

Genetic confirmation of Red cornetfish, *Fistularia petimba* (Syngnathiformes: Fistulariidae) occurrence in Turkish marine waters

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Abstract

There is a controversy whether *Fistularia petimba* or *F. commersonii* are same species or distinct, since it is difficult to distinguish them based on morphological characters. In the present study, the COI and Cyt b gene regions of the mitochondrial DNA were used to address this question as well as to clarify the presence *F. petimba*. Based on the results, genetic distance between *F. petimba* and *F. commersonii* based on COI and Cyt b sequences were found to be 0.9536 and 0.95500, respectively. Average genetic diversity between species based on COI and Cyt b were found to be 0.00487, 0.04651 respectively. According to the results, *F. petimba* and *F. commersonii* are found genetically distinct from each other. The occurrence of red cornetfish *F. petimba* from Turkey marine waters was also confirmed showing its rapid range expansion to the Eastern Mediterranean Sea.

Keywords: Red cornetfish, mtDNA, Mediterranean Sea, Turkey.

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Introduction

The red cornetfish, *Fistularia petimba* Lacepede, 1803 belonging to the family Fistulariidae, are found in tropical waters of the east Atlantic to the Indian and Pacific oceans (Fritzsche 1990). This species is also found in the Red Sea (Dor 1984), from East Africa to the Hawaiian and Tuamoto islands, and to the south in Victoria, Australia in Indo-Pacific Ocean (Fricke 1999). The first report of *F. petimba* was from west part of the Mediterranean Sea (Cardenas et al. 1997) indicating its extension to the east by entering to the Mediterranean Sea from the eastern Atlantic via Gibraltar Strait. However, Dor (1984) and Yasemi (2012) reported *F. petimba* from Red Sea and Oman Sea, respectively. Stern et al. (2017) reported its occurrence from Israel Coast, Unluoğlu et al. (2018) from Antalya and Iskenderun Bays, the Mediterranean Sea, and Çiftçi et al. (2019) from Mersin Bay, the North-Eastern Mediterranean Sea.

In this study, the presence of *F. petimba* is confirmed by a rapid range expansion to the Eastern Mediterranean Sea. Since two invasive cornetfish i.e. *F. commersonii* and *F. petimba* are found in the Eastern Mediterranean, hence to clarify the presence *F. petimba*, its morphological examination along with mitochondrial DNA analysis using COI and Cyt b genes were performed.

Material and Methods

Collection details: A single specimen of *F. petimba* (650 mm TL; 455 mm SL) was captured by trawling at depth of 95 m in Mersin Bay (36°07'730"N, 33°31'240"E) on 22 November 2017 and two specimens of *F. commersonii* and *F. petimba* were captured by trawling at depth of 70 m in Antalya Bay (36°03'712"N, 32°32'054"E) on 09 January 2018 (Fig. 1). All measurements were made to the nearest 0.01 mm using digital caliper. The diagnostic features and morphometric characters were followed Fritzsche (1976) (Table 1).

Genetic analysis: Genomic DNA was extracted using phenol-chloroform isoamyl alcohol technique (Sambrook et al. 1989), total DNA was visualized by gel electrophoresis (1.5%) and quantified by spectrophotometric assay. After DNA extraction, mtDNA COI and Cyt b genes were amplified using PCR (Saiki et al. 1988).

The partial COI and Cyt b nucleotide sequences were aligned using Clustal W embedded in BioEdit (Thompson et al. 1994; Hall 1999). For further analysis, MEGA v5 (Tamura et al. 2011) and dnaSP (Librado

and Rozas 2009) software packages were used.

Table 1. Morphometric and meristic measurements of the captured *Fistularia petimba* compared with the previous studies.

	Present study	Cárdenas et al. (1997)	Azevedo et al. (2004)	Banon and Sande (2008)	Yasemi (2012)	Stern et al. (2017)	Ünlüoğlu et al. (2018)
Number of Samples	N=1	N=1	N=1	N=1	N=1	N=1	N=1
characters							
Total weight (gr)	34.5-55.2	950	-	1580	1760	-	-
Total length (mm)	410-650	1240	-	1400	1525	415	398
Standard length	393-455	1040	1007	1182	1233	280	383
Head length	131-171	365	373	429	456	-	142.5
Head height	128-143	36	-	-	-	-	-
pre-orbital length	110.8-133	-	-	321	-	-	112.06
post-orbital length	127.3-171	-	65	77	82	14.6	21.27
Snout length	110-134	264	284	-	-	85.5	-
Interorbital width	11-12.65	20	-	-	-	8.7	4.78
Body height	14.5-18	41	-	53	-	-	15.08
Pre-dorsal length	325-385	851	830	-	1016	235	315
Base of dorsal fin	15.76-17.6	36	-	34	65	-	13.78
Dorsal fin height	29.35-35.2	61	-	-	-	23.1	-
Pre anal length	324-381	849	-	-	1027	-	314
Base of anal fin	18-19.54	37	32	-	58	-	13.76
Anal fin height	29.95-36	62	-	-	-	20.5	-
Pectoral fin length	17.5-18.62	54	-	48	62	-	15.94
Caudal fin length	20.18-23.1	57	-	-	-	-	20.5
Meristic characters							
Dorsal fin rays	14-15	15	15	16	16	15	16
Anal fin rays	14-17	15	14	16	15	14	15
Pectoral fin rays	15	15	15	15	15	15	15
Pelvic fin rays	7-8	6	6	6	7	6	6
Branchiostegal rays	7-8	-	-	7	9	-	7

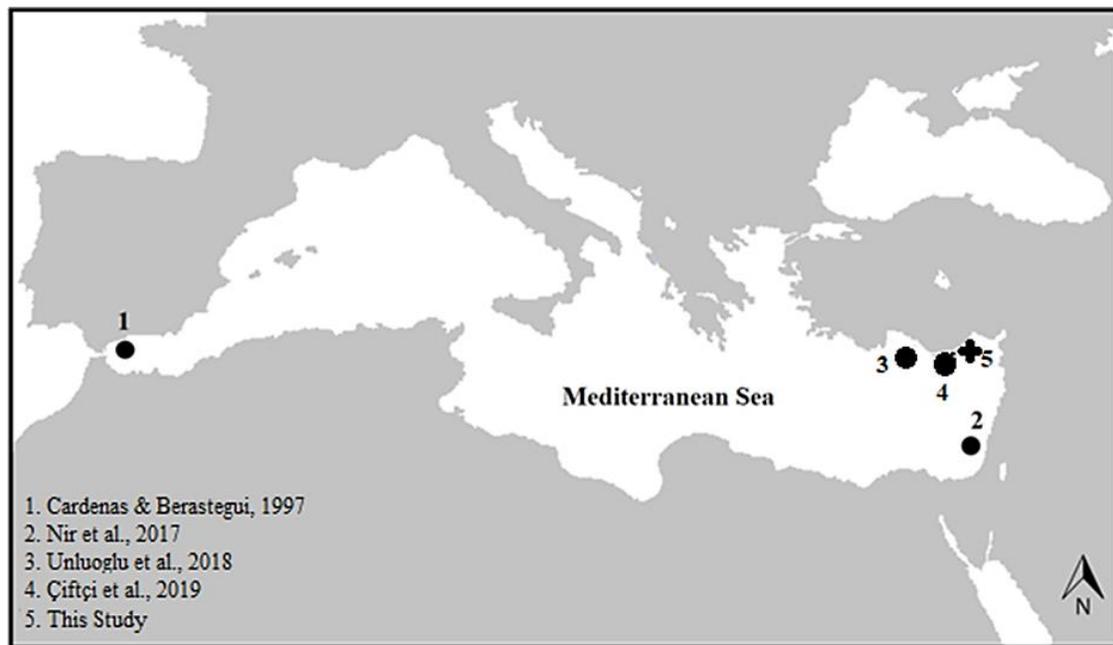


Figure 1. Sampling location of *Fistularia petimba* from the Mersin Bay (3), and the previous records from the Mediterranean Sea (1-2). 1- Mediterranean Spain (Cárdenas and Berastegui 1997); 2 - Israel (Nir et al. 2017); 3 - Antalya Bay and Iskenderun Bay (Ünlüoğlu et al. 2018); 4- North-Eastern Mediterranean Sea (Mersin Bay) (Çiftçi et al. 2019); 5- This study.



Figure 2. The captured specimen of *Fistularia petimba* from Antalya Bay (Eastern Mediterranean Sea, Turkey).



Figure 3. Distinguishing morphological characters of *Fistularia petimba*, row of bony plates throughout the dorsal midline.

Table 2. Variable nucleotide regions of COI haplotypes in *Fistularia* species.

Haploid	Variable nucleotide region
Hap_1	TGCGTTTCCCTCATCTCGACACCTGCAACCTCTCCCGCGCTAAAGG
Hap_2	TGCGTTTCCCGCATCTCGACACCTGCAACCTCTCCCGCGCTAAAGG
Hap_3	GTTTAGCATGTGTCTGGATCTGATCATGTATCTTTTTCTATCGGGAA
Hap_4	GTTTAGCATGTGTCTGGATCTGATCATGTATCTCTTTCTATCGGGGA

Results

Diagnosis: All measurements, morphological descriptions and colorations of the captured specimen were in agreement with the descriptions of Fritzsche (1976), and the diagnostic features of the examined *F. petimba* were compared with the previous studies (Cárdenas et al. 1997; Azevedo et al. 2004; Banon and Sande 2008; Yasemi 2012; Stern et al. 2017) which were similar (Table 1). The captured specimens of *F. petimba* were between 440-650 mm in total length, 393-455 mm in standard length and 34.5-55.2 g in total weight. It distinguishes from *F. commersonii* by having a row of bony plates throughout the dorsal midline (Fig. 3) and parallel ridges along the top of the snout (Fritzsche and Thiesfeld 1999).

COI gene: The partial COI gene sequences consists of 665 bp fragments in which there were 618 bp conserved, 47 bp variable, 44 bp parsimony informative and 3 bp singleton. The average nucleotide composition of adenine (A), thymine (T), cytosine (C), and guanine (G) were 24.1, 26.8, 30.6 and 18.5%, respectively. Four different haplotypes were found out of 4 COI sequences, and there was not shared haplotypes between this two cornetfish species. Variable nucleotide positions of COI sequences in cornetfish species are shown in Table 2.

For phylogenetic tree reconstruction, the best model based on our data was Kimura 2 (BIC: 5562.650) (Kimura 1980). The average value of genetic diversity within species was 0.00226. The smallest and highest genetic diversities were observed in *F. petimba* (0.0015055) and *F. commersonii* (0.0030166). Average genetic diversity between species was found to be 0.00487. Average divergence between *F. petimba* and *F. commersonii* was 0.04651. For inter-specific comparison, the genetic distance value found as 0.95364. Both Neighbour-Joining and Maximum parsimony algorithm for COI sequences showed distinction of *F. petimba* from *F. commersonii* (Figs. 4, 5).

Cyt b gene: The partial Cyt b gene sequences consists of 359 bp fragments in which there were 299 bp conserved sites, 60 bp variable, 56 bp parsimony informative and 4 bp singleton. The average nucleotide composition of A, T, C and G were 22.5, 28.8, 28.8 and 19.9%, respectively. Four different haplotypes were found out of 4 Cyt b sequences, and there were not shared haplotypes between two cornetfish species. Variable nucleotide positions of Cyt b sequences in cornetfish species are show in Table 3.

The best model based on our data was Kimura 2 (BIC: 5562.650) (Kimura 1980). The average value of genetic diversity within species were 0.00226. The smallest and highest genetic diversities were observed in

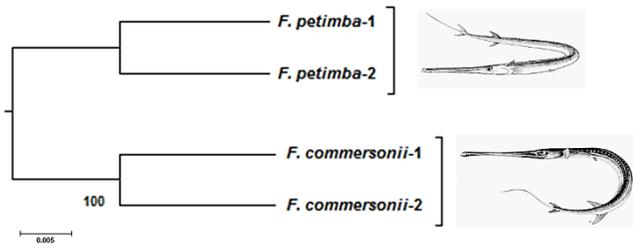


Figure 4. Neighbour-joining dendrogram of at mtDNA analysis of COI region.

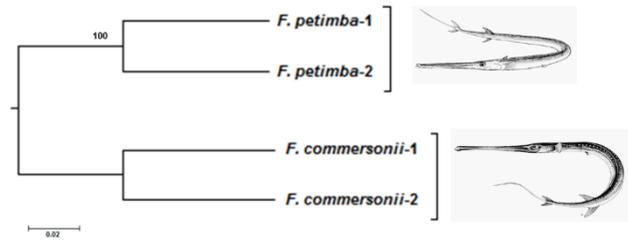


Figure 5. Maximum parsimony dendrogram of mtDNA analysis of Cyt b region.

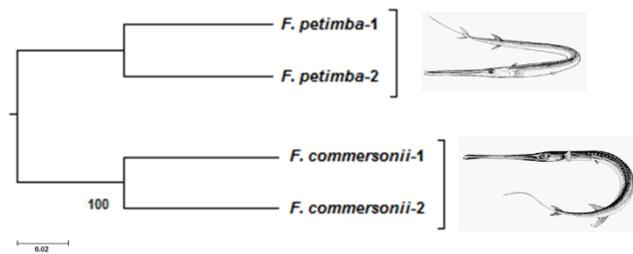


Figure 6. Neighbour-joining dendrogram of mtDNA analysis of Cyt b region.

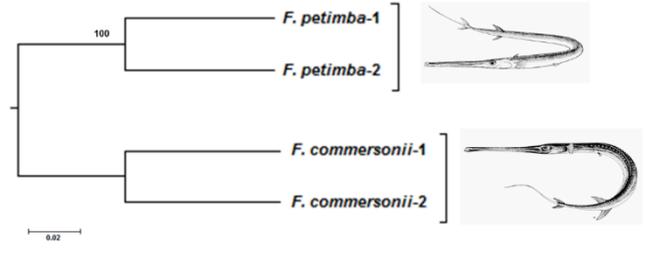


Figure 7. Maximum parsimony dendrogram of analysis of Cyt b region.

Table 3. Variable nucleotide regions of Cyt b haplotypes in *Fistularia* species.

Haploid	Variable Nucleotide Region
Hap_1	TCGGATCCGAAAAAACCTCCCCCCTATTTTTCTACAAGTTATACAGCACGCATTCCACGA
Hap_2	TCGGATCCGAAAAAACCTCCCCCCTAGTTTTCTACCAGTTATACAGCACGCATTCCACGG
Hap_3	CACTCCTTAGGCTGCTTCATTATTCGGACCCACGTCTCACACGTCCTCTCTCCGTCGCA
Hap_4	CACTCCTTAGGCTGCTTCATTATTCGGACCCACGTCTCACCCGTCCTCTCTCCGTCGCA

F. commersonii (0.0027914) and *F. petimba* (0.0084034). Average genetic diversity between species was found to be 0.04651. Average divergence between *F. petimba* and *F. commersonii* were 0.12439. Genetic distance between species found as 0.95500. Both Neighbour-Joining and Maximum parsimony algorithm for Cyt b sequences showed that *F. petimba* is distinct from *F. commersonii* (Figs. 6, 7).

Discussion

The red cornetfish, *F. petimba*, is a large fish, typically found throughout the soft bottoms of coastal areas and usually at depths over 10 m (Fritzsche 1978; Sommer et al. 1996). In this study, we captured the this species by trawling at depths of 70 and 95 m. The genetic confirmation of the two cornetfish species i.e. *F. petimba* and *F. commersonii* which are distributed in the Turkish marine waters were investigated. Two species were clearly separated in the NJ and MP trees with a high bootstrap value. The universal COI and Cyt b primers were successfully amplified the target region in two species, generating 4 COI sequences of 665 bp and 4 Cyt b sequences of 359 bp. Common haplotypes was not detected between species for both genes.

For inter-specific comparison, the genetic distance between *F. petimba* and *F. commersonii* based on COI sequences was 0.95364. Stern et al. (2017) studied genetic characterization of *F. petimba* inferred from COI sequences and found inter-specific divergence of their *F. petimba* as 0.159 from *F. commersonii*.

To date, there are two *Fistularia* species viz. *F. commersonii* and *F. petimba* in Turkish marine waters. The increase in water temperature may favor to migration of *F. petimba* via Suez Canal (Turan et al. 2016). In that circumstance, *F. petimba* should also be considered as a lessepsian species in addition to the Atlantic migrated.

On the other hand, the both ways might be suitable for entering to the Mediterranean Sea.

Acknowledgments

Thanks to Republic of Turkey Ministry of Food, Agriculture and Livestock General Directorate of Agricultural Research and Policies (Project no: TAGEM-16/AR-GE/21) for financial support.

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