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Determination of genetic diversity between natural and cultured populations of Common Dentex (*Dentex dentex*) fish in the East Aegean Sea

by

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Abstract

Common dentex (*Dentex dentex*) is a commercial species of fish that is a highly valuable food source living naturally near Mediterranean and Atlantic Coasts. Therefore, monitoring and maintaining common dentex habitats are of high importance. A total of 53 specimens were collected from 6 natural habitats (from the Eastern Aegean and from the Antalya coast, which is the closest coast to the Western Mediterranean), and one aquaculture facility in the Eastern Aegean Sea. The mitochondrial Cytochrome Oxidase I (*COI*) gene was used to detect a total of 26 haplotypes. Along with the *COI* gene sequence, real-time PCR and high-resolution melting analyses were performed as rapid and inexpensive alternatives. Sequence analysis showed that the highest haplotype diversity was obtained from the aquaculture facility in Karaburun and Antalya Locations, Turkey. Analysis of Molecular Variance (AMOVA) based on the haplotype frequencies resulted in 92.54% genetic variation within localities and 7.46% genetic variation between/among localities. The mean fixation index (*Fst*) was calculated as 0.0746 ($p < 0.001$). Genetic distances were primarily in collaboration with geographical distances and were efficiently confirmed by high resolution melting (HRM) analysis. The results will be valuable in monitoring and maintaining natural habitats as well as aquaculture facilities where common dentex are grown.

Key words: mtDNA, Common Dentex (*Dentex dentex*), Genetic Diversity, Aegean Sea

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1. Introduction

Sustaining a high biodiversity of established marine animals' populations, the Aegean Sea is considered as oligotrophic. It has an area larger than 215,000 km^2. Therefore, it has been of great interest for the capture fishing and aquaculture sector. The Aegean Sea is indeed historically an important fishing region involving vast coastlines, with more than 1,450 islands and islets, and has very rich ichthyofauna involving 449 species for the Turkish coast and 510 species for the Greek Coast including the Aegean Sea (Bilecenoğlu et al. 2014; Papaconstantinou 2014). A total of 130 commercial taxa (70 from Greece, 60 from Turkey) have been reported from this sea (GFCM 2021). It sustains two of the largest fishing fleets in the Mediterranean, which together account for more than 18% of the entire Mediterranean commercial fishing fleet, and a sizable population of artisanal fishers. In addition, about 8% of all Mediterranean and Black Sea catches between 2016 and 2018 came from the Aegean Sea (FAO 2020). A total of approximately 200 fish cage farms and 480 shell fish farms are located in the area of the Aegean Sea (Greek territorial waters; EMODNET) and approximately 210 cage fish farms in the Turkish territorial waters (Candan et al. 2007). These farms cover an area of over 400,000 ha (FGM, 2018).

The Aeagean Sea also harbors one of the economically significant sparid fish, the common dentex, *Dentex dentex* (Linnaeus, 1758) which is exploited by commercial fishing activities, the aquaculture industry and recreational fishing (Morengo et al. 2014). Therefore, understanding the genetic makeup of exploited populations, the common dentex, *per se*, is crucial for the conservation and sustainable management of fisheries.

The common dentex belongs to the Sparidae family and is one of the most commercially caught fish species especially in the Mediterranean Sea (Hanel et al. 2011). The common dentex, also known as synagrite, has considerable commercial and organoleptic value due to its large size and flesh quality (Marengo et al. 2015); however, it is classified as "vulnerable" in the Red List of Threatened Species by the International Union for the Conservation of Nature (IUCN). Due to low market supply, the potential for selling these fish from aquaculture operations is thought to be high (Frimodt 1995). Therefore, more detailed information and continuing updates about the population's genetic structure as well as the morphology of the common dentex would be valuable. Analysis of mitochondrial genomes is one of the most suitable methods to dissect the genetic structure of the common dentex as well as many other species. The complete mitochondrial genome of the common dentex revealed that the sequence is comprised of 16,652 bp and consists of 13 protein-coding genes, 2 rRNA genes, 22 tRNA genes and two non-coding regions (D-loop and L-origin, Mascolo et al. 2018). Among these, polymorphism information from the part of the *COI* gene has been successfully used to identify closely related species in animals and other higher taxa (Hebert et al. 2003). It has been widely utilized to analyze the biodiversity by discriminating ambiguous species, subspecies, hybrids and anonymous species (Mytilineou et al. 2016). The polymorphic region of the *COI* gene is also used as the DNA barcoding (Folmer et al. 1994) not only to dissect the genetic structure but also for possible food frauds (Ceruso et al. 2021).

The DNA barcode is the sequence of the ''Folmer fragment'' (Folmer et al. 1994), a polymorphic part of the mitochondrial *COI* gene, which can be used to identify closely related species as well as higher taxa in many animal phyla (Hebert et al. 2003). It can help in discerning between similar species, allowing an accurate analysis of biodiversity, differentiating between ambiguous species, subspecies, hybrids and species still unknown (Mytilineou et al. 2016).

There are a few published studies, some are indicated in this paragraph, on common dentex genetic diversity using *COI* sequences. In one study on the biodiversity of fish species including common dentex, the genetic structure of fish populations in Italian and Egyptian coastal regions was examined and updated by using *COI* gene sequences, with the result that the genetic classification was in collaboration with that of the previous morphological classification (Guerriero, 2017). They used the *COI* gene along with two other genes and microsatellite markers, and reported the absence of a population genetic structure from the Bay of Biscay to the eastern Mediterranean Sea (Viret et al. 2018). In another study, including common dentex, 89 commercially important freshwater and marine fish species in Turkey were barcoded with the *COI* gene to their corresponding taxonomic classification, which is significant for efficiently monitoring, conserving, and managing fish and fisheries (Keskin & Atar, 2013). Since sequencing analysis of the *COI* gene for biodiversity is expensive and time consuming, HRM analysis was also used in barcoding a few fish species genetic diversity for rapid results. High resolution melting analysis is as sensible as one base changing and was successfully used for species differentiation and geographical variation (Valente et al. 2013; Behrens-Chapuis et al. 2018). To the best of our knowledge, no HRM study has been performed to dissect genetic variation on common dentex in the East Mediterranean.

The objective of this study is to dissect the genetic diversity of common dentex populations in their natural habitat as well as fishery and aquaculture along the East Aegean. For this purpose, we used mitochondrial DNA sequences of *COI* gene parts. As a rapid and inexpensive alternative, HRM analysis was also performed for species' identification and geographical distinction. The results will likely be useful for the conservation of common dentex in natural habitats and the management of commercial fisheries and aquacultures.

2. Materials and methods

In this study, natural and cultural samples of common dentex (*Dentex dentex*) were obtained from the pre-determined stations in the Aegean Sea. In order to reveal the genetic interaction as well as intraspecies natural and cultural interactions within the species, the mtDNA *COI* region was targeted and the tissue samples were taken from the subcutaneous muscle tissue of the fish samples from the region close to the caudal fin. Purification and isolation of the targeted mtDNA *COI* region were performed for PCR (polymerase chain reaction), and finally a sequence analysis with the Sanger method, which was applied to amplified regions thereby phylogenetic analysis was carried out accordingly.

2.1. Fish material used in the study

Six different locations were selected to represent the natural common dentex species distributed in the Aegean Sea and other seas (Fayazi et al. 2006; Grant 2005; Ergüden 2006; Ergüden 2002; Eroğlu et al. 2008). The sampling locations and the number of individuals sampled with corresponding pictures are depicted in Figures 1, 2, and 3. In addition, wild fish samples were obtained from local fishermen, and samples of cultural stocks were obtained from net-cage enterprises and hatcheries in the region – shown in Tables 1, and 2.

The number of specimens used for the analysis was changed between 4 (Güllük Natural IV-W) to 10 (Kuşadası Natural III-W). Previously, a lower number of samples than in our study was used in *COI* barcoding of Atlantic cod (Fernandes et al. 2017) and Indian fresh water fish studies (Lakra et al. 2016). In addition, genetic identification of the blue crab Callinectes sapidus and other *Callinectes* spp. was also performed using as few samples as 1 for a particular location (Lee et al. 2020). Although more than 25 species would need to be collected in order to achieve maximum accuracy, Goodall-Copestake et al. (2012) note that a sample size of five individuals per species population is adequate to differentiate between extremes in assessing the haplotype and nucleotide *COI* variation across wide-ranging animal taxa. In addition, DNA barcodes are presumed to be of sufficiently high quality, such that they are free of both ambiguous and missing nucleotide bases, which can lead to the overestimation of observed and total haplotype numbers through creating artificial haplotype variation within species (Phillips et al. 2019, and references within).

2.2. Genetic Analysis

As the first step of genetic analysis, DNA samples were taken from fish using the chelex 100 method

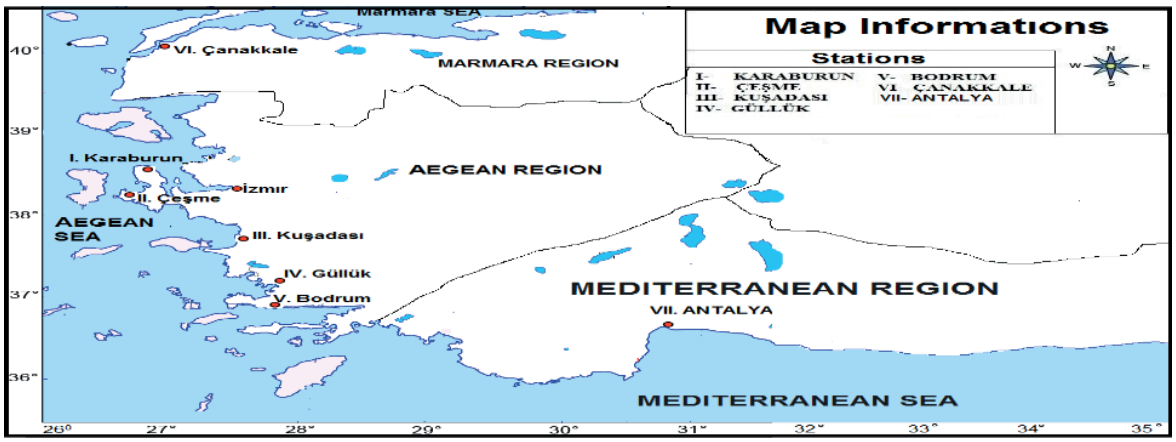

Figure 1

Six different locations where fish samples were obtained

Figure 2

Some pictures of the common dentex samples obtained from the locations

(Singer-Sam et al. 1989; Walsh et al. 1989). Briefly, tissue samples were removed from the freezer by cutting epithelial tissue with the help of a 1.5 × 1.5 cm scalpel in the upper skin part of the lateral region near the tail. The muscle tissue in the lower part was chopped into small pieces on the sample by the grid method, and 1-2 g of muscle tissue (2 mm) was cut and placed in a 1.5 ml microcentrifuge tube (Keskin & Atar 2013; Eroğlu et al. 2008). Then, 300 µl of 10% chelex 100

Table 1

Sampling locations of common dentex and number of the samples per location

No.	Sampling Location	Sample Code	Number of samples
1	Karaburun- Natural	I-W	9
2	Karaburun- Culture	I-C	9
3	Çeşme-Natural	II-W	5
4	Kuşadası-Natural	III-W	10
5	Güllük-Natural	IV-W	5
6	Bodrum-Natural	V-W	4
7	Çanakkale-Natural	VI-W	6
8	Antalya-Natural	VII-W	5
TOTAL			53

Table 2

Map coordinates of the stations where common dentex samples were obtained

Stations	Common Dentex Natural	Common Dentex Culture
(I) Karaburun	Foça offshore 38°48'34.8"N 26°38'09.4"E	Akvatur Fish Farm 38°30'29.3"N 26°23'12.4"E
(II) Çeşme*	Çeşme fish pond 38°22'07.1"N 26°19'39.6"E	
(III) Kuşadası**	Çağlar fishery agency 37°48'51.1"N 27°08'58.4"E	
(IV) Güllük	Güllük Gulf 37°14'25.0"N 27°35'32.8"E	
(V) Bodrum	Bodrum Şenol Fishery 37°02'08.2"N 27°25'54.7"E	
(VI) Çanakkale	Engin Fishery 40°08'38.5"N 26°24'19.6"E	
(VII) Antalya	Antalya offshore 36°50'4.98"N 30°38'47.14"E	

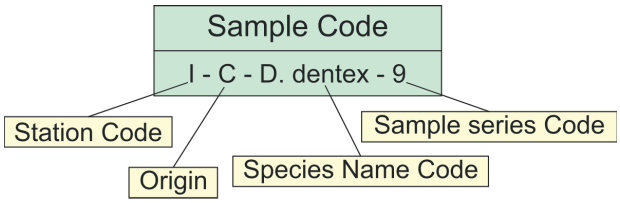

Figure 3
Sample coding system used in the present study

solution was added into the tube. The mixture was homogenized by a vortex for 2 minutes. After the homogenization step, the mixture was precipitated by centrifugation and incubated at 98°C for 10 minutes. Following to the incubation step, the mixture was homogenized again by vortexing for 2 minutes, and then centrifuged at 10000 g for 1 minute. Finally, 100 µl of liquid was withdrawn from the upper phase and transferred to a new tube.

For the real time PCR reaction of 10 µl total volume: 2 × qPCR Mix (3 mM $MgCl^2$, 0.4 mM dNTP mix, 0.2 U High Fidelity Hot Start Taq DNA Polymerase, 2 × EvaGreen), 5 µl Forward primer (10 µM), 0.5 µl Back primer (10 µM), 0.5 µl molecular scale water and 3 µl DNA 1 µl were used. The primer design was constructed using Primer 3 software (Untergasser et al. 2012) with 0.5°C heat sensitivity. The forward primer was 5'-CGAGCTGAACTTAGCCAACC-3', reverse primer was 5'- GGTCGAAGAAGGTGGTGTTT-3' and the total length of the target product was 553 bps.

Starting denaturation 98°C for 5 min (1 cycle), denatutarion 98°C for 20 s and elongation 55°C for 40 s (40 cycles) were used for the PCR reactions. High resolution melting (HRM) was performed using 5s/step, 72°C - 95°C (0.2 C s^{-1} rate of increase and continuous reading; 1 cycle).

The Roche LightCycler® Nano Real-Time PCR system was used to perform the reactions. QPCR, and the HRM results were evaluated using the software of the Roche LightCycler® Nano Real-Time PCR system.

2.3. Sequence analysis and phylogenetic analysis

Sequence analyses of partial *COI* gene amplicons obtained from PCR products were determined by the Sanger method (ABI prism Big Dye Terminator Cycle Sequencing Ready Reaction Kit on an ABI Prism 377 DNA sequencer, Applied Biosystems, USA). The sequences were analyzed with the CHROMAS software 2.6.6 program (http://technelysium.com.au/wp/chromas/). The sequence similarities in the database were determined using the NCBI BLAST (http://blast.ncbi.nlm.nih.gov/) program.

DNA sequences were compared and aligned using ClustalW software (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Only clearly compatible base positions were used in the analysis. MEGA software (http://www.megasoftware.net/) was used for drawing phylogenetic trees based on sequence comparisons. The phylogenetic trees were obtained with four different algorithms (Neighbor-joining, maximum likelihood, maximum parsimony, UPGMA). The DNAsp v5 software program (http://www.ub.edu/dnasp/) was used to determine the nucleotide (π) and haplotype diversity (H) among intraspecies samples (Rozas et al. 2003). MEGA (v.4) and Arlequin (v.3.5.5) applications (Excoffier & Lischer, 2010) were used to determine the genetic distances between species.

The nucleotide sequences (in base pairs) of the target mtDNA *COI* region were separated according to their stations and types, edited and aligned with the Chromas® program. The sequences obtained after the editing of the mtDNA sequences were determined by the Bioedit® software program (Hall 1999) as a reference for 1 (one) of the samples, the similarities of the other samples with the reference sample sequence were shown as dots (.), different bases (A, G, T, C) in the form of Bioedit® visualized by the program. The DNA sequences of the target species were ordered separately according to the stations determined in the project.

2.4. Statistical Analysis

Nucleotide diversity, which is used to measure the degree of polymorphism within a population, was assessed by the following formula:

$$P_S = \frac{S}{N}$$

where P_s is nucleotide diversity, S is the segregating sites and N is the total number of sequences examined (Nei & Kumar 2000).

The distances within species and between species were calculated using the Kimura-2-parameter (K2P) model (Kimura 1980); a phylogenetic tree was constructed using the neighbor-joining (NJ) method. The clade credibility in the tree that was obtained using the NJ method was tested by bootstrapping, in which 1,000 repeated sampling tests were performed to obtain the support values of the clade nodes.

The nucleotide diversity was calculated by determining the average number of nucleotides between any two randomly selected DNA sequences from the studied population and is represented by

the Symbol π. This statistical value is often used to see variation within and between ecological populations and to determine evolutionary relationships. If the π value is calculated for all possible sequence pairs, the equation given below is obtained (Nei ve Kumar, 2000):

$$\hat{\pi} = \frac{n}{n-1}\sum_{ij}\hat{x}_i\hat{x}_j\pi_{ij}$$

In the equation, n is the total number of samples, *xi* and *xj* values *i*, with n being different from each other for a particular DNA region, and j stands for the frequencies of the strings. Moreover, π *ij* is the number of nucleotide differences for each nucleotide region between the i^{th} and j^{th} sequences.

Haplotype Diversity (*h*) was calculated by the formula:

$$h = (1-\sum xi^2).n.(n-1)^{-1}$$

Here, the frequency xi value of a haplotype is expressed by the gene copy number n and is accepted as 1 for mtDNA (Nei & Kumar, 2000; Jobling et al. 2004)

Nei's "Standard Genetic Distance" measurement:

$$I_N = \frac{\sum_{t=1}^{m}(p_{ix}.p_{iy})}{\sqrt{(\sum_{t=1}^{m}p^2{}_{ix})(\sum_{t=1}^{m}p^2y)}}$$

In populations *x* and *y*, *Pix* and *Piy* values were used to express the frequency of the i^{th} allele. The *I* value can take a value between 0 and 1. If the *I* value is 0, there are no alleles shared between populations, if equal to 1, the population can be interpreted as having the same allele (Jobling et al. 2004; Freeland, 2005; Hedrick, 2005).

Wright's *F* statistic (F_{ST}), also called the fixation index, is a frequently used statistic based on allele frequencies and used to measure the genetic distance between subpopulations with the help of classical markers.

$$\frac{V_p}{p(1-\overline{p})} = F_{st} = 1-(\frac{Hs}{Ht})$$

The *Hs* value in the formula is calculated as the expected heterozygosity for the subpopulation, and the *Ht* value is calculated as the expected heterozygosity for the whole population. In addition, the following equation can be used to calculate the F_{ST} value: Here, the *p* value represents the mean of the gene frequencies between two populations, and the *Vp* value represents the variance of the gene frequencies between two populations (Jobling et al. 2004, Hedrick 2005).

3. Results and discussions

Corresponding primer pairs successfully amplified the polymorphic *COI* gene region in all specimens and sequence analysis results confirmed the 654 bp long region of interest (KJ012372.1) in the nucleotide database (https://www.ncbi.nlm.nih.gov/nuccore/KJ012327.1/).

The threshold cycle numbers (*Ct* values) and melting temperatures (*Tm* values) obtained by the *COI*-targeted QPCR of common dentex fish samples are given in Figure 4. The *Ct* and *Tm* values also indicated that the target DNA was amplified successfully from all samples. Primer dimers next to the target *COI* gene were eliminated before the analysis by using buffers in which 100 bp and above DNAs were purified, during the PCR product purification stage in the DNA sequence analysis stage. All the common dentex samples gave an amplification with a *Ct* (Cycle threshold) between 19.4 and 26.1 (a low Ct indicates the existence of a specific fragment used in the experiment) and *Tm* between 81.8°C and 83.2°C as expected. Although there was no statistically significant difference in the *Ct* value among the groups because of variance analysis ($p > 0.05$), the lowest *Ct* value was 18.5 in the *I-W* location and the highest Ct value was 26.1 in the *III-W* and *V-W* locations. The general average of all the data was found to be 23.5. While the highest Ct change was seen in the *IV-W* location, the least change was observed in the *VII-W* location (Figure 4).

There was no difference among locations in terms of *Tm* values regarding the *COI* target gene of the *D. dentex* species ($p > 0.05$). However, interestingly, the lowest 81.8°C *Tm* value and the highest 83.2°C *Tm* value were seen in the *VI-W* location. The highest *Tm* change was observed in the *VI-W* location, while the least change was observed in the *II-W* and *IV-W* locations (Figure 4). There was also no significant relationship found between the *Tm* values and *Ct* values ($r = 0.132$; $p > 0.05$).

Our ANOVA analysis of *Tm* and *Ct* values resulted in an insignificant association among locations although some individuals had a clear distinction from

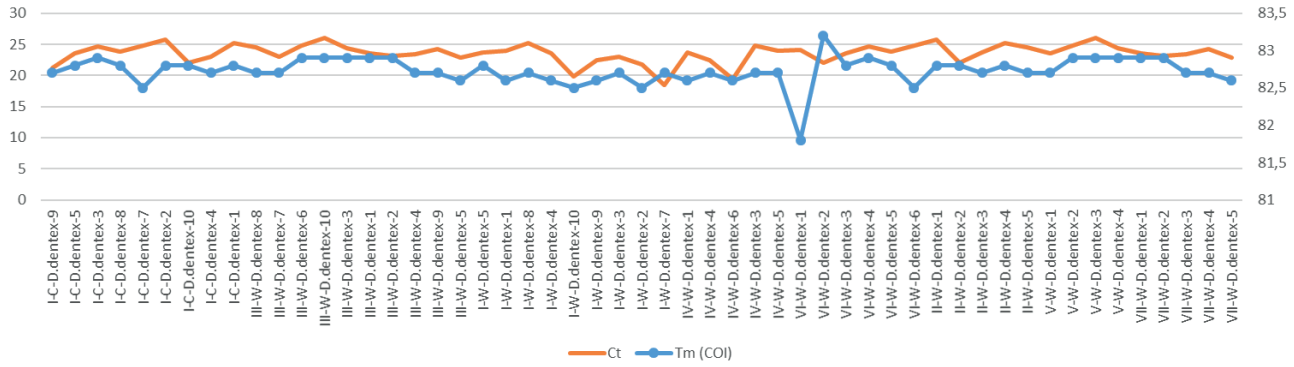

Figure 4

On the left axis is the Cycle number (*Ct*, orange line) and on the right axis is the melting temperature (*Tm*, blue line) data of the QPCR experiments for the *COI* target gene of the *D. dentex* samples collected from different locations.

others (Figure 4). The results of *Tm* and *Ct* values were previously efficiently used to discriminate species (Ceruso 2021); however, the use of real-time PCR for intraspecific variation may require more efforts. We also performed an HRM analysis to dissect the genetic diversity of the *D. dentex* population in the East Aegean Sea. After the analysis, individuals were separated under two haplogroups (HRM groups). A total of eight individuals were involved in haplogroup HRM1 (*II-W-D.dentex-1*, *II-W-D.dentex-3, V-W-D.dentex-3, VII-W-D.dentex-1, VII-W-D.dentex-2, VII-W-D.dentex-3, VII-W-D.dentex-4,* and *VII-W-D. dentex-5*) while the rest of individuals were within haplogroup HRM2.

We conducted an HRM analysis to see if the discrimination of populations or individuals was as successful as it was in the sequence analysis. Although HRM analysis is easy to perform and far more inexpensive and time saving than is sequence analysis, its discrimination power was not as effective. However, the HRM analysis clearly distinguished the population from Antalya VII-W which is far from the other location while it was inefficient discriminating intraspecific variation. The results are in collaboration with previous studies, which also indicated similar findings (Behrens-Chapuis et al. 2018, Valente et al. 2013).

The HRM studies on fish biodiversity are very few. Manipulating the HRM protocol especially by changing different sequence sites in the primer construction, different temperature regimes in PCR protocols etc. will likely help improve the HRM method for biodiversity studies more efficiently. Our study is one of the first to reveal the biodiversity of common dentex using HRM coupled with real-time and *COI* sequence analysis results.

The sequence analysis results obtained after the sequencing of PCR products, which belong to all *D. dentex* specimens, were aligned and examined one after the other. All the DNA sequences examined are most similar to the mtDNA *COI* gene sequence of the relevant species in the gene bank. This indicates that the DNA sequence analysis has been carried out successfully. The range of similarity is between 77-100%. Through the sequence analysis, a total of 477 nucleotides were examined, of which 31% T, 29% C, 24%A and 17%G.

The sequence of 477 nucleotide sites of the *COI* region obtained from 53 specimens of *D. dentex* was found to be highly variable and was efficient to dissect the genetic diversity of the fish population in the region. There were a total of 27 variable positions or nucleotide loci (6% of 477 bp) which revealed 26 different haplotypes (Table 3). According to the *COI* sequence analysis results of 53 samples belonging to *D. dentex*, 26 different haplotype groups were formed. A total of 25 samples were in the H1 haplotype group (Table 4).

When the sequence matches of *D. dentex* haplotypes are examined, the most nucleotide changes are observed to be in the start site of the *COI* region. Nearly half of the samples (47%) did not show any nucleotide diversity; however, base changes in other samples ranged from 1 to 6 bases. The most nucleotide diversity was observed in the H13 (i.e. *II-W-D.dentex-2*) group (6 base changes). The nucleotide diversity in the culture samples (*I-C* region) was higher than the other samples, and 28 nucleotide variations were observed in a total of 9 samples. The highest nucleotide conversion in the *COI* region was in the form of A→C (7 nucleotide positions) transversion and T→C (7 nucleotide positions) transitions (Table 3). There were no G→T and C→G transversions in the *COI* region of the *D. dentex* samples.

Table 3

Haplotype structure of *D. dentex* samples with respect to nucleotide variation in the *COI* region

Nucleotide positions in *COI* region																												
Haplotype Number	Frequency	19	20	37	47	54	65	80	98	111	126	129	131	150	165	171	192	238	247	284	342	398	399	406	407	441	447	455
H 1	25	T	A	G	A	A	T	A	C	T	T	A	A	C	T	T	G	T	A	A	A	C	T	T	C	T	T	G
H 2	1	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 3	1	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.
H 4	1	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	C	.
H 5	1	.	.	.	.	.	.	.	A	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 6	1	G	.	.	C	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 7	1	G	C	A	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 8	1	G	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.
H 9	2	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 10	1	G	C	A	.	.	G	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.
H 11	1	G	C	A	C	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 12	1	G	C	A	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 13	1	G	C	A	.	G	G	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.
H 14	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.
H 15	2	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 16	2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.
H 17	1	G	.	A	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
H 18	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.
H 19	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.
H 20	1	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 21	1	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	C	.	.	.	.	G	.	.	.	.
H 22	1	G	C	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 23	1	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.
H 24	1	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
H 25	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	.	.	.	.	.	A	.	C
H 26	1	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.

Table 4

Haplotype groups and sample codes in the same haplotype group

Haplotypes	Sample Code		
H 1	*I-W-D.dentex-1, I-W-D.dentex-2, I-W-D.dentex-3, I-W-D.dentex-7, I-W-D.dentex-8, I-W-D.dentex-9, I-W-D.dentex-10, I-C-D.dentex-9, II-W-D.dentex-4, II-W-D.dentex-5, III-W-D. dentex-2, III-W-D.dentex-4, III-W-D.dentex-8, III-W-D.dentex-9, III-W-D.dentex-10, IV-W-D.dentex-2, IV-W-D.dentex-4, IV-W-D.dentex-5, IV-W-D.dentex-6, V-W-D.dentex-2, V-W-D. dentex-4, VI-W-D.dentex-2, VI-W-D.dentex-4, VI-W-D.dentex-5, VII-W-D.dentex-5*		
H 2	*I-W-D.dentex-4*	H 15	*III-W-D.dentex-1, III-W-D.dentex-5*
H 3	*I-W-D.dentex-5*	H 16	*III-W-D.dentex-3, VI-W-D.dentex-3*
H 4	*I-C-D.dentex-1*	H 17	*III-W-D.dentex-6*
H 5	*I-C-D.dentex-2*	H 18	*III-W-D.dentex-7*
H 6	*I-C-D.dentex-3*	H 19	*V-W-D.dentex-1*
H 7	*I-C-D.dentex-4*	H 20	*V-W-D.dentex-3*
H 8	*I-C-D.dentex-5*	H 21	*VI-W-D.dentex-1*
H 9	*I-C-D.dentex-7, IV-W-D.dentex-1*	H 22	*VI-W-D.dentex-6*
H 10	*I-C-D.dentex-8*	H 23	*VII-W-D.dentex-1*
H 11	*I-C-D.dentex-10*	H 24	*VII-W-D.dentex-2*
H 12	*II-W-D.dentex-1*	H 25	*VII-W-D.dentex-3*
H 13	*II-W-D.dentex-2*	H 26	*VII-W-D.dentex-4*
H 14	*II-W-D.dentex-3*		

When the sequence conversion of all populations was examined with respect to the Kimura 2P (K2P) parameter, A←→G transversions (10.3) and C←→T (10.3) transitions were the higher ones compared to other base conversions (7.35). Transversion pairs are 56% more common than transitional ones. The low level of the transition/transversion rate is also reported in the *COI* gene in common dentex (Bargelloni et al. 2003). The total transition/transversion bias for the common dentex samples was calculated as R = 0.70.

Our results in the nucleotide substitution were similar to other ones studying sparid fish. In sparid genomes, transversion rates tend to be higher than transition rates (Hanel et al. 2000). However, transition/transversion rates in the fish *COI* region changed with respect to the codon position, while the average frequency of each nucleotide was similar (Keskin & Atar, 2013). The average G-C content was found to be 45.5%, which is close to those of other fish *COI* genomes (Keskin & Atar, 2013; Lakra et al. 2011; Ward et al. 2005).

The highest haplotype diversity values were obtained from the *I-C* and *VII-W* locations, while the lowest were in *I-W* and *IV-W*. The highest polymorphic sites were found in the *COI* gene of the *I-C* samples while *III-W, IV-W, V-W, VI-W* and *VII-W* samples did not contain any polymorphic sites at all. However, the *V-W* and *VI-W* samples had highly informative regions in terms of constructing suitable phylogenetic trees (Table 5).

When investigating the stations, the *I-C* (Karaburun culture) region rendered the highest genetic diversity values and each individual differed from one another by at least one nucleotide in the *COI* region. Although the number of samples was less, the genetic diversity was also at the highest level in fish caught from the *VII-W* Antalya natural environment.

Compared to the culture type, the haplotype diversity was found to be less than half of those in the culture in the common dentex populations grown in the natural environment in *I-W* (Karaburun). The least haploid diversity was observed in the *IV-W* Güllük Natural region (Table 5).

Twenty-six haplotypes were identified in the Median-Joining Network of haplotypes created for the 53 sample *COI* sequences of the *D. dentex* population. The network clearly indicated a central haplotype (H1), which implies an evolutionary relation (Figure 5). Central haplotype H1 involved 25 individuals from all locations, while most others were represented by only one or two individuals. Notably, five different haplotypes from Karaburun Culture *I-W* (H6, H7, H8, H10, and H11), two from Çeşme *II-W* (H12 and H13), one from Kuşadası *II-W* and one from Çanakkale *VI-W* (H22) separately branched from two haplotypes (H2 and H15 from Karaburun *I-C* and Kuşadası *II-W*, respectively, Figure 5).

A total of 53 individuals resulted in 26 haplotypes (50%) which indicates a large genetic variation. As high as a 66% haplotype diversity was also reported in other sparid *COI* genomes including that of the common dentex; however, absence of genetic structure is also common (Bargelloni et al. 2003; Viret et al. 2018). High levels of haplotype diversity (*Hd*) and low levels of nucleotide diversity (π) were observed in the populations, suggesting rapid population growth according to the regions (Zink et al. 2008).

Table 5

Genetic diversity and neutrality tests of the *D.dentex* populations with respect to locations

Population	Sample size (*n*)	Haplotype number (*N*)	Haplotype Diversity (*h*)	PS	PIR	Nucleotide diversity (π)	Taijm'a's D	Fu's Fs
I-C. D.dentex	9	9	1.000	6	13	0.0097(± 0.0012)	-1.513	-0.380
I-W. D.dentex	9	3	0.417	4	7	0.0014 (± 0.0007)	-0.176	-5.174**
II-W. D.dentex	5	4	0.900	1	6	0.0076 (± 0.0022)	0.498	-0.036
III-W. D.dentex	10	5	0.756	0	1	0.0031 (± 0.0005)	-1.276	-1.320
IV-W. D.dentex	5	2	0.400	0	2	0.0008 (± 0.0007)	-0.817	0.090
V-W. D.dentex	4	3	0.833	0	7	0.0021 (± 0.0007)	-0.710	-0.887
VI-W. D.dentex	6	4	0.800	0	7	0.0049 (± 0.0014)	-1.390*	-0.219
VII-W. D.dentex	5	5	1.000	0	3	0.0059 (± 0.0014)	-1.162	-2.371*

PS: Polymorphic site, PIR: Number of parsimonious informative regions

*$p < 0.05$, **$p < 0.01$

Table 6

Genetic distance calculation of *D. dentex* populations with respect to where the stations' samples were collected from (Nei's dxy and Da method)

Da									
Dxy*		I-C	I-W	II-W	III-W	IV-W	V-W	VI-W	VII-W
	Karaburun I-C	0	0.00148	-0.00045	0.00080	0.00141	0.00169	0.00060	0.00160
	Karaburun I-W	0.00701	0	0.00056	0.00009	0.00000	0.00000	-0.00008	0.00000
	Çeşme II-W	0.00815	0.00503	0	0.00014	0.00050	0.00084	0.00000	0.00084
	Kuşadası III-W	0.00717	0.00233	0.00545	0	0.00006	0.00014	-0.00028	-0.00004
	Güllük IV-W	0.00666	0.00112	0.00470	0.00201	0	0.00000	0.00000	0.00000
	Bodrum V-W	0.00757	0.00175	0.00566	0.00273	0.00147	0	0.00000	0.00000
	Çanakkale VI-W	0.00788	0.00307	0.00622	0.00370	0.00287	0.00349	0	-0.00042
	Antalya VII-W	0.00936	0.00363	0.00755	0.00444	0.00335	0.00398	0.00496	0

*All *Dxy* values within and between groups are statistically significant ($p < 0.01$)

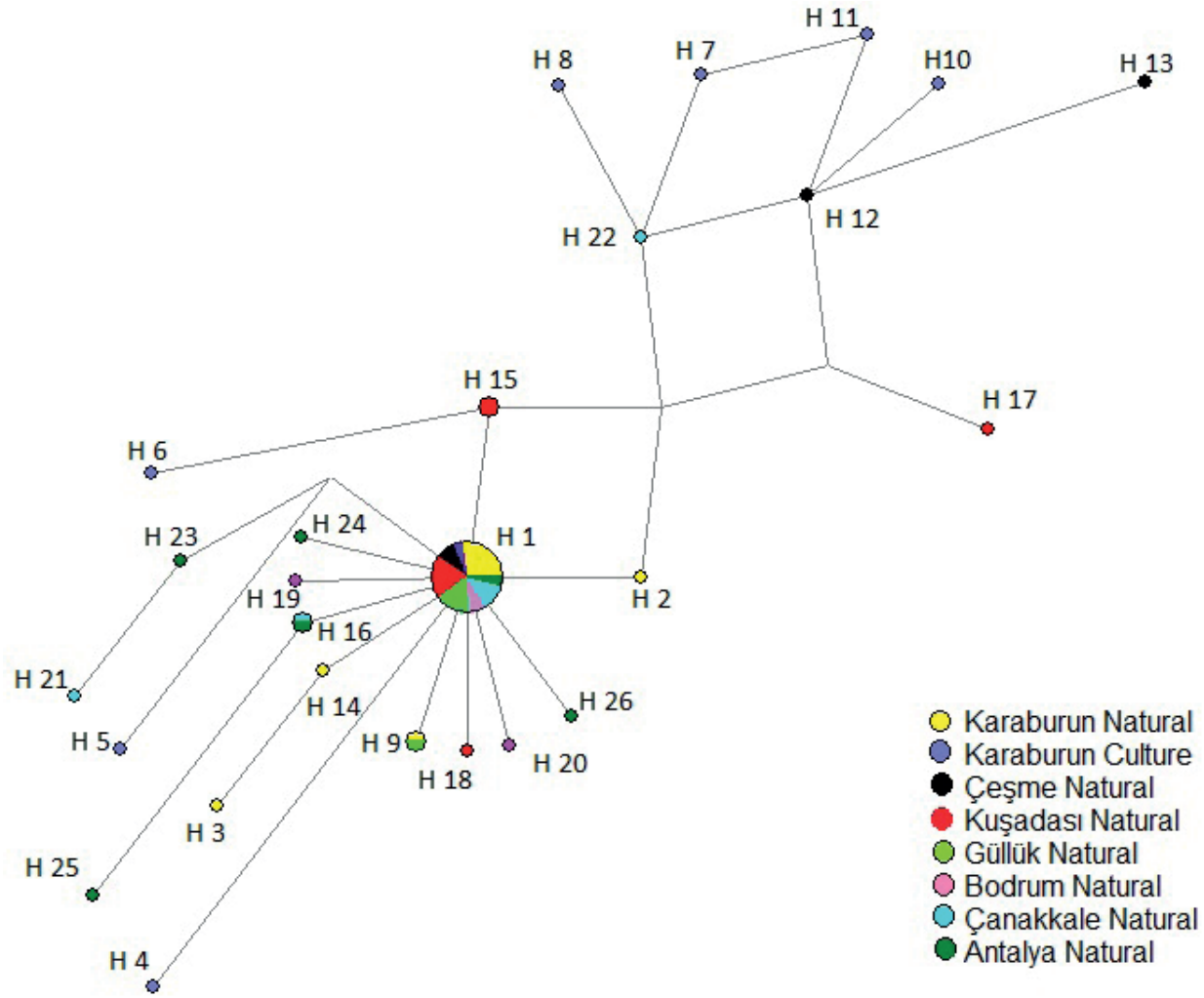

Figure 5

Median Joining network to sample haplotypes and representation of haplogroups of Common Dentex

Tajima's *D* (1996) and Fu's *FS* (Fu 1997) belonging to neutrality tests were evaluated which revealed the presence of selection for an allele in the population (Tajima 1989; Fu 1997). Tajima's *D* value had a positive value (0.498) in the *II-W* population and a negative one in other populations, while the sum was negative and found to be statistically insignificant ($p > 0.05$). In Fu's *Fs* tests, *IV-W* populations had positive values (0.090); the values were negative for other populations, with the sum being negative and therefore found to be mostly statistically insignificant ($p > 0.05$, Table 4). Therefore, there is rarely selective pressure for any allele studied in this research. Negative values in populations are considered to be associated with the growth of the population, while the positive value as in the *II-W* population would have resulted from a decline in the population size (Parmaksız & Ekşi 2017). Below the diagonal, *Dxy* indicates the average number of nucleotide substitutions per site between populations (percentage), and above the diagonal, *Da* indicates the average number of net nucleotide substitutions per site between populations (Nei 1987). Nei's corrected distance values (*Dxy* and *Da*) was used to estimate the genetic distances among populations collected from the regions. All the genetic distances are statistically significant ($p < 0.01$, Table 6).

The dendogram based on Nei's corrected distance parameters shows that the closest distance was between Güllük *IV-W* and Karaburun *I-W*, which are clearly separated from others (Figure 6). The cladogram obtained using Wright's *Fst* matrix (Table 7) is also in corroboration with Nei's phylogram, and the topology of the trees is identical (Figure 6). Both trees indicate that common dentex populations in Güllük *IV-W* and Karaburun *I-W* are closely related and probably descended from the same ancestral lineage and clearly separated from the others. Interestingly, the natural common dentex in Karaburun fall far apart from the cultured ones in Karaburun, which indicates that their ancestral lineage is very different, probably due to the artificial selection methods in fish cultures in Karaburun.

We also performed an AMOVA based on the haplotype frequencies and found that 92.54% of the genetic variation was within localities and 7.46% of the genetic variation was between/among localities. The mean fixation index (*Fst*) was calculated as 0.0746 ($p < 0.001$, Table 8). Probably due to the geographic barriers in the sea, the variation among haplotypic groups remained low. Close proximity geographies resulted in low variation in common dentex populations as the Atlantic coastal *D. dentex* formed a clearly distinct haplotypic group from those of the Mediterranean coasts (Bargelloni et al. 2003, Viret et al. 2018).

The genetic distance value is a value scale which describes the similarity of the base sequence in the *COI* gene fragment; the smaller the value, the closer the kinship relationship between the two populations (Nei 1987). In our study, the genetic distance values of locational groups are in agreement with the physical locations of the fish population. Such similarities are also common in other fish species such as *Siganus fuscescens, Acanthurus triostegus* as well as sparid fish (Bramandito et al. 2018; Planes et al. 2002).

In order to visually reveal the intraspecies interactions and haplotype structures of common dentex species, the neighbor joining tree and minimum evolution trees of the data obtained by DNA sequencing, a pairwise deletion feature was activated and phylogenetic trees were created by making 1000 bootstraps (Figure 8).

The genetic relationships of the individuals were also analyzed by constructing the NJ tree in which they are assigned to different groups with respect to reference sequences (Figure 4). A total of 53 individuals including common dentex (KJ01237.1) and

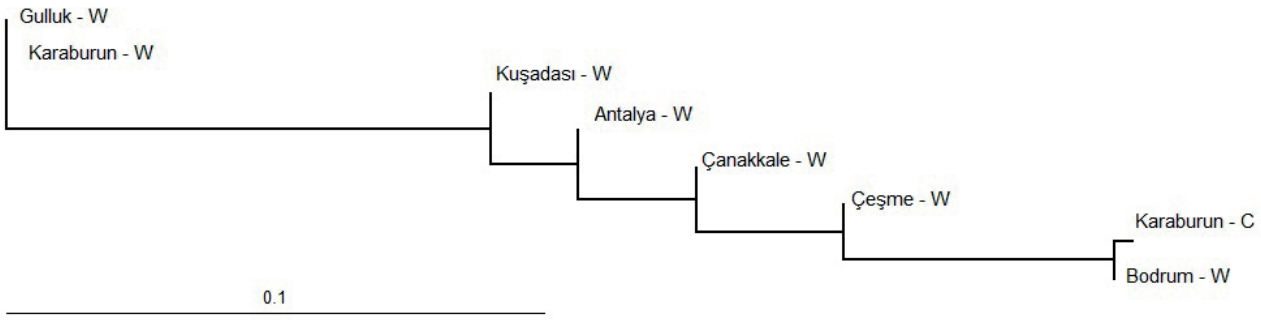

Figure 6

Phylogenetic reconstruction of 53 Common Dentex COI sequences grouped with respect to locations using Nei's Dxy distance matrix. W: wild, C: culture.

Table 7

Distance matrix obtained according to Wright's F statistics for the Common Dentex (*Dentex dentex*) sample at the stations in the study

	I-C	*I-W*	*II-W*	*III-W*	*IV-W*	*V-W*	*VI-W*	*VII-W*
Karaburun I-C	0.00000							
Karaburun I-W	0.22466*	0.00000						
Çeşme II-W	0.08522	0.00077	0.00000					
Kuşadası III-W	0.03716	0.07255	-0.02564	0.00000				
Güllük IV-W	-0.08257	0.16667	0.04412	0.00474	0.00000			
Bodrum V-W	0.21178	-0.02273	-0.03261	0.04085	0.16667	0.00000		
Çanakkale VI-W	0.03226	0.01562	-0.08541	-0.05174	-0.00737	-0.02362	0.00000	
Antalya VII-W	0.02463	0.04000	-0.06047	-0.05882	-0.01205	0.00415	-0.08711	0.00000

*$p < 0.01$

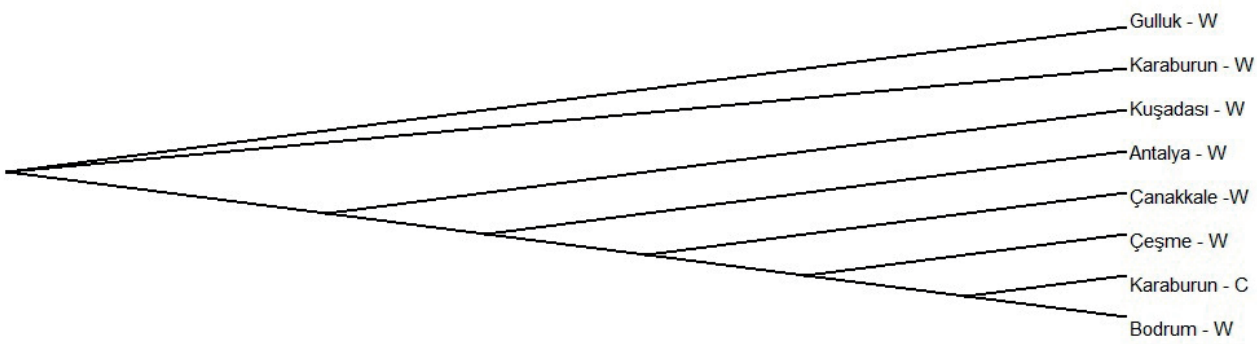

Figure 7

The cladogram NJ tree created with the Treeview obtained according to Wright's *F* statistic for the common dentex samples at the stations in the study. W: wild, C: culture.

sea bass (JQ623929.2) reference *COI* sequences formed seven haplogroups. The largest, Clade A, involved 44 individuals under two subgroups containing 17 (Subgroup A1) and 27 (Subgroup A2) individuals, respectively. Clade B and D included four and two individuals, respectively. Other clades (C, E, F, and G) contained only one individual. Although subgroup A2 contained individuals from all locations, Subgroup A1 did not include the Karaburun culture *I-C*, hence A1 is a better representative of the natural haplogroup (Figure 8).

The grouping placement of individuals on the NJ tree indicates that the *COI* genetic structure is irrespective of the location where samples were collected. The lack of special genetic structure of common dentex, as palmitic fish, was also reported earlier by Viret et al. (2018).. While the NJ tree discriminates species efficiently, some discrepancies are not uncommon within species also in other fish species (Amir et al. 2022).

4. Conclusion

Common dentex is a commercially and organoleptically valuable fish species. Yet it has become more valuable since the natural habitats of this threatened species have been diminishing. Aquaculture production also depends on sea life; therefore, monitoring common dentex in their natural and artificial habitats will be more valuable in terms of conserving this species for marine life as well as for the human food supply. Genetic diversity studies have been helpful in understanding the gene flow among and within species. Mitochondrial genome segments such as *COI* is proved to be one of the best methods for genetic dissection and barcoding of the higher organisms and has been extensively used at species and subspecies levels. Our results suggest that sequencing is the best way to analyze intraspecific genetic diversity, while HRM

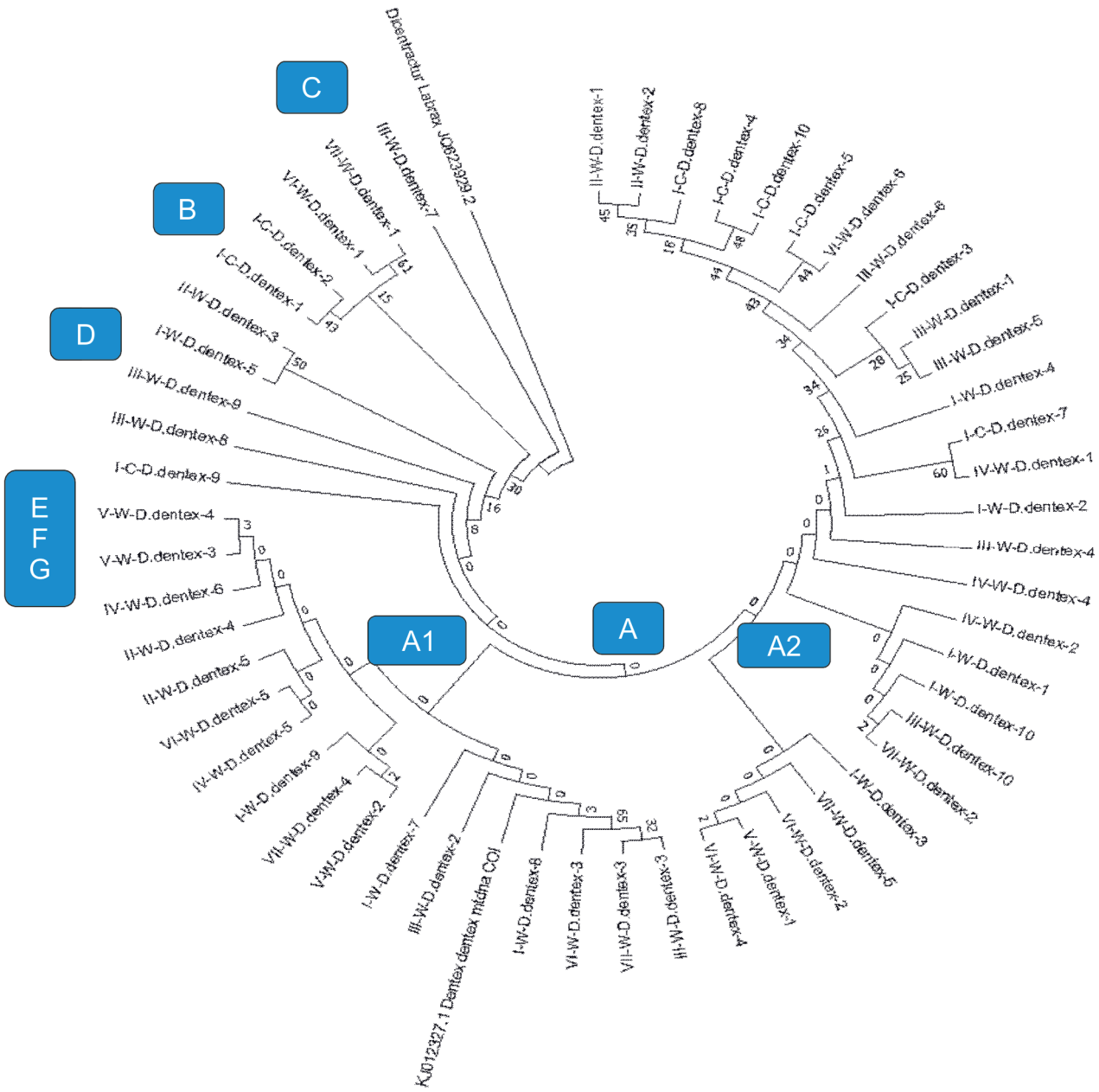

Figure 8

The cladogram NJ tree created with the Treeview obtained according to Wright's *F* statistic for the common dentex samples at the stations in the study. W: wild, C: culture.

analysis may be a good alternative for interspecific and geographically distant species. The high level of haploid diversity combined with the low level of nucleotide diversity indicates rapid population growth but may slow adaptation characteristics. It should be also noted that the diversity level in aquaculture facilities is more than in natural habitats. Genetics as well as other parameters of rapid population growth could be further researched to support aquaculture facilities while natural habitats could be enriched with common dentex with adaptable and compatible genetic backgrounds. Our study will be one of the first using polymorphic regions of *COI* gene sequencing coupled with real-time PCR and HRM analysis. Results will be useful for conserving natural habitats as well as maintaining the aquaculture facilities of common dentex.

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