grouped in the second branch with sister group to *S. sarda*. In Maximum Parsimony phylogenetic tree (Figure 2), two phylogenetic nodes were detected; in the first node, *S. scombrus* and *S. colias* grouped together. In the second node two main branches were observed while the first branch is composed of *S. commerson*, and the second branch is composed two another branches. One of them is composed of *S. sarda* while *T. thynnus* and *T. alalunga* and *K. pelamis* are grouped together with sister group *E. alletteratus* and another branch is composed of *A. rochei*.

Discussion

Generation of DNA barcoding of nine Scombrid species which are distributed in the Turkish Seas were investigated in the present study. All the species under the six genera were clearly separated by different clusters in the NJ and MP trees with a high bootstrap value. The universal primers amplified the target region in all nine species, generating 35 COI barcodes of 634 bp. No shared haplotypes was detected betwen species, and the barcode sequences clearly discriminated taxonomic status of all nine Scombrid species examined.

Genetic diversity within species was calculated zero for *K. pelamis* and *E. alletteratus*. This low genetic diversity may be explained with overfishing of these species in Turkish waters, but the number of samples sequenced for these species was low which most probaly caused the detected low genetic diversity. A similar result reported by Keskin and Atar (2013) using DNA barcoding to identify 89 commercially important freshwater and marine fish species found in Turkish ichthyofauna and COI reffered the lowest genetic distance (except intraspecific distances) between *Thunnus alalunga–Thunnus thynnus* (0.012). Chow *et al.* (2006) studied the Intra and inter specific nucleotides sequence variation of rDNA (ITS1) and analyzed the genus *Thunnus*. Intraspecific

nucleotide sequence variation was ranging from 0.003 to 0.014 (K2P) whereas variation between species within the genus *Thunnus* ranged from 0.009 to 0.05. Chow *et al.* (2006) also reported the genetic distance as 0.029 between *T. alalunga* and *T. thynnu*s and 0.013 while it is 0.005 in the present study.
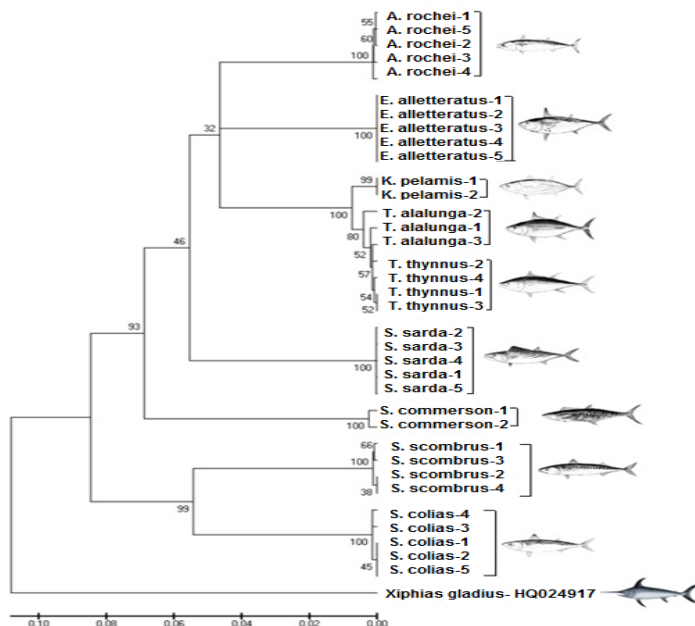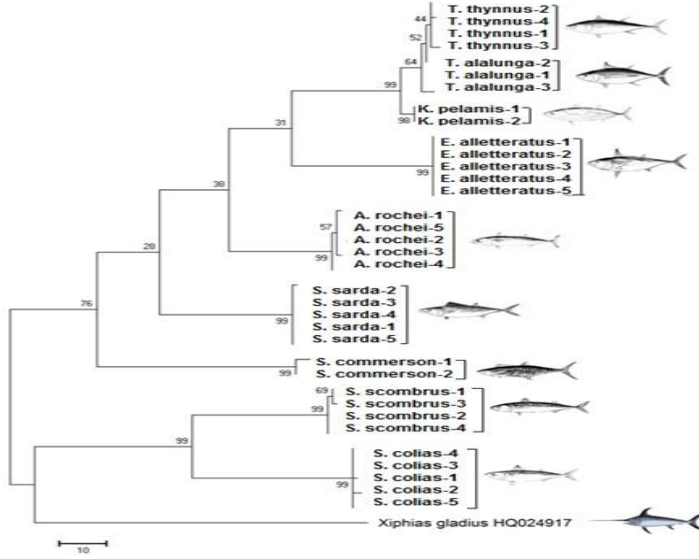

Figure 1. Neigbour joining phylogenetic tree based on COI sequences. *Xiphias gladius* was used as outgroup. Numbers on nodes indicate the bootstrap values.
Fish drawings: Froese and Pauly (2015)

A similar result was reported by Mudumala *et al.* (2011) the genetic distance values between *T. alalunga* and *K. pelamis* but contrarily, the genetic distance values between *T. alalunga* and *T. thynnu*s is very low in the present study. Vinas and Tudela (2009) studied genetic identification of eight Scombrid species (*T. atlanticus, T. tonggol, T. albacares, T. obesus, T. maccoyii, T. alalunga, T. orientalis* and *T. thynnus*) using mtDNA control region, mtDNA COI gene and nuclear DNA ITS1 region and reported that reliablity of COI gene is questionable. Vinas and Tudela (2009) also reported that COI gene is not a good marker for inferring evolutionary relationships in *Thunnu*s species. In the prsent study, T (28.9%) and C (28.7%) content were highest in the COI region.

The present finding is in accordance with many studies. Mudumala *et al.* (2011) studied phylogenetic relationships of *A. rochei, A. thazard, E. affinis* and *T. tonggol* species inferred from mitochondrial DNA sequences in the COI gene and reported the nucleotide compositions as A 24.0%, T 30.2%, G 18.4% and C 27.4%. Kochzius *et al.* (2010) aimed to evaluate the applicability of the three mitochondrial genes 16S rRNA (16S), cytochrome b (cyt b), and cytochrome

oxidase subunit I (COI) for the identification of 50 European marine fish species (incluluding *S. Japonicus* and *S. Scombrus*) by combining techniques of DNA barcoding and microarrays.

Figure 2. Maximum Parsimony phylogenetic tree based on COI sequences. *Xiphias gladius* was used as outgroup. Numbers on nodes indicate the bootstrap values.
Fish drawings: Froese and Pauly (2015)

As a result, while cyt b and COI are equally well suited for DNA barcoding of fishes. On the other ahnd, 16S has drawbacks in discriminating closely related species. This study was also first DNA barcoding attemt on marine fish species of Turkey. Al these studies and many further have shown that genetic identification by ''COI barcodes'' can provide a useful tool to identify seafood for consumer protection to control fisheries, and to detect possibly cryptic species, and even to describe new species.

In conclusion, the present study has strongly authenticated the efficacy of COI in identifying the scombrid species with designated barcodes. The present results also suggest that COI barcoding can be taken up as pragmatic approach for resolving unambiguous identification of scombrid species in marine waters of Turkey with applications in its management and conservation.

DNA Barkodlama ile Türkiye Denizlerinde bulunan Scombrid türlerinin ayırımı

Özet

Türkiye denizlerinde bulunan *Scombridae* familyasına ait dokuz türün (*Thunnus alalunga, Thunnus thynnus, Euthynnus alletteratus, Auxis rochei, Katsuwonus pelamis,*

Sarda sarda, Scomber colias, Scomber scombrus, Scomberomorus commerson) barkodlama tekniği ile tanımlanması için mtDNA COI bölgesi kullanılmıştır. 634 bç olarak incelenen COI bölgesinin 457 bç'lik kısmı evrimsel süreçten etkilenmemiş bölgelerden oluşurken, 177 bç'lik bölge ise türler arasında çeşitli sebeplerden ötürü değişen bölge olarak tespit edilmiştir. 175 bç'lik bölge ise türler arasında belirteç görevi görmüştür. Türiçi ve türler arası genetik çeşitlilik sırasıyla 0.002 ve 0.117 olarak belirlenmiştir. Toplamda 22 farklı haplotip belirlenirken haplotip çeşitliliği ise 0.96 olarak tespit edilmiştir. En yüksek türiçi genetik çeşitlilik *S. commerson* bireylerinde gözlmenlenirken (0.005) en düşük türiçi genetik çeşitlilik ise *K. pelamis* ve *E. alletteratus bireyleri* arasında (0.000) gözlmenlenmiştir. En yüksek ve en düşük nükleotid farklılığı ise sırasıyla *S. commerson - S. colias* (0.201) ve *T. alalunga - T. thynnus* (0.005) arasında tespit edilmiştir.

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