GENETIC STRUCTURE OF MODERN AND ANCIENT SWORDFISH POPULATIONS FROM COASTS OF TURKEY

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ABSTRACT

GENETIC STRUCTURE OF MODERN AND ANCIENT SWORDFISH POPULATIONS FROM COASTS OF TURKEY

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In this study, partial mitochondrial DNA (mtDNA) and 8 microsatellite loci of swordfish collected from Northern Levantine Sea and Northern Aegean Sea were analyzed. Also same mtDNA region of ancient swordfish samples unearthed from Yenikapı excavation were sequenced.

Obtained sequences were evaluated comparatively with the sequences from Balearic Sea, Ligurian Sea, Ionian Sea and Southern Aegean Sea, available in databases. Analysis of the molecular variance revealed a significant differentiation between Western (Balearic, Ligurian) and Eastern (Ionian, Southern Aegean, Northern Aegean, and Northern Levantine) Mediterranean. Eastern Mediterranean had lower diversity and lower long term effective population size (Ne) than Western Mediterranean. Generally, migration from Eastern Mediterranean to Western Mediterranean was higher. Ancient DNA (aDNA) population was slightly different from modern populations based on F_{ST}. However, continuity between aDNA/ Southern Aegean, aDNA/ Northern Aegean and aDNA/ Northern Levantine could

not be rejected. Long term Ne and genetic diversity of aDNA were higher than Eastern Mediterranean and nearly equal to Western Mediterranean.

F_{ST}, PCA and STRUCTURE results showed Northern Aegean and Northern Levantine were not differentiated based on microsatellites. All short term Ne's calculated with various methods were less than 150 and MSVAR results showed dramatic Ne reduction in swordfish of both Northern Aegean and Northern Levantine, started 1240 and 599 years ago respectively.

Mediterranean swordfish is considered as a single stock and only one conservation plan is offered. According to results, different conservation plans must be implemented for Eastern and Western Mediterranean and Eastern population, harboring the swordfish of Turkish coasts, is at great risk.

Keywords: Swordfish, Mitochondrial DNA, Microsatellites, Ancient DNA, Coasts of Turkey

ÖZ

TÜRKİYE KIYILARINDAKİ MODERN VE ANTİK KILIÇ BALIĞI POPÜLASYONLARININ GENETİK YAPISI

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Bu çalışmada Kuzey Levant Denizi ve Kuzey Ege Denizi'nden toplanan kılıç balıklarının kısmi mitokondriyal DNA (mtDNA) ve 8 mikrosatelit lokusu analiz edilmiştir. Ayrıca Yenikapı kazılarından ortaya çıkarılan antik kılıç balığı örneklerinin aynı mtDNA bölgesi dizilenmiştir.

Elde edilen diziler veri tabanlarında bulunan Balear Denizi, Ligurya Denizi, İyon Denizi ve Güney Ege Denizi'ne ait diziler ile birlikte değerlendirilmiştir. Moleküler varyans analizi, Batı Akdeniz (Balear, Ligurya) ve Doğu Akdeniz (İyon, Güney Ege, Kuzey Ege ve Kuzey Levant) arasında anlamlı bir fark olduğunu göstermiştir Doğu Akdeniz, Batı Akdeniz'e göre daha düşük genetik çeşitliliğe ve etkin popülasyon büyüklüğüne (Ne) sahiptir. Genel olarak doğudan batıya doğru göç daha yüksek orandadır. Antik DNA (aDNA) popülasyonu F_{ST}'ye göre modern popülasyonlardan farklıdır. Ancak aDNA/ Güney Ege, aDNA/ Kuzey Ege ve aDNA/ Kuzey Levant arasında süreklilik ret edilememiştir. aDNA'nın uzun dönem Ne ve genetik çeşitliliği Doğu Akdeniz'den yüksek ve Batı Akdeniz ile yaklaşık olarak aynı bulunmuştur.

F_{ST}, PCA ve STRUCTURE sonuçları Kuzey Ege ve Kuzey Levant'ın mikrosatelitlere göre farklı olmadığını göstermiştir. Faklı yöntemlerle hesaplanan kısa dönem Ne sonuçlarının hepsi 150'den küçük bulunmuştur ve MSVAR sonuçlarına göre, hem Kuzey Ege hem de Kuzey Levant'ın kılıç balığı Ne değerleri, sırasıyla 599 ve 1240 yıl önce, çarpıcı bir azalma göstermiştir.

Akdeniz kılıç balığı tek bir stok olarak ve kabul edilmektedir ve sadece tek bir koruma planı önerilmektedir. Bu sonuçlara göre, Doğu ve batı Akdeniz için farklı koruma planları yapılmalıdır. Türkiye kılıç balıklarının da bir parçası olduğu Doğu popülasyonu, ciddi olarak tehlikededir.

Anahtar Kelimeler: Kılıç balığı, Mitokondriyal DNA Mikrosatelit, Antik DNA, Türkiye Kıyıları

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A: Number of Alleles
AD: Anno Domini
aDNA: Ancient DNA
AMOVA: Analysis of Molecular Variance
AMS: Accelerator mass spectrometry
Ar: Allelic Richness
Atl: Atlantic
bp: Base Pair
BSA: Bovine Serum Albumin
CI: Clade I
CII: Clade II
cm: Centimeter
CR: Control Region
D-Loop: Displacement Loop
dNTP: Deoxynucleotide triphosphate
Et-Br: Ethidium Bromide
g: Gravitational Acceleration
H: Haplotype Diversity
HE: Heterozygosity Excess
He: Expected Heterozygosity
Ho: Observed Heterozygosity
IATTC: Inter-American Tropical Tuna Commission
ICCAT: International Commission for the Conservation of Atlantic Tunas
kg: Kilogram
km: Kilometer
LD: Linkage Disequilibrium

m: Meter

MAD: Modern and Ancient Dataset

Max: Maximum

MC: Molecular Coancestry

MD: Modern Dataset

Med: Mediterranean

min: Minute

mg: Milligram

MJ: Median Joining

ml: Milliliter

mM: Millimole

mtDNA: Mitochondrial DNA

N. Levantine: Northern Levantine

ng: Nanogram

NJ: Neighbor Joining

Nm: Nanomole

PCA: Principle Component Analysis

pmol: Picomole

r: Raggedness Index

RFLP: Restriction Fragment Length Polymorphism

sec: Second

SNP: Single Nucleotide Polymorphism

TAC: Total Allowable Catch

UV: Ultraviolet

°C: Degrees Celsius

°N: Degrees North

°S: Degrees South

°W: Degrees West

μ: Mutation Rate

μl: Microliter

π: Nucleotide Diversity

CHAPTER 1

INTRODUCTION

In the present study, swordfish (*Xiphias gladius* Linnaeus, 1758) populations from the coast of Turkey were examined by means of different genetic markers mainly to understand their conservation status.

1.1 Swordfish (*Xiphias gladius* Linnaeus)

Swordfish is a highly migratory, large epipelagic billfish species which can be found in all tropical, subtropical and temperate seas between latitudes of 45°N – 45°S (Palko et al., 1981). It is the only member of the family Xiphiidae and usually characterized with its long bill shaped like a sword (Palko et al., 1981). Picture of a swordfish can be seen in figure 1.1.1.

Figure 1.1.1: Picture of an adult swordfish captured from Antalya Bay during sampling from Mediterranean shores of Turkey.

Swordfish can be described in three developmental stages, larval, juvenile and adult (Palko et al., 1981). Adults reach a total length of around 445 cm and total weight about 540 kg, most of the time females are larger than males (Nakamura, 1985). They start spawning around age of 5-6 and have max. age of at least 9 years (Palko et al., 1981). Swordfish can be found in tropical, temperate, and sometimes cold waters (Nakamura, 1985) and inhabit temperatures between 10.1°C to 28.6°C, but they also have great temperature adaptability, shown to be capable of tolerating temperatures between 4°C-28°C (Abascal et al., 2015). Larvae are more frequently can be seen at temperatures above 24°C (Nakamura, 1985). Generally, swordfish migrates toward temperate or cold waters for feeding in summer and to warm waters in autumn for spawning and they feed primarily on other pelagic fishes for example bluefishes, tunas, dolphin fishes, barracudas (Nakamura, 1985).

Swordfish is highly migratory and can travel for long distances (Palko et al., 1981). Average recorded distance for the swordfish traveled is 27.71+14.99 km per day for Pacific Ocean (Abascal et al., 2009) and 24.7±19.5 km per day (averaged up to 100 km per day in some months) for Atlantic Ocean (Abascal et al., 2015).

They stay in 400-600 m deep water during the day, can go deeper than 900 m for hunting with maximum recorded depth 1136 m (Abascal et al., 2009; Abascal et al., 2015). At night they stay close to surface 0-100 m (Abascal et al., 2009; Abascal et al., 2015). Vertical movements of swordfish also change according to the phases of moon, quarter of the year and sea surface temperature. They tend to stay in deeper during full moon and/or last quarter of the year and close to surface during new moon and/or low sea surface temperatures (Abascal et al., 2015).

Swordfish show strong natal homing behavior (Alvarado Bremer et al., 1996). These species are segregated into feeding, spawning and transitional areas (Mejuto et al., 1991; Mejuto et al., 1995; Mejuto et al., 1998; Mejuto and García-Cortés, 2003; Alvarado Bremer et al., 2005b). Spawning areas are defined by the existence of larval stages, presence of spawning females and discrepancy in the sex ratio of adult swordfishes. In spawning areas, larvae and spawning females are more abundant and

males are encountered more frequently than normal (Alvarado Bremer et al., 1995; Alvarado Bremer et al., 2005a; Mejuto et al., 1998). Feeding areas are defined by abundance of prey population hunted by swordfish (Alvarado Bremer et al., 2005a; Young et al., 2006). Transitional areas are routes where swordfish followed to feeding and spawning areas (Mejuto et al., 1998; Mejuto and García-Cortés, 2003).

1.2 Swordfish Stocks

In order to understand possible sub-structuring of swordfish, swordfish living in different oceans were previously analyzed with different genetic markers, using different methods.

Significant differentiations were observed between Indo-Pacific, South Atlantic, North Atlantic and Mediterranean, by mitochondrial DNA (mtDNA) control region (CR) sequencing (Alvarado Bremer et al., 1996; Chow et al., 1997; Chow and Takeyama, 2000), mtDNA CR restriction fragment length polymorphism (RFLP) data (Alvarado Bremer et al., 1996; Chow et al., 1997; Chow and Takeyama, 2000), variants in the calmodulin gene intron 4 (CaM) gene (Chow and Takeyama, 2000) intron variation in the aldolase C (aldC) and lactate dehydrogenase A (Idh-A) genes (Greig et al., 1999) and microsatellites (Kotoulas et al., 2007).

In addition, South Atlantic, North Atlantic and Mediterranean were differentiated by mtDNA CR sequencing (Viñas et al., 2007) and 26 single nucleotide polymorphisms (SNP)'s in 10 nuclear loci (Smith et al., 2015). South Atlantic and North Atlantic were differentiated by mtDNA CR sequencing (Alvarado Bremer et al., 2005a), variants in CaM gene (Chow and Nohara, 2003), a single nucleotide polymorphisms (SNP) at the CAM intron (Chow et al., 2007), 26 SNP's in 10 nuclear loci (Smith and Alvarado Bremer, 2010) and microsatellites (Kasapidis et al., 2007). Finally, North Atlantic and Mediterranean were differentiated by mtDNA CR sequencing (Kotoulas et al., 1995; Alvarado Bremer et al., 1999; Kotoulas et al., 2003; Alvarado Bremer et al., 2005b) and microsatellites (Reeb et al., 2003).

According to those studies swordfish populations were separated into four major stocks (Alvarado Bremer et al., 1995). These stocks are given below and shown in figure 1.2.1.

- Indo-Pacific Stock
- North Atlantic Stock
- South Atlantic Stock
- Mediterranean Stock

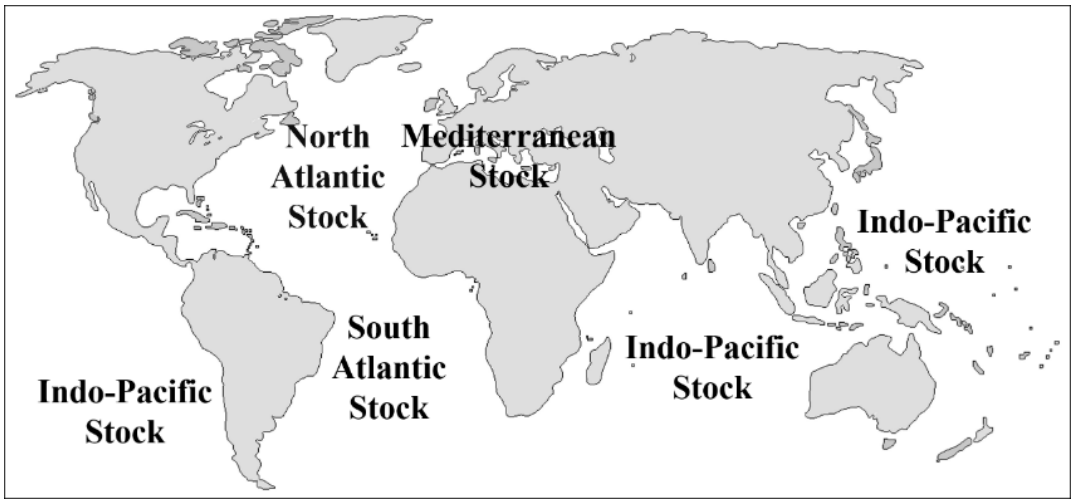

Figure 1.2.1: Locations of 4 major swordfish stocks.

Natal homing behavior of the swordfish, presence of the separate spawning areas and possible geographical barriers because of oceanic currents and streams are the reasons proposed for the existence of different stocks (Alvarado Bremer et al., 1996; Chow et al., 2007; Smith et al., 2015).

1.2.1 Indo-Pacific Stock

Some studies showed that Indo-Pacific stock is highly heterogeneous among itself (Alvarado Bremer et al., 2006; Hinton and Alvarado Bremer, 2007; Lu et al., 2016). This heterogeneity was observed with genetic studies conducted by sequencing of mtDNA control region (Reeb et al., 2000), sequencing of ldh-A intron 6 (Alvarado Bremer et al., 2006), sequencing of NADH-dehydrogenase subunit 2 (ND2) (Bradman et al., 2011a), allozyme analysis (Grijalva-Chon et al., 1996), microsatellite analysis (Kasapidis et al., 2008), SNP analysis (Lu et al., 2016) along with the fishery stock structure analysis (Hinton, 2003). Even though borders of the differentiated areas are controversial, it is suggested that differentiation is between Central North Pacific, Northeast Pacific, Southeast Pacific, Southwest Pacific and Indian Ocean (Hinton and Alvarado Bremer, 2007; Lu et al., 2016).

Data about the spawning areas and time in the Indo-Pacific stocks are sparse but according to the ichtyoplankton surveys larvae can be seen at the Northwest Pacific nearly 150°W off Taiwan in April and June, at the Southwest Pacific near Northeast Australia in October to December and in the Central South Pacific around 10°S all year as shown in figure 1.2.1.1 (Lu et al., 2016).

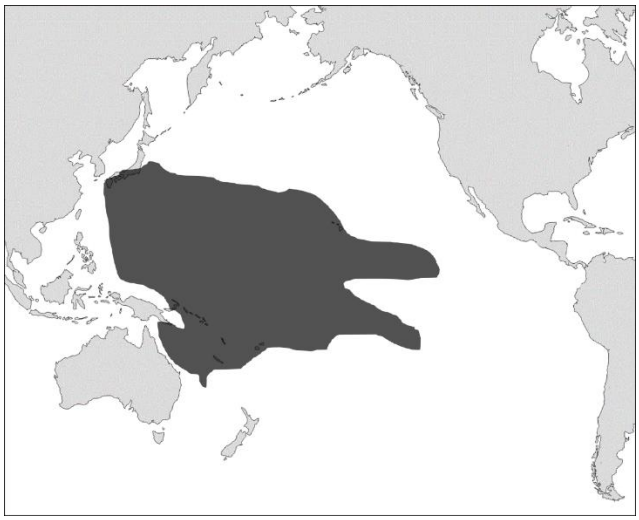

Figure 1.2.1.1: Swordfish larvae distribution in Pacific Ocean shown in black, according to the ichtyoplankton surveys (Lu et al., 2016).

1.2.2 North Atlantic and South Atlantic Stocks

Both North Atlantic and South Atlantic stocks are considered as very well defined with little or no heterogeneity within themselves (Alvarado Bremer et al., 2005a). Spawning area of North Atlantic is near Caribbean Islands and spawning area of South Atlantic is near Brazil and Uruguay, as shown in figure 1.2.2.1 (Alvarado Bremer et al., 2005a). In the North Atlantic, spawning occurs from December to June and in the South Atlantic spawning from April-June and October-March (Arocha, 2007).

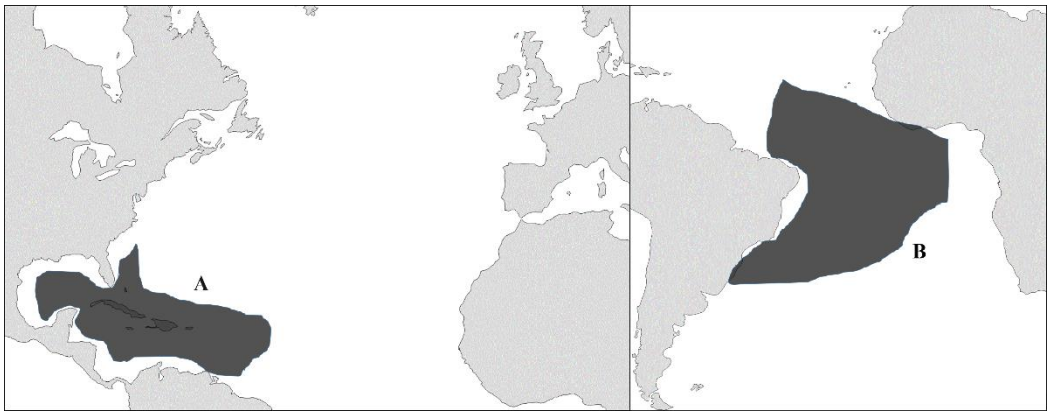

Figure 1.2.2.1: Swordfish spawning areas in the Atlantic Ocean shown in black. A) Near Caribbean Islands at the North Atlantic. B) Near Brazil and Uruguay at South Atlantic (Alvarado Bremer et al., 2005a).

1.2.3 Mediterranean Stock

Mediterranean stock is defined as "not well managed" (Collette et al., 2011) mainly because there is little information about the structure of the sword populations in Mediterranean. There are only a few genetic studies on Mediterranean swordfish. Studies based on mtDNA CR sequences (Chow et al., 1997; Alvarado Bremer et al., 2005b) and microsatellites (Kotoulas et al., 2007; Kasapidis et al., 2009) indicated that, Mediterranean has the lowest genetic variation when compared to the rest of the stocks. Studies that analyzed samples from both Eastern and Western Mediterranean, by using allozymes (Pujolar et al., 2002) RFLP of mtDNA (Kotoulas et al., 1995; Chow et al., 1997), failed to detect any differentiation within Mediterranean basin. On the other hand, a study conducted with CR sequences proposed a possible genetic differentiation between Eastern (Aegean Sea, Ionian Sea) and Western (Balearic Sea, Ligurian Sea and Tunisian Coast) Mediterranean swordfish populations (Viñas et al., 2010).

Discrete spawning areas for swordfish are proposed in Mediterranean Sea; a known area around the Straits of Sicily, Messina and Tyrrhenian Sea (Cavallaro et al., 1991; Romeo et al., 2009) and another one that is suggested to be area around the

northwest of the Levantine Sea (Tserpes et al., 2001; Tserpes et al., 2008) as shown in figure 1.2.3.1. Spawning in Mediterranean occurs in spring and summer, generally culminates in June and July (Di Natale et al., 2002).

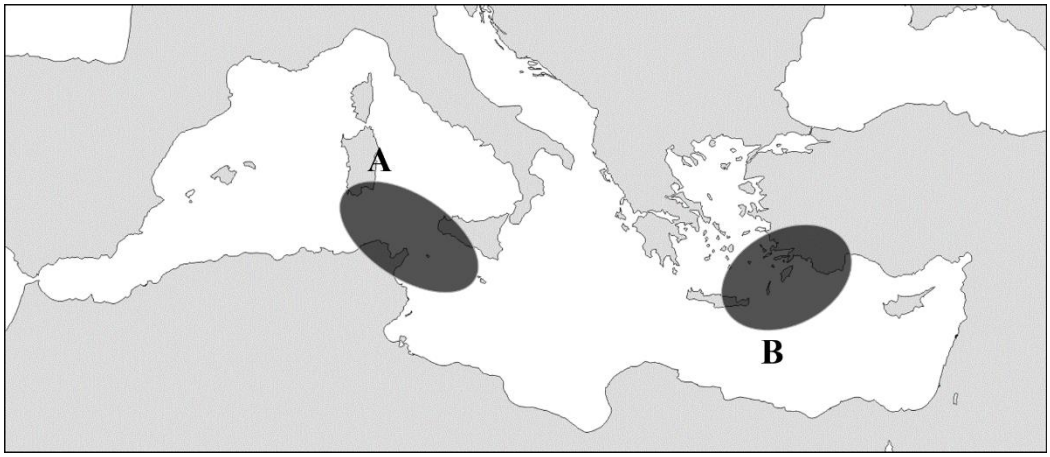

Figure 1.2.3.1: Suggested swordfish spawning areas in the Mediterranean. A) area around the Straits of Sicily, Messina and Tyrrhenian Sea (Cavallaro et al., 1991; Romeo et al., 2009) B) area around the northwest of the Levantine Sea (Tserpes et al., 2001; Tserpes et al., 2008).

1.3 Conservation and Management of Swordfish

Atlantic and Mediterranean swordfish stocks are managed by the International Commission for the Conservation of Atlantic Tuna (ICCAT) whereas Indo-Pacific stock is managed by Inter-American Tropical Tuna Commission (IATTC), these commissions are responsible from protecting tuna and tuna like species including their close relative, swordfish.

Swordfish showed 28% global decline over the past three generations (20 years). International Union for Conservation of Nature (IUCN), categorized swordfish as 'Least Concern' but with the population trend "decreasing" (Collette et al., 2011). Meanwhile, regional evaluation of the Mediterranean swordfish classified this stock

as “Near Threatened” (Abdul Malak et al., 2011), because it is the only stock that is NOT well managed (Collette et al., 2011).

Swordfish less than 3 years old (most probably never spawned) generally comprise 50-70% of the total yearly catches (Anonym, 2017a). Thus, stock is currently overfished and unless some measures will be taken it will continue to suffer from overfishing. So harvest and the volume of juvenile catches should be reduced in order to rebuilt the Mediterranean swordfish stock (Anonym, 2017a).

Currently, ICCAT implements series of rules for the management of Mediterranean swordfish, including 3 months of fishing ban (1 October - 30 November and 15 February-30 March) minimum landing size of 90 cm, registration of list of authorized vessels and ports, gear specification (Anonym, 2017a). Finally, in 2017 total allowable catch (TAC) was targeted to be as 10,500 tons with a further 15% reduction between the years of 2018-2022 (3% each year) in order to preserve 75% of the stock. Whereas, for the Mediterranean countries present TAC that is recommended are as follows: 5.238% for the Algeria, 70.756% for the European Union, 9.952% for the Morocco, 9.597% for the Tunisia, 4.200% for Turkey based on their previous catch rates (Anonym, 2017b). Therefore, recommended total catch for Turkey is 441 tons in 2017.

1.4 Swordfish Populations in Turkey

In Turkey, swordfish can be seen in the Aegean and the Mediterranean shores of Turkey. Although current literature describes Black Sea and Sea of Marmara among the habitat of swordfish it could hardly be caught in those areas in the last 50 years (Akyol and Ceyhan, 2011).

Total swordfish catches between 1970-2010 based on data from ICCAT (Anonym, 2017a) are given in figure 1.4.1. According to this data, swordfish catches were more or less stable between 1970-1980, reached a peak in 1988, started to decrease until 1992, re-increased until 2010 and decreased again after 2010.

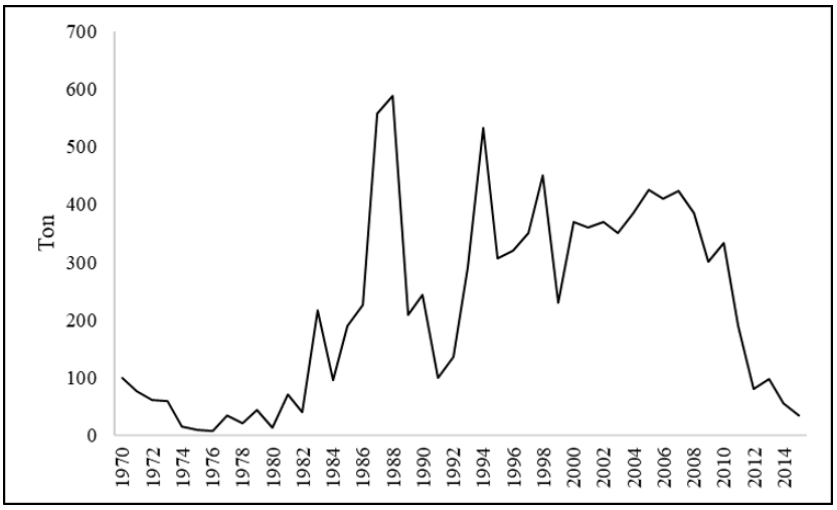

Figure 1.4.1: Graphic of swordfish total catch of Turkey between years of 1970-2015 (prepared with data obtained from ICCAT (Anonym, 2017a).

Sharp increase in swordfish catches between 1980-1988 is attributed to the possible overestimation of total catches prior to the change in catch statistics in 1990 (Anonym, 2017a). In 2011, Turkey banned all types of driftnets, including gillnets and decreased the number of registered vessels, resulting a sharp decrease in yearly catches (Anonym, 2017a; Anonym, 2017b). Current catch in Turkey is 35 tons which is under the recommended amount of catch by ICCAT (Anonym, 2017b).

1.5 Genetic of Studies of Swordfish Populations in Turkey

Genetic studies were not conducted on the swordfish of Northern Levantine part of the Mediterranean Sea and north of the Aegean Sea. As mentioned above, it is suggested that one of the spawning areas in the Mediterranean Sea is in the Northeast Levantine (Tserpes et al., 2001; Tserpes et al., 2008) This information further emphasizes the importance of the area.

In this study, contemporary (modern) swordfish samples were collected from the Northern Levant area of the Mediterranean Sea and from Northern Aegean Sea. Partial mtDNA CR of those samples were sequenced and 8 loci of microsatellite

markers were analyzed. Also, ancient DNA from ancient swordfish bones unearthed from Marmara-Yenikapı excavations were extracted and partial mtDNA CR of ancient samples were sequenced.

1.6 Swordfish Mitochondrial DNA (mtDNA)

Mitochondrial DNA (mtDNA) is a circular and haploid DNA that is found in every eukaryotic cell. It is an important marker that is regularly used in population genetic studies (Bruford et al., 2003; Bruford, 2004). It has high rate of evolution and because it is a haploid molecule it can be easily differentiated within the isolated gene pools. Therefore, it can readily be used to analyze the differentiation between recently diverged species or populations of the species. Differences on the mtDNA sequences can only arise by mutations so it is easy to work on polymorphisms statistically. However, data obtained from mtDNA are hardly used for making inferences about diversity of genomic DNA because mtDNA is a single gene and only informative about maternal lineages (Bruford et al., 2003; Bruford, 2004).

Control region (CR) is the fastest evolving region of the mtDNA and contains the displacement loop (D-Loop). Because of it fast evolution rate, CR is used for measuring genetic diversity and differentiation studies (Bruford et al., 2003).

Swordfish mtDNA is approximately 16520 bp long (Kadoyama et al., 2008) and CR is approximately 835 bp long (Bradman et al., 2011a). There is 1-3 TACA tandem repeats near 5' of the L-strand (Alvarado Bremer et al., 1995; Alvarado Bremer etal., 2005a). There are two maternal lineages in the swordfish which are named as Clade I and Clade II (Alvarado Bremer et al., 1996). Clade I (CI) has higher haplotype diversity, it is found in every ocean and it is the only clade that can be seen in the Indo-Pacific Stock (Alvarado Bremer et al., 1996; Alvarado Bremer etal., 2005a; Chow et al., 1997; Chow and Takeyama, 2000). It also has two different subclades named as CI_α and CI_β. CI_β lineage is more abundant in the Pacific but its frequency is lower in North Atlantic, South Atlantic and Mediterranean than Indo-Pacific (Alvarado Bremer et al., 2005a). Clade II (CII) has the highest frequency in

the Mediterranean (Alvarado Bremer et al., 2005a; Viñas et al., 2010). It is composed of two sub-clades named CII_θMed and CII_θAtl, former is exclusively seen in the Mediterranean and latter can only be seen in South and North Atlantic (Alvarado Bremer et al., 2005a). Figure 1.6.1 summarizes the clade and sub-clade distribution in all stocks (Alvarado Bremer et al., 2005a).

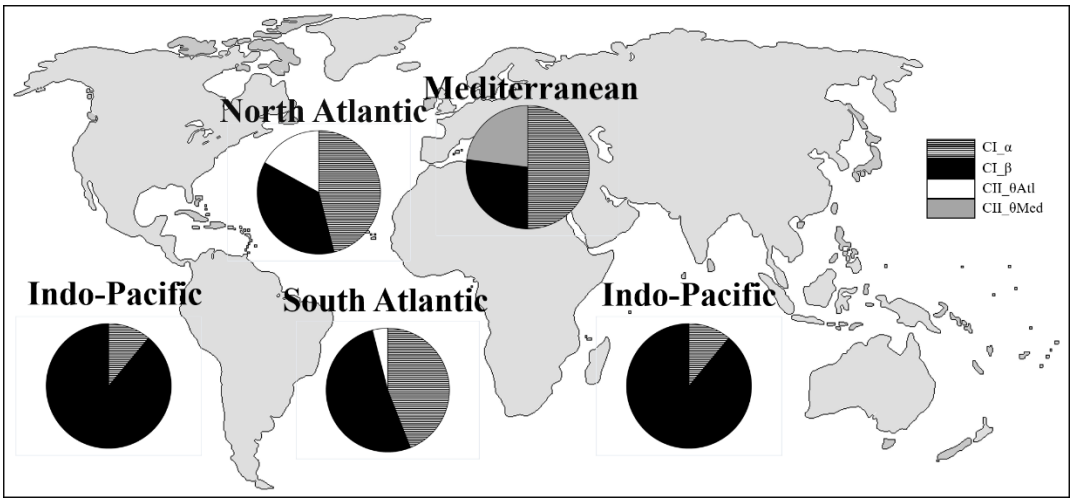

Figure 1.6.1: Frequency distribution of mtDNA clades and sub-clades of swordfish based on stocks (data taken from Alvarado Bremer et al., 2005a).

1.7 Swordfish Microsatellites

Microsatellites are tandem repeats that are usually consist of 1 to 6 long sequence motifs. Number of times a motif repeated at a polymorphic locus can vary and they are inherited in a Mendelian fashion (Allendorf and Luikart, 2007).

Microsatellites are extensively used in population genetics, molecular ecology and conservation studies. They are highly polymorphic and well distributed across the genome, so inferences can be made about the diversity of whole genome (Allendorf and Luikart, 2007). They are non-coding, neutral as long as they are not closely

linked to the coding regions and not affected by natural selection. Hence, microsatellite loci provide unbiased information about the level of genetic diversity of a genome (Jobling et al., 2014). Beside their advantages, microsatellites also have some disadvantages. They may have null alleles (explained in detail at section 2.6.2.1.1) and size homoplasy (convergence of sizes in different alleles). Because of subjective genotyping, direct comparisons of data from different studies is not possible (Jobling et al., 2014).

In genetic studies of swordfish microsatellite markers are both used to analyze differentiation between stocks (Kotoulas et al., 2003; Kotoulas et al., 2007; Reeb et al., 2003; Kasapidis et al., 2007) and within stock (Kasapidis et al., 2008; Kasapidis et al., 2009; Bradman et al., 2011b). So far, 52 microsatellite loci are defined for swordfish and 18 of them are defined for Atlantic and Mediterranean stocks. According to those microsatellites North Atlantic stock has higher number of alleles and haplotype diversity than west of the Mediterranean (Kasapidis et al., 2009).

1.8 Ancient DNA (aDNA)

Ancient DNA (aDNA) is type of DNA that is extracted from museum specimens and archeological specimens like, bones, seeds, coprolites, fossil remains. Ancient DNA is used in population genetics and conservation genetics to gather information about changes in genetic diversity and hence about evolutionary history of populations (Pääbo et al., 2004). Most of the time, information obtained from aDNA cannot be acquired from modern DNA, e.g. Larson et al., (Larson et al., 2007).

After death, DNA molecules start to fragmentize (Pääbo, 1989). Heat and water in the environment accelerates this fragmentation and after a while, DNA is destroyed completely (Allentoft et al., 2012). Furthermore, some nucleotide changes start to occur because of nuclease activities, hydrolytic processes, oxidative processes and microbial activities, which are referred as postmortem modifications/nucleotide misincorparations (Hofreiter et al., 2001).

Nucleotide changes can be transitions or transversions. Transitions are categorized as Type 1 (T-C and A-G) and Type 2 (C-T and G-A) (Hansen et al., 2001). Most of the postmortem modifications are type 2 transitions and indels and although other type of changes can also be seen, they can be considered as amplification artifacts (Olivieri et al., 2010). Short fragment length (Pääbo, 1989), and Type 2 transitions (Briggs et al., 2007; Brotherton et al., 2007) are considered as the evidence for the authenticity of aDNA. In the studies, in order to detect Type 2 transitions, aDNA is extracted and amplified more than once and differences between the repeated aDNA sequences from the same specimen are examined to confirm the authenticity of the aDNA sequences.

1.9 Marmaray – Yenikapı Excavations

During the construction of Marmaray-Yenikapı subway station in İstanbul, number of shipwrecks and animal skeletons were discovered. In 2004, an archeological excavation is initiated on a 58.000 m^2 wide area, located 3 m above sea level, by Ministry of Culture, General Directorate of Cultural Heritage and Museums and under the direction of İstanbul Archaeology Museums. During excavations, Theodosius harbor (Portus Theodosiacus), the biggest port of the early Byzantium, was unearthed (Onar et al., 2013). It is located at a natural cove at the mouth of the currently dried Lykos brook in Bayrampaşa (Onar et al., 2013) as shown in figure 1.9.1.

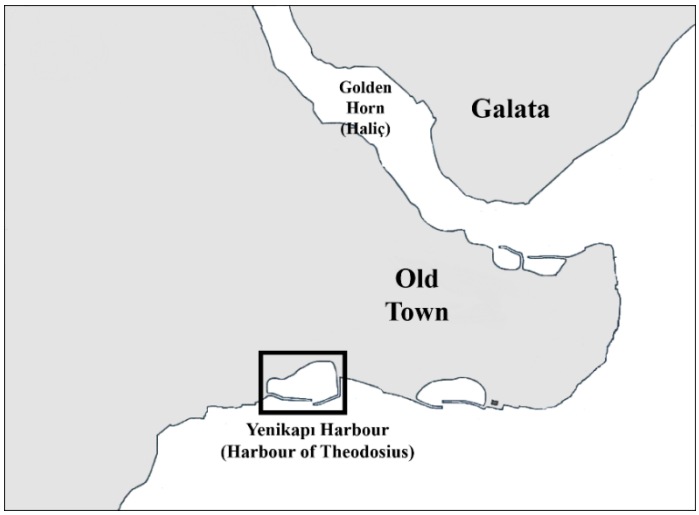

Figure 1.9.1: Location of the ancient Theodosius harbor (Onar et al., 2013).

Substantial number of animal bones were excavated from Theodosius harbor. Six of those bones (5 vertebrates and 1 sword bone) were identified as swordfish and dated between 4^{th} - 10^{th} century AD by context (Onar et al., 2008; Onar et al., 2013).

1.10 Aims of the Study

In the present study, by employing the examined populations: Northern Levantine (N. Levantine) and Northern Aegean (Aegean 2) and aDNA samples from Yenikapı excavation site of Istanbul, the following objectives were targeted.

On the basis of mtDNA data, aims were:

- To re-analyze genetic diversity between Mediterranean swordfish by adding two populations from the east and northeast of the basin.

- To evaluate changes in genetic variation by examining ancient samples along with the modern samples.

- To contribute to the understanding of demographic history of swordfish populations in the Mediterranean.

- To calculate effective population sizes and maternal migration rates of swordfish populations in the Mediterranean.

On the basis of microsatellite data, the aims were:

- To obtain baseline genetic data belonging to Turkish swordfish populations by means of neutral nuclear markers.

- To characterize past demographic events like declines in the population sizes.

- To estimate effective population sizes of the populations inhabiting the Turkish coasts.

CHAPTER 2

MATERIALS AND METHODS

2.1 Sampling

2.1.1 Modern Samples

In order to genetically characterize swordfish from Northern Aegean Sea, 26 muscle and fin samples were collected and analyzed from the coasts of Çanakkale and Gökçeada. Additionally, 41 muscle samples were collected from the coasts of Antalya Bay and 1 muscle sample was collected and analyzed from coast of Karşıyaka-Cyprus, to represent Turkish shores of Mediterranean Sea. In this study, in total 42 swordfish samples were analyzed from Mediterranean Sea. Sampling locations are given in figure 2.1.1.1.

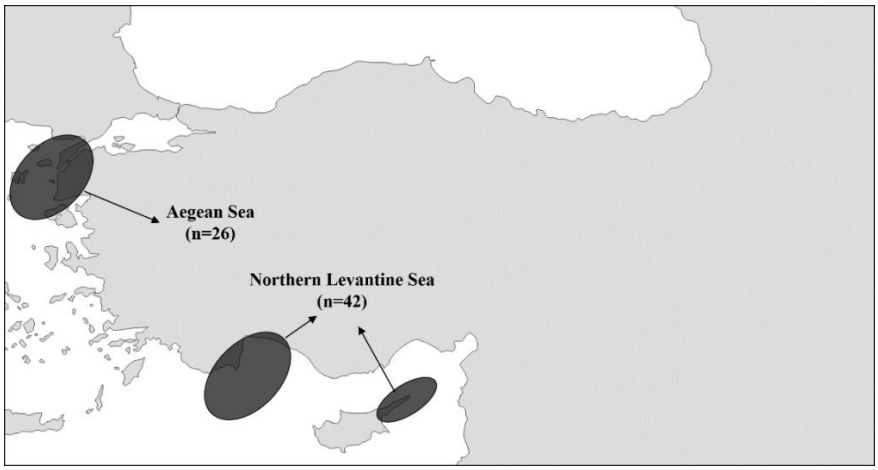

Figure 2.1.1.1: Sampling locations of modern swordfish samples which are shown in black ellipsis.

2.1.2 Ancient Samples

Six ancient swordfish bone samples, constituting 5 vertebrate and 1 rostral bone, as shown in figure 2.1.2.1 were obtained from Marmaray-Yenikapı excavations (Onar et al., 2013).

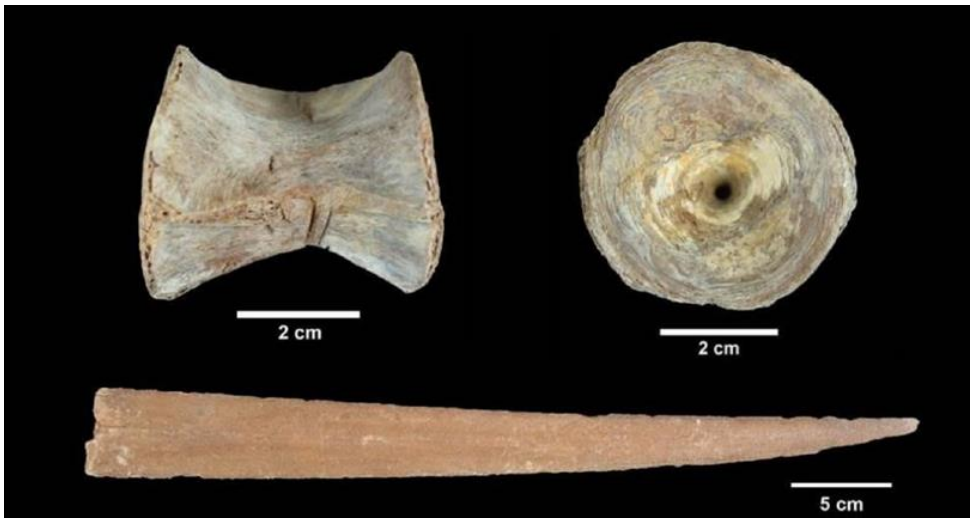

Figure 2.1.2.1: Swordfish bones excavated from Marmaray-Yenikapı (Onar et al., 2013).

Approximate date of each bone sample was determined based on the AMS 14C radiocarbon dating of other animal bones excavated from the same archeological context as presented in Onar et al.'s (2008) study. Date of each bone is given in table 2.1.2.1:

Table 2.1.2.1: Date of each bone, which is determined by AMS 14C radiocarbon dating of other animal bones excavated from the same archeological context as presented in Onar et al.'s (2008) study.

Sample Lab ID	Sample Excavation ID	Date
YK1	Met16319	4^{th}-6^{th} century
YK2	Met12951	8^{th}-9^{th} century
YK3	Mry3065	6^{th}-7^{th} century
YK4	Mry5364	8^{th}-9^{th} century
YK5	Met13042	9^{th}-10^{th} century
YK6	Met15650	7^{th}-8^{th} century

The date of the most ancient sample was between 4^{th}-6^{th} century/early meanwhile the date of the most recent sample was between 9^{th}-10^{th} century. Visible blade marks on the bones suggests that these swordfish were captured, dissected and sold at the Theodosius port unearthed from Marmaray-Yenikapı (Onar et al., 2013).

2.2 DNA Extraction

2.2.1 DNA Extraction from Modern Samples

DNA was extracted from muscle and fin tissues by using commercially available Genejet DNA Purification Kit (Fermentas). Quality and quantity of samples were first controlled by agarose gel electrophoresis with 1% polymerized agarose gel. Gel was stained by ethidium bromide (Et-Br) solution and visualized under UV light by a gel imaging system. Concentration of the DNA was checked by observing presence and the thickness of the corresponding bands on the gel. Quality of the DNA was controlled by observing if there are smears on the bands. Quality and quantity of DNA concentration were also measured by NanoDrop spectrophotometer by examining the absorbance values at 260/280 and 260/230 nm ratios (Desjardins and Conklin, 2010).

2.2.2 DNA Extraction from Ancient Samples

Ancient DNA was extracted from ancient bone samples by using the column-based extraction method modified from Rohland et al.'s (2010) method. Details of this method is given below:

A piece of bone from each sample was grinded completely to 230-250 mg powder. Bone powders were placed in to 15 ml conical centrifuge tubes and 5 ml extraction buffer was added to each tube. Tubes were sealed with parafilm and rotated overnight (16-20 hours) at 37°C. After incubation, tubes were centrifuged for 2 minutes at 5000g. Supernatants of the samples were transferred to new 15 ml conical centrifuge tubes and 2.5 ml binding buffer was added to each tube (pellets of the samples were kept at -20°C for using in another extraction). Silica buffer was vortexed for 15 seconds and 100 µl silica buffer was added to each tube. Tubes were sealed with parafilm and rotated for 3 hours at room temperature. After incubation, supernatants were discarded and 450 µl binding buffer was added to each tube. Pellets were re-suspended in the binding buffer and solutions were transferred to 2 ml micro centrifuge tubes with assembled columns. Tubes were centrifuged for 2 minutes at 13.300g. Supernatants in the collection tubes were discarded, columns were placed to the same collection tube and 450 µl washing buffer was added to each tube. Tubes were centrifuged for 2 minutes at 13.300g and the supernatants in the collection tubes were discarded. The washing step was repeated once more. In order to remove remaining buffer from column, empty columns were centrifuged for additional 2 minutes at 13.300g after last washing step. Collection tubes were discarded and columns were placed to new 1.5 ml centrifuge tubes. Finally, 50 µl TE buffer was added and the tubes were incubated for 10 minutes with closed lids. After incubation tubes were centrifuged for 2 minutes at 13.300g. Extracted DNA is stored at 4°C for immediate use and -20°C for long term storage (Rohland et al., 2010). Ingredients of chemical solutions used in the extraction are presented in appendix A.

DNA extraction was repeated twice for each ancient sample with one-month gap between two extractions.

Since DNA extracted from ancient samples usually have low yield (Hofreiter et al., 2001) quality and quantity of the DNA were not measured with agarose gel electrophoresis or NanoDrop spectrophotometer.

2.3 PCR and Sequencing of the mtDNA CR

2.3.1 PCR and Sequencing of Modern Samples

Partial mtDNA CR of the modern samples, which is 450 bp long, was amplified by primers defined in Viñas et al.'s (2004) study. Sequences of forward and reverse primers used for amplification are given in table 2.3.1.1:

Table 2.3.1.1: Sequences of forward and reverse primers that are used for amplification of partial mtDNA CR of the modern samples (Viñas et al., 2004).

Primers	Primer Sequence (5'-3')
L15998 (Fwd)	TACCCCAAACTCCCAAAGCTA
CSBDH (Rev)	TGAATTAGGAACCAGATGCCAG

Concentrations of the reagents used in the PCR mix and PCR conditions are given in table 2.3.1.2 and table 2.3.1.3 respectively.

Table 2.3.1.2: Concentration of the reagents in the PCR mix for partial mtDNA CR of modern samples.

Solution	Concentration
dH_2O	13.2 µl
PCR Buffer	1 X
$MgCl_2$	2.0 mM
dNTP	0.4 mM
Fwd Primer	15 pmol
Rev Primer	15 pmol
BSA	5 ng
Taq Polymerase	1 unit
DNA	3 ml
Total Volume	**25 µL**

Table 2.3.1.3: PCR conditions for the amplification of partial mtDNA CR of modern samples.

Step	Temperature	Duration	Number of Cycles
Initial Denaturation	95°C	5 min	1X
Denaturation	94°C	45 sec	35X
Annealing	57°C	45 sec	
Extension	72°C	60 sec	
Final Extension	72°C	15 min	1X

Results of the PCR amplification were checked by visualizing the presence of the PCR products on 2% polymerized agarose gel. Gel was stained by Et-Br solution and visualized under UV light by a gel imaging system.

Successfully amplified DNA samples were purified with commercially available Genejet DNA Purification Kit (Fermentas) in order to remove unused PCR buffer, $MgCl_2$, primers, dNTP, BSA, Taq polymerase and genomic DNA remains. After purification, partial mtDNA CR of each sample were sequenced in both forward and reverse directions, at REFGEN Gene Research and Biotechnology Limited Inc. (Ankara, Turkey). Resulted peaks were analyzed by Geneious v8 (Kearse et al.,

2012; www.geneious.com) and obtained sequences were aligned with each other and with other swordfish sequences from NCBI database with BioEdit v7.2 (Hall, 1999) by using ClustalW multiple alignment algorithm (Thompson et al., 1994) with 1000 bootstraps.

2.3.2 PCR and Sequencing of Ancient Samples

The same 450 bp long mitochondrial DNA partial CR of aDNA samples were also amplified, but not with the same primers that were used for amplification of modern samples. Since aDNA is more fragmented than modern DNA (Pääbo, 1989) this 450 bp long region was amplified in the form of 5 smaller overlapping fragments. Five pairs of primers were designed for amplification by using Primer 3 (Koressaar and Remm, 2007; Untergasser et al., 2012). Sequences of these primers and lengths of the 5 fragments are given in table 2.3.2.1.

Table 2.3.2.1: Sequences of the primers that are designed for amplification of 5 partial CR aDNA fragments and lengths of these fragments.

Fragment Number	Primer Sequence (5'-3')	Length
Fragment 1	Fwd: TACCCCAAACTCCCAAAGCTA Rev: TGATGTATAAACGTGAATGGTTACAT	177 bp
Fragment 2	Fwd: GCACATAACATGTAATTCGAC Rev: GCTAGGTCTTAAGTTTCTGCTTG	138 bp
Fragment 3	Fwd: AATGTAACCATTCACGTTTATACATC Rev: TCGGAATTTGAGTCCTGGTA	150 bp
Fragment 4	Fwd: CAAGCAGAAACTTAAGACCTAGCA Rev: ATGCTGGTTGGTGGTCTCTT	111 bp
Fragment 5	Fwd: CCAGGACTCAAATTCCGATT Rev: TGAATTAGGAACCAGATGCCAG	150 bp

All fragments were amplified with the same PCR conditions and PCR reagent concentrations. Concentrations of the reagents in the PCR mixes and PCR conditions are given in table 2.3.2.2 and table 2.3.2.3 respectively.

Table 2.3.2.2: Concentration of the reagents in the PCR mixes for each partial fragment.

Solution	Concentration
dH_2O	8.1 µl
PCR Buffer	1 X
$MgCl_2$	2.25 mM
dNTP	0.2 mM
Fwd Primer	10 pmol
Rev Primer	10 pmol
BSA	0.05 ng
Taq Polymerase	3 unit
DNA	5 ml
Total Volume	**20 µL**

Table 2.3.2.3: PCR conditions for each fragment of partial mtDNA CR of aDNA samples.

Step	Temperature	Duration	Number of Cycles
Initial Denaturation	94°C	15 min	1X
Denaturation	94°C	45 sec	60X
Annealing	52°C	45 sec	
Extension	72°C	60 sec	
Final Extension	72°C	15 min	1X

Three sets of PCRs were made for each of the 5 fragments. PCR products were run with 3% polymerized agarose gel, stained by Et- Br, checked with UV light, purified with commercially available Genejet DNA Purification Kit (Fermentas) and sequenced for forward and reverse directions as described in section 2.3.1. Peaks

obtained from sequencing were analyzed by Geneious v8 (Kearse et al., 2012; www.geneious.com).

2.4 Authenticity of Ancient Samples and Fragment Overlapping

Authenticity of ancient samples were determined by checking postmortem misincorparations described in section 1.8. Misincorparations were determined by comparing same nucleotide of 6 replica obtained from 3 different PCRs (3 forward, 3 reverse) for each sample. Frequencies of nucleotides seen in each position were calculated and the nucleotide with higher frequency is assigned to that position. For example, in the case where C was seen at one position for 5 of the sequences and T was seen at the same position for the 6^{th} sequence, nucleotide at that position was assumed to be C and the T that was observed in 6^{th} sequence assumed to be a misincorparation.

After determining misincorparations, fragments were aligned and overlapped in order to obtain one continuous sequence for each sample by using ClustalW multiple alignment algorithm (Thompson et al., 1994) implemented in BioEdit v7.2 (Hall, 1999) with 1000 bootstraps.

2.5 PCR and Genotyping of Microsatellite Loci

Among all microsatellite loci defined in the study of Kasapidis et al.'s (2009), eight of them that have the highest variation in the Western Mediterranean were chosen for genotyping modern samples.

Forward primers of all microsatellite loci were labeled with fluorescent dye. In order to analyze all loci in one read, loci that have overlapping allelic range were labeled with different fluorescent dye. Meanwhile loci that have different allelic range were labeled with the same fluorescent dye.

Names, repeat motifs and allelic ranges of the 8 microsatellite loci used in this study (Kasapidis et al., 2009) are given in table 2.5.1. Sequences of forward and reverse primers used for amplification of these 8 microsatellite loci (Kasapidis et al., 2009) and type of the fluorescent dye that was used to label forward sequences are given in table 2.5.2.

Table 2.5.1: Names, repeat motifs and allelic ranges of the 8 microsatellite loci used in this study (Kasapidis et al., 2009).

Locus	Repeat Motif	Allelic Range
XgSau98R1	$(CA)_8$	156-188
Xg31	$(TG)_{12}$	206-226
VBC201	$(TGA)_7$	241-271
Xgl-561	$(CA)_6GA(CA)_7$	127-151
Xg41b	$(CT)_{20}N_6(GT)_{21}$	172-246
Xgl-65b	$(CT)_{16}$	259-289
Xg51	$(CA)_{16}$	122-202
Xgl-536	$(CT)_7N_{51}(CT)_{16}$	226-284

Table 2.5.2: Sequences of forward and reverse primers used for amplification of 8 microsatellite loci (Kasapidis et al., 2009) and type of the fluorescent dye that was used to label forward sequences (Fwd: Forward, Rev: Reverse).

Locus	Primer Sequence (5'-3')	Fluorescent Dye
XgSau98R1	Fwd: CCACAATGTTTACGCTTCATC Rev: CGAATACTTTGTGTAGATGGCACA	HEX
Xg31	Fwd: CAGGTTTCCCTCGTTCTTCA Rev: CCATGTCTTCGTCTGTGTCG	HEX
VBC201	Fwd: GATGAGTCATACTGCCGACG Rev: GAGGCAGGTGAGAGTATATTGC	HEX
Xgl-561	Fwd: GCATGCTGGGAAGACAATTC Rev: AATCCTGCTCGTCTTTATAGG	FAM
Xg41b	Fwd: CACTTGGTGGAGGGATTTCA Rev: CATCAGACCGACTGTCCTCA	FAM
Xgl-65b	Fwd: TGCACTGCATTCAACAGTGTC Rev: GCAAACCATCGTGTGAGTTG	FAM
Xg51	Fwd: GTAAAGAGCAGCTTACTAGG Rev: ACACGTACATCTAGCTGAG	TAMRA
Xgl-536	Fwd: TCCAAGCGTGAACATCAGAG Rev: GCAACAGAGCGAGGAGTGAC	TAMRA

All of the loci have same PCR conditions and PCR solution concentrations which are given in table 2.5.3 and table 2.5.4 respectively.

Table 2.5.3: Concentration of the solutions in the PCR mixes for 8 microsatellite loci used in this study.

Solution	Concentration
dH_2O	8.1 µl
PCR Buffer	1 X
$MgCl_2$	2.25 mM
dNTP	0.25 mM
Fwd Primer	15 pmol
Rev Primer	15 pmol
Taq Polymerase	1 unit
DNA	3 ml
Total Volume	**20 µL**

Table 2.5.4: PCR conditions for 8 microsatellite loci used in this study.

Step	Temperature	Duration	Number of Cycles
Initial Denaturation	94°C	5 min	1X
Denaturation	94°C	45 sec	35X
Annealing	53°C	45 sec	
Extension	72°C	45 sec	
Final Extension	72°C	10 min	1X

All PCR results were controlled by 2.5% polymerized agarose gel stained with Et-Br under UV light as described in section 2.3.2. Successful amplifications were sized at REFGEN Gene Research and Biotechnology Limited Inc. (Ankara, Turkey) by using ROX GS500 internal size standard. Microsatellite peaks were analyzed with Peak Scanner (Applied Biosystems).

2.6 Statistical Analysis

2.6.1 Statistical Analysis of mtDNA Region

2.6.1.1 mtDNA Datasets

Partial mtDNA CR sequences obtained for this study were analyzed along with Mediterranean swordfish sequences from Alvarado Bremer et al.'s (2005b) with NCBI Accession No: AY650761-64, AY650768, AY650776-78, AY650781, AY650793-94, AY650805-06, AY650809-10, AY650812-17, AY650820-23, AY650825-29, AY650831-33, AY650836-44, AY650846-47, AY650849, AY650851, AY650866 and Viñas et al.'s (2010) with NCBI Accession No: EU827746, EU827749, EU827759, EU827762-71, EU827784-96, EU827798. Locations and sample sizes of all sequences used in this study are given in figure 2.6.1.1.1.

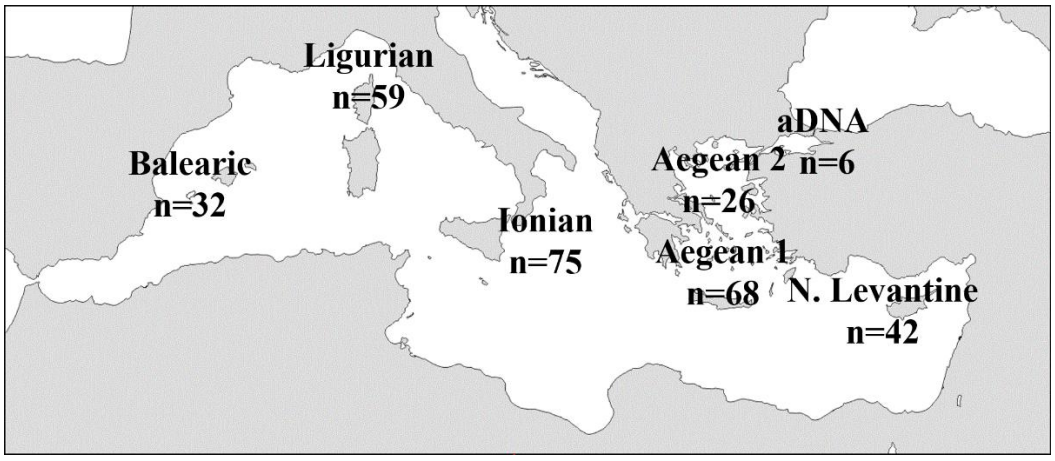

Figure 2.6.1.1: Locations and sample sizes of all sequences used in this study (n=sample size).

Sequences from Southern Aegean Sea, which were obtained from NCBI-GenBank were referred as Aegean 1 and samples collected for the present study, from the Northern Aegean Sea were referred as Aegean 2. Samples collected from Northern Levantine Sea was referred as N. Levantine.

Two different dataset were prepared with all sequences used in this study. First dataset was constructed by modern sequences, named as modern dataset (MD) and consists of 6 populations (total n=302). Second dataset was constructed by using both modern and ancient sequences, named as modern and ancient dataset (MAD) and consists of 7 populations (total n=308).

2.6.1.2 Determination of Nucleotide Substitution Model

Nucleotide substitution is the process of sequence evolution in time by nucleotide mutations. In order to determine the best nucleotide substitution model 6 core models were tested for with/without gamma distributed rate variation (G) and/or proportion of invariable sites (I). They are described below:

Jukes-Cantor Model (JC): This model assumes equal rate of substitution and equal base frequencies for all nucleotides. Higher rates of transitional substitutions are not corrected in this model (Jukes and Cantor, 1969).

Kimura 2-Parameter Model (K2): This model also assumes equal rate of substitution and equal base frequencies for all nucleotides, but it accounts different rates between transitions and transversions (Kimura, 1980).

Hasegawa-Kishino-Yano Model (HKY): This model assumes also variable base frequencies for all nucleotides and accounts different rates between transitions and transversions (Hasegawa et al., 1985).

Tamura-Nei Model (TN93): This model also assumes variable base frequencies all nucleotides and accounts different rates between transitions and transversions. In addition, it differentiates purine to purine and pyrimidine to pyrimidine transitions (Tamura and Nei, 1993).

General Time Reversible Model (GTR): This is the most general independent model. This model assumes symmetric variable rate of substitution and variable base frequencies for all nucleotides (Tavaré, 1986).

Tamura 3-Parameter Model (T92): This model assumes equal rate of substitution and equal base frequencies for all nucleotides, but it accounts different rates between transitions and transversions, similar to K2. Additionally, it also takes G+C-content bias into consideration (Tamura, 1992).

All these models were tested based on Bayesian Information Criterion (BIC) (Schwarz, 1978) and Akaike Information Criterion (AIC) (Akaike, 1974). Most appropriate model was the one with the smallest AIC and/or BIC.

In this study, most appropriate nucleotide substitution models were determined by MEGA v6 (Tamura et al., 2013).

2.6.1.3 Neighbor Joining (NJ) Tree and Clade Determination

Neighbor joining (NJ) tree is a type of phylogenetic tree that is constructed by fast clustering algorithm (Saitou and Nei, 1987). NJ trees are unrooted trees and they do not assume constant rate of evolution.

In the present study, NJ trees were constructed with MEGA v6 (Tamura et al., 2013) by using 1000 bootstraps. Clades of the samples sequenced in this study were determined by locating each sample on NJ tree and identifying its cluster.

2.6.1.4 Median Joining (MJ) Network

Median Joining (MJ) network (Bandelt et al., 1999) is a network that connects haplotypes to each other based on number and location of mutations that separates these haplotypes. It uses Kruskal's (1956) algorithm in order to determine minimum spanning trees by preferring short connections and Farris's (1970) maximum-parsimony (MP) heuristic algorithm order to adds new median vectors (in this case sequences).

In this study, haplotypes were identified by DnaSP v5 (Librado and Rozas, 2009), networks were constructed by NETWORK 4.6.1.0 (http://www.fluxus-engineering.com). Default values were used for MJ network construction (ε=0; weight of the sites=10).

2.6.1.5 Diversity Indices

2.6.1.5.1 Nucleotide Diversity (π)

Nucleotide diversity (π) is the average number nucleotide differences between two sequences per site, a genetic diversity measure that is independent from the length of the sequences. Its value and standard deviation can be calculated by the equations given in Nei (1987).

In this study all nucleotide diversities and their standard deviations were calculated by DnaSP v5 (Librado and Rozas, 2009).

2.6.1.5.2 Haplotype Diversity (H)

Haplotype diversity (H) is the measure of rareness of a haplotype, it is measured by calculating the probability of randomly selecting sequences with different haplotypes from a dataset. Its value and standard deviation can be calculated by the equations given in Nei (1987).

In this study haplotype diversities and their standard deviations were calculated by DnaSP v5 (Librado and Rozas, 2009).

2.6.1.6 Population Differentiation

2.6.1.6.1 Pairwise F_{ST}

Pairwise F_{ST} is an F-statistic that is used to measure proportion of total variance in allele/clade frequencies between populations. It can be calculated by the equation given in Nei (1977). F_{ST} has a value between '0-1'. If two populations are completely the same F_{ST} is equal to '0'. If two populations are completely different F_{ST} is equal to '1' (Allendorf and Luikart, 2007).

In this study, pairwise F_{ST} values and their significances were calculated by Arlequin v3.5 (Excoffier and Lischer, 2010). Their possible significant deviations from "0" were calculated with 10000 permutations.

2.6.1.6.2 PCA

Principal component analysis (PCA) is a type of multivariate analysis method that calculates genetic similarities between set of observations, within a space constructed by independent vectors (principle components), where vectors are combinations of

variables with different weights. As a result, total variation can be summarized in two or three dimensions, where first dimension explains the highest proportion of total variation, second dimension explains the next highest variation and the method keeps extracting new dimensions sequentially and independently, number of dimensions is related with the number of variables (Dytham, 2011; Jobling et al., 2014).

In this study, PCA graphs were constructed based on both genetic distances between individuals and mean genetic distances between populations by GenAlEx v6.4 (Peakall and Smouse, 2006).

2.6.1.6.3 Analyses of Molecular Variance (AMOVA)

Analyses of molecular variance (AMOVA) method that is analogous to analyses of variance (ANOVA), that is used to partition the total variance into various hierarchical levels and to test the significance of the differentiation within hierarchical levels by using ϕ statistics, an analogue of the F-statistics (Excoffier et al., 1992).

In this study, populations of MD were first assigned to one gene pool and then assigned to two gene pools as Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine). Proportion of variation and significance of the differentiation within regions and among regions were calculated with AMOVA, by using Arlequin v3.5 (Excoffier and Lischer, 2010) based on Tamura-Nei (Tamura and Nei, 1993) and pairwise differences with 10000 permutations.

2.6.1.7 Long Term Effective Population Sizes Parameter (Ɵ) and Migration Rates

Long term (historical) effective population size can be defined as effective population size of population based on the past tens to thousands of generations (Wang, 2005). Long term effective population size parameter Ɵ of populations and

migration rates (M) between populations can be estimated by applying coalescent approaches based on probability of the polymorphism patterns (Beerli and Felsenstein, 2001).

Long term Ne parameter Θ and M between populations based on mtDNA data were estimated by Bayesian inference (Beerli, 2006) of Migrate-n v 3.6 (Beerli and Felsenstein, 2001). UPGMA based start genealogy is used as starting genealogy with transition/transversion values calculated based on Tamura 3-Parameter (Tamura, 1992) model and empirical base frequencies. One long chain with 3 replicates were run with 100000 recorded steps, 100 increment and 100 000 burn in for each replicate. Standard Metropolis-Hastings algorithm (Metropolis et al., 1953; Hastings, 1970) was used for posterior distributions and priors were drawn from uniform distributions. Generation time of the swordfish is assumed to be 5 years (Palko et al., 1981). Since mtDNA mutation rate (μ) for swordfish is not available, a range of 10^{-6} to 10^{-8} per nucleotide per generation, that covers mtDNA μ estimates of bluefin tuna (Suzuki and Chow, 2015), was used to calculate a long term Ne with the equation $\theta=2Ne$, specifically indicating Ne calculated with μ of $2.45x10^{-7}$ per nucleotide per generation that has been conservatively defined for mtDNA CR-I of skipjack tuna and yellowfin tuna (Ely et al., 2005).

2.6.1.8 Demographic Analyses

2.6.1.8.1 Mismatch Distribution

Mismatch distribution is distribution of the of number of pairwise nucleotide differences between sequences under infinite sites model. Infinite sites model assumes a population at equilibrium with population size N_0 instantaneously expands t years ago and reaches to population size N_1 and remains in that size after that (Rogers and Harpending, 1992). Information about population demography, mainly about population expansion, can be inferred from the shape of this distribution of number of pairwise difference sequences. A bell shaped distribution shows a sudden population expansion from a single haplotype, meanwhile ragged multimodal

distribution shows a constant population size for long time period (Rogers and Harpending, 1992). Congruence of observed mismatch distribution to expected distribution based on sudden expansion model can be evaluated by raggedness index (r) calculated with equation given in (Rogers and Harpending, 1992), Significant r shows observed distribution does not fit to the sudden expansion model (Rogers and Harpending, 1992).

Parameters of the expansion, which are the tau (τ) and the magnitudes θ_0, and θ_1 of the expansion, can also be calculated by the mismatch distribution with the equation given in (Schneider and Excoffier, 1999). Tau (τ) estimates the time of expansion as mutation unit. θ_0, and θ_1 are the parameters of population size N_0 and N_1 respectively, magnitude of expansion can be assessed by the difference between these two parameters (Schneider and Excoffier, 1999).

In the present study, observed mismatch distributions, expected mismatch distribution based on sudden expansion model and its r value and demographic expansion parameters (θ_0, θ_1 and τ) were calculated by Arlequin v3.5 (Excoffier and Lischer, 2010). Significance of r value is examined by 10000 bootstraps.

2.6.1.8.2 Neutrality Tests

Neutrality tests are estimations of the departures from neutrality by observing diversity that is expected under neutral evolution. In the case for neutral markers it can be used to determine possible past expansions or contractions (Jobling et al., 2014). There are several neutrality test, Tajima'D (Tajima, 1989) Fu'Fs (Fu, 1997) and R2 (Ramos-Onsins and Rozas, 2002) were used in the present study.

Tajima's D test compares number of segregating sites and average number of nucleotide differences, two genetic variation measures, which are moments estimates that are expected to be the same in neutrally evolving populations that is at equilibrium between mutation and genetic drift, with constant size (Tajima, 1989). When Tajima's D is calculated with known neutral markers, significantly negative

Tajima's D indicates population expansion and significantly positive Tajima's D indicates decrease in population size (Tajima, 1989).

Fu's FS test evaluates the probability of observing a random sample with a number of alleles equal to or smaller than the observed value under the assumption of infinite-site model without recombination (Fu, 1997). Similar to the Tajima's D, when Fu's Fs is calculated with known neutral markers, significantly negative values indicate population expansion and significantly positive values indicate decrease in population size (Fu, 1997).

R2 estimates the differences between the number of singleton mutations and the average number of nucleotide differences with the equation given in (Ramos-Onsins and Rozas, 2002). Significant deviations from "0" indicates population expansion (Ramos-Onsins and Rozas, 2002).

In the present study, Tajima's D, Fu's Fs and R2 statistics and their statistics were calculated by DnaSP (Librado and Rozas, 2009) with 10000 coalescent simulations.

2.6.1.9 Continuity Analyses

Continuity analysis refers to testing possible continuity between sets of ancient and contemporary populations by generating coalescent simulations of serial samples drawn from populations with given complex evolutionary scenarios based on different type of genetic markers (Excoffier and Foll, 2011). The continuity between the populations can be determined by comparing F_{ST} values of actual populations and the F_{ST} values of simulated populations with the assumption: when most of the (for instance 95%) simulated F_{ST} values were lower than actual F_{ST}, actual F_{ST} cannot arise solely by drift.

Genetic continuity between ancient samples and the geographically closest modern populations (Aegean1, Aegean2 and Northern Levantine) was tested by serially simulating 1000 populations by using Fastsimcoal v2 (Excoffier et al., 2013) through

time under the assumption of exponential population decline. Negative exponential growth rate was calculated as the natural logarithm of the ratio of the population sizes of aDNA and modern populations. Ancient samples were dated over a wide range from 4th century AD, Early Byzantium to 10th century AD, Late Byzantium. Three different continuity simulations were run using 4th century for the earliest sample, 10th century for the latest sample and 7th century for weighted average of all samples. For mutation rate (μ), a range of 10^{-6} to 10^{-8} per nucleotide per generation that spans the mutation rate estimates of bluefin tuna (Suzuki and Chow, 2015), and $2.45x10^{-7}$ per nucleotide per generation that was defined for mtDNA CR-I of skipjack tuna and yellowfin tuna (Ely et al., 2005). Ne of the ancestral population (aDNA) is assumed to be 25000 based on the mtDNA long term effective population size estimates for aDNA, whereas Ne of the modern populations assumed to be between 50-100 based on Ne calculated from microsatellite data. Generation time of the swordfish is assumed to be 5 years (Palko et al., 1981). F_{ST} values of simulated populations were calculated by Arlsumstat v3.5 (Excoffier and Lischer, 2010) and calculated F_{ST} values were plotted by using MATLAB v8.0 (The MathWorks, Inc., Natick, Massachusetts, United States).

2.6.2 Statistical Analysis Microsatellite Loci

2.6.2.1 Reliability of the Data

2.6.2.1.1 Null Allele Frequencies

Null alleles emerge as genotyping errors that are usually caused by mutations at the binding site of the primer site. As a result, individuals those were falsely genotyped as homozygotes cause excess of homozygotes in the populations (Allendorf and Luikart, 2007). Therefore, diversity within a population can be underestimated (Paetkau and Strobeck, 1995) and genetic differentiation between populations can be overestimated (Paetkau et al., 1997). Null alleles can be detected by their own specific allelic features like deficiencies and excesses of particular genotypes (Chapuis and Estoup, 2007).

In this study, frequency of microsatellite null alleles is calculated with FreeNA (Chapuis and Estoup, 2007) by using expectation maximization algorithm (Dempster et al., 1977) and 10000 iterations.

2.6.2.1.2 Linkage Disequilibrium

Linkage disequilibrium is commonly described as "nonrandom association between pairs of alleles at different loci". Two loci are in linkage disequilibrium if their alleles are linked with each other and they are in linkage equilibrium if they are inherited independently (Allendorf and Luikart, 2007). Linkage disequilibrium can be measured by a coefficient which can be calculated by the equation given in Lewontin and Kojima, (1960). When D=0 it means alleles are in linkage equilibrium and D is not zero, it means alleles are in linkage disequilibrium (Allendorf and Luikart, 2007).

Linkage disequilibrium coefficient D of each pair of loci and its possible significant deviations from "0" were calculated by Genepop v4.5 (Rousset, 2008) with Markov chain parameters of 100000 dememorisation, 1000 batches and 5000 iterations per batch. Bonferroni correction was applied to critical significance levels (p) by dividing them with number of tests.

2.6.2.2 Genetic Diversity Indices

2.6.2.2.1 Number of Alleles (A) and Allelic Richness (Ar)

Total number of alleles in each locus is a commonly used measure of genetic diversity. This measure is more sensitive to loss of genetic variation than the loss of heterozygosity and also highly dependent to sample size. Because of that comparing the genetic diversity of populations with different sample sizes is not appropriate (Allendorf and Luikart, 2007).

Allelic richness (Ar) is another measure of allelic diversity which is defined as number of discrete alleles expected in a random sub-sample of size g drawn from the population (Petit et al., 1998). It uses rarefaction method to estimate number of alleles based on typically smallest sample size if there is more than one population with different sample sizes. Thus, dependence of A value to sample size can be avoided by using Ar (Allendorf and Luikart, 2007).

Number of alleles and allelic richness of 8 loci used in this study were calculated by FSTAT v2.9.3 (Goudet, 2001) for both populations.

2.6.2.2.2 Heterozygosity

Average expected heterozygosity (He) under the Hardy-Weinberg equilibrium state, is the most common measure within genetic diversity of a population. Expected heterozygosity is independent from sample size and can be measured with very low number of samples as long as high number of loci are genotyped (Allendorf and Luikart, 2007).

In this study, observed and expected heterozygosities of 8 loci were calculated by Arlequin v3.5 (Excoffier and Lischer, 2010) for both populations. Significance of differences between observed and expected heterozygosities were calculated with 1000000 Markov chain and 100000 dememorization steps. Bonferroni correction was applied to calculate critical significance levels (standard significance levels were divided by the number of tests).

2.6.2.3 Population Differentiation

2.6.2.3.1 Pairwise F_{ST}

Pairwise F_{ST} between Aegean 2 and N. Levantine based of microsatellite data was calculated by Arlequin v3.5 (Excoffier and Lischer, 2010). Its possible significant deviation from “0” was calculated with 10000 permutations.

2.6.2.3.2 Principal Component Analysis (PCA)

A PCA is constructed based on genetic distances between individuals based on microsatellite data by using GenAlEx v6.4 (Peakall and Smouse, 2006) for microsatellite data.

2.6.2.3.3 STRUCTURE Analysis

Genetic differentiation among and within populations can be searched by assigning individuals probabilistically to K (sometimes unknown) probable ancestral clusters with the assumption that when there is admixture, one individual may be assigned to two or more clusters (Mank and Avise, 2003).

There are different methods for estimating most probable K. One is the method proposed by, Evanno et al. (Evanno et al., 2005) where most probable K is the maximum value of the second order rate of change of the likelihood function with respect to K. Another method is proposed by Rosenberg et al. (2002) and according to this method the most probable K is the K where the similarity of the independent runs is at the highest.

In this study, individuals of Aegean 2 and N. Levantine were analyzed by STRUCTURE v2.3.4 (Pritchard et al., 2000). for 2-6 ancestral K with 30.000 MCMC steps, 30 000 burn-in length and 20 replications Replication were averaged and maximum value of the second order rate of change of the likelihood functions were calculated by Harvester v0.6 (Earl and VonHoldt, 2012). The similarity coefficients were calculated by the software CLUMPP v1.1 (Jakobsson and Rosenberg, 2007). The graphical images were constructed by Distruct v1.1 (Rosenberg, 2004).

2.6.2.4 Long Term Effective Population Size Parameter Θ

Long term Ne parameter θ of Aegean 2 and N. Levantine based on microsatellite data were estimated by Bayesian inference (Beerli, 2006) of Migrate-n v 3.6 (Beerli

and Felsenstein, 2001) with Brownian motion model. One long chain with 3 replicates were run with 100000 recorded steps, 100 increment and 100 000 burn in for each replicate. Standard Metropolis-Hastings algorithm (Metropolis et al., 1953; Hastings, 1970) was used for posterior distributions and priors were drawn from uniform distributions. Similar to the mtDNA, μ estimate for microsatellite are not available for swordfish. So, a range of 10^{-2} to 10^{-6} per nucleotide per generation that spans the mutation rate estimate used for Atlantic bigeye tuna (Gonzalez et al., 2008) is used to calculate a long term Ne range using the equation $\theta=4Ne\mu$.

2.6.2.5 Short Term Effective Population Size

Short term (contemporary) effective population size can be described as Ne of a population including the past one-to-few generations (Wang, 2005). It can be estimated by various different methods, for instance employing one of the following measures: linkage disequilibrium (LD), heterozygosity excess (He) and molecular coancestry (MC).

Linkage disequilibrium (LD) method estimates short term Ne based on level of nonrandom associations among alleles at different loci with the assumption that this non randomness can be unrevealed by genetic drift (Hill, 1981; Waples and Do, 2010). This method is susceptible to bias because different other process, such as sub-structuring, migration, and admixture can also generate linkage disequilibrium (Luikart et al., 2010).

Heterozygosity excess (HE) method estimates short term Ne based on the amount of observed heterozygosity excess with the assumption that finite sizes of populations will cause slight differences from the expected heterozygosities because of binomial sampling error (Pudovkin et al., 1996).

Molecular coancestry (MC) method estimates short term Ne based on the parameter of allele sharing among samples (Nomura, 2008).

In this study, short term Ne of Aegean 2 and N. Levantine based on LD, HE and MC methods were calculated using the program NeEstimator v2 (Do et al., 2014).

2.6.2.6 MSVAR Analysis

MSVAR method uses coalescent theory to estimate posterior probability distribution of the short term Ne, long term Ne and time since the demographic change based on full-likelihood with Bayesian and with the following assumption; population varies in size either linearly or exponentially (Beaumont, 1999; Storz and Beaumont, 2002).

In this study, ancestral and contemporary Ne values, possible population expansions and/or declines and the approximate date of these possible demographic events were estimated by the Bayesian based MSVAR method (Storz and Beaumont, 2002). Analyses were run with 2000000 step and 10,000 burn-in. Generation time of the swordfish is assumed to be 5 years (Palko et al., 1981) and μ was assumed to be 10^{-4} per nucleotide per generation used for Atlantic big eye tuna on microsatellites (Gonzalez et al., 2008).

CHAPTER 3

RESULTS

3.1 Results of DNA Extraction

3.1.1 Modern Samples

DNA from 43 modern swordfish samples were extracted as described in section 2.2. Presence and the quality of the extracted DNA were controlled with agarose gel electrophoresis. Result of 6 samples is given in figure 3.1.1.1.

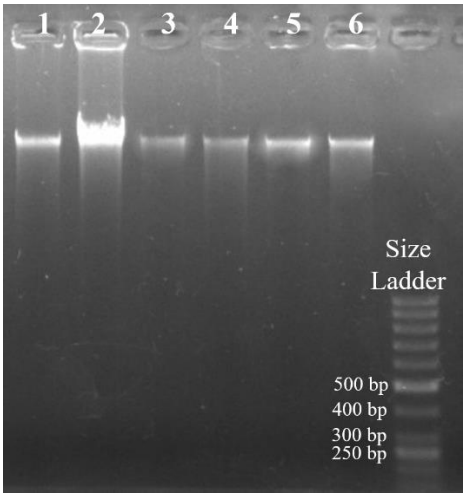

Figure 3.1.1.1: Agarose gel image of extracted DNA. Numbers above the wells are sample numbers. Last well contains DNA size ladder.

On the gels, bands of samples with no smears were considered as DNA bands presenting potentially successful DNA extractions. Their quality and concentrations were further measured by Nanodrop Spectrophotometer. Samples that have values of 1.80 for 260/280 nm ratio and value of 2.00 for 260/230 nm ratio (Desjardins and Conklin, 2010) were used for PCR.

3.1.2 Ancient Samples

Since amount of DNA is very low in ancient samples, extracted DNA was not controlled with agarose gel electrophoresis, they were directly used for PCR.

3.2 Results of PCR

3.2.1 Partial mtDNA CR of Modern Samples

Partial mtDNA CR of modern samples, which is approximately 420 bp long, were amplified by PCR and results were visualized by agarose gel electrophoresis. A blank- negative control that has PCR mixture without DNA was also used, in order to identify possible contaminations in the PCR reaction. Figure 3.2.1.1 represents PCR results of 8 samples.

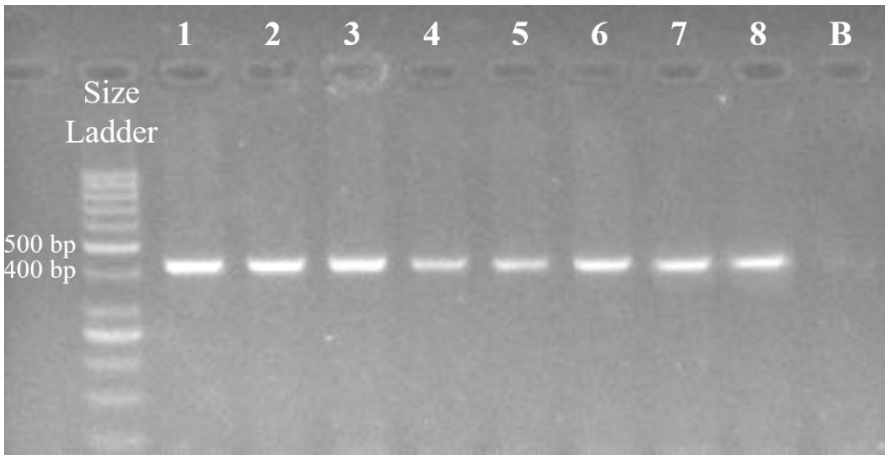

Figure 3.2.1.1.: Agarose gel image of modern partial mtDNA CR PCR. Numbers above the wells are sample numbers. First well contains DNA size ladder, last well contains blank- negative control (B).

Samples that have bands with length between 400-500bp based on DNA size ladder with clean negative control were considered successfully amplified and used for sequencing.

3.2.2 Partial mtDNA CR of Ancient Samples

Partial mtDNA CR of ancient samples were amplified by PCR in 5 overlapping fragments with primers given in section 2.3.2 and designed for this study. Results were controlled by agarose gel electrophoresis. Two blank negative controls were used during PCR in order to identify possible contaminations in the PCR reaction and during DNA extraction. First one was named extraction blank, it has extraction chemicals but no sample and the other one was named PCR blank, it has PCR mixture but no DNA. Figure 3.2.2.1 represents PCR results of 6 ancient samples for 5 fragments.

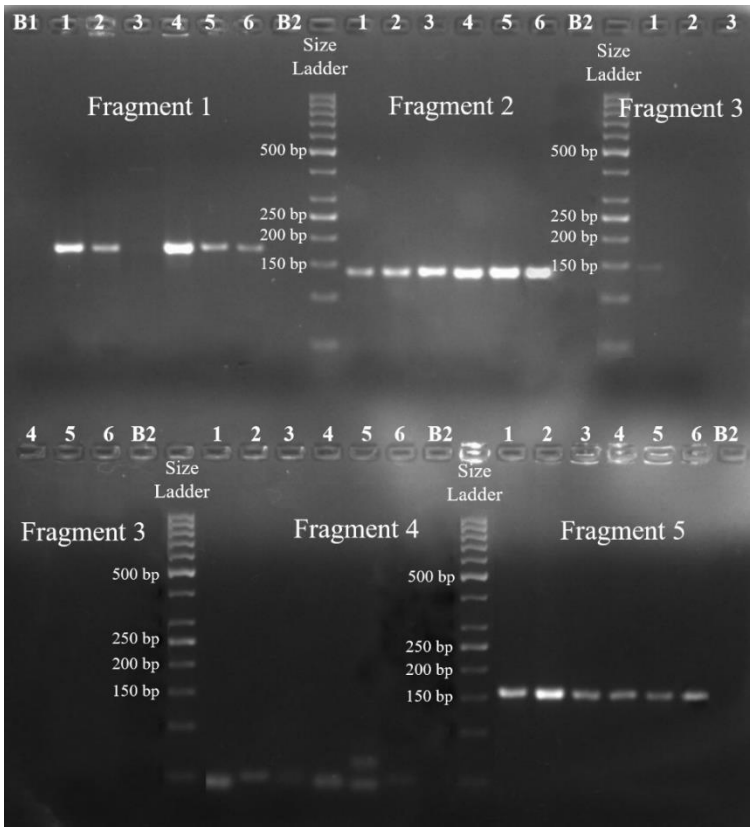

Figure 3.2.2.1: Agarose gel image of 5 ancient partial mtDNA CR fragments. Numbers above the wells are sample numbers. Well labeled as B1 is extraction blank and wells labeled as B2 are PCR blanks. Wells at the end of each fragment set contain DNA size ladder.

Samples that have bands with lengths given in section 2.3.2 based on DNA size ladder with clean negative controls were considered successfully amplified and used for sequencing.

3.2.3 Microsatellite Loci of Modern Samples

Eight microsatellite loci of modern samples were amplified by PCR and results were controlled by agarose gel electrophoresis A blank negative control that have PCR mixture without DNA was also used, in order to identify possible contaminations in the PCR reaction. Figure 3.2.3.1 represents PCR results of 5 microsatellite loci (Xg41b, Xgl-561, Xgl-51, Xgl-536 and XgSau98R1) for 2 samples.

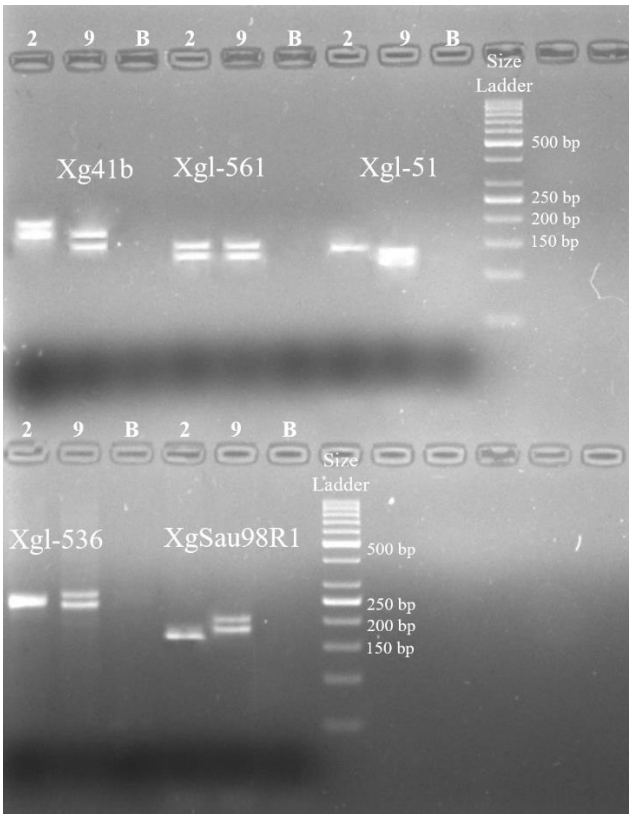

Figure 3.2.3.1: Agarose gel image of 5 microsatellite loci for 2 samples. Numbers above the wells are sample numbers. Wells labeled as B are blank negative control, the last wells in each row contain DNA size ladder.

3.3 Sequencing

After amplification partial mtDNA CR of modern and ancient samples were sequenced in both forward and reverse directions. Resulted forward and reverse chromatograms were assembled by Geneious v8 (Kearse et al., 2012, www.geneious.com). Part of the assembled forward and reverse chromatograms of a modern N. Levantine sample is shown in figure 3.3.1.

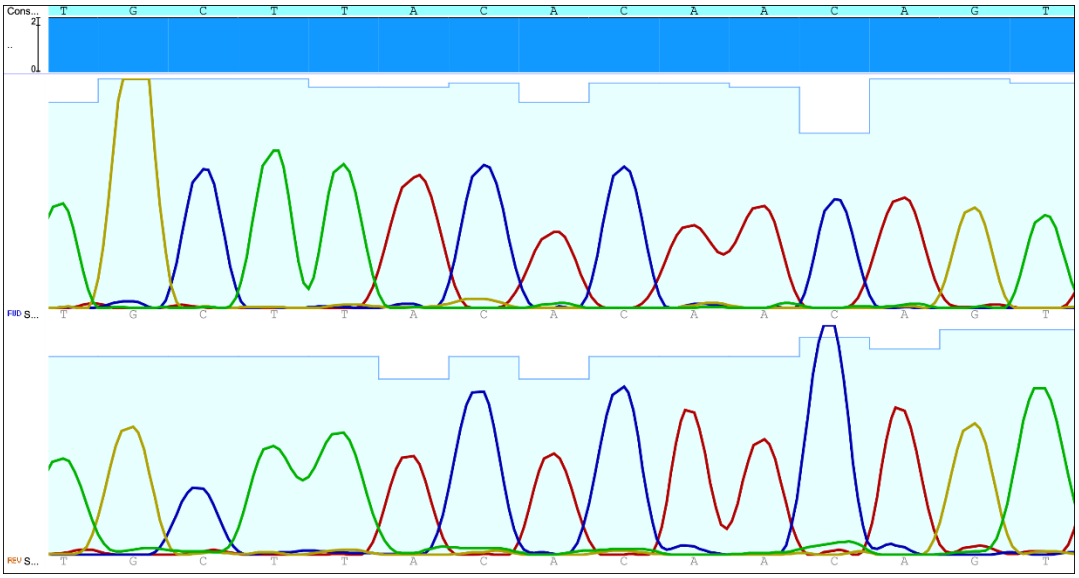

Figure 3.3.1: The part of assembled chromatograms of forward and reverse sequences of a N. Levantine sample. Consensus sequence is represented at the top the chromatogram.

For aDNA analyses, three forward and three reverse sequences of each fragment, obtained from three PCR's of two separate aDNA extractions were not assembled together, they were examined separately in order to detect possible postmortem modifications (explained in more detail at section 1.8).

3.4 Authenticity of aDNA Samples

In order to verify authenticity of the aDNA sequences, nucleotide substitutions between different PCR amplicons of the same sample that indicate postmortem misincorparation were searched and a few of them were observed. These nucleotide substitutions are summarized in table 3.4.1.

Table 3.4.1. Types of nucleotide changes that were observed between different PCR amplicons of the same sample.

Sample No	C-T	G-A	Indels	Other
YK1	1	-	-	1
YK2	1	1	-	-
YK3	1	-	1	-
YK4	-	-	-	1
YK5	-	-	1	-
YK6	-	1	1	-

3.5 Alignment and Trimming of mtDNA

All sequences used in this study were aligned together and trimmed based on the shortest sequence by using BioEdit v7 (Hall, 1999). For MD, sequences were trimmed to 301 bp. For modern and ancient dataset (MAD), fragment 1 of CR could not be amplified from 2 ancient samples, so this fragment was trimmed from all samples. Since 6 bp of the fragment 2 in sample YK6 and 25 bp of the fragment 4 in samples YK2 and YK6 were low in quality, these regions were completely removed from the analyses. Consequently, final length of the mtDNA sequences for ancient dataset were reduced to 194 bp.

3.6 Microsatellite Genotyping

Eight microsatellite loci analyzed in this study were genotyped in one read by Peak Scanner v1.0 (www.appliedbiosystems.com). Figure 3.6.1 represents 8 electropherogram of microsatellite loci of one sample.

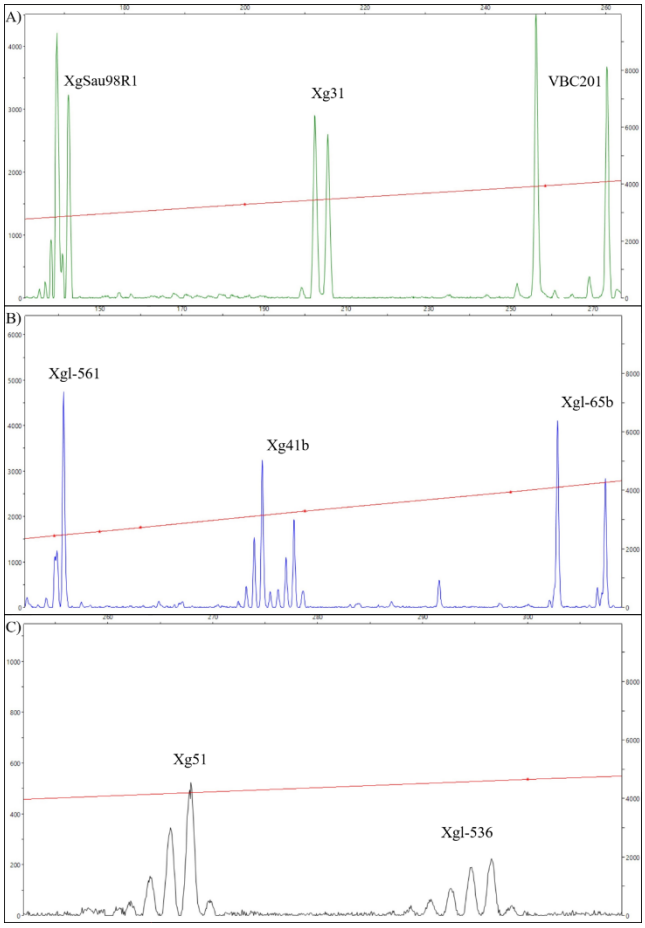

Figure 3.6.1: Microsatellite electropherogram representing microsatellite loci: A) XgSau98R1, Xg31, VBC201 B) Xgl-561, Xg41b, Xgl-65b C) Xg51, Xgl-536. Names of the loci are indicated above their corresponding peaks.

According to the figure 3.6.1, loci XgSau98R1, Xg31, VBC201, Xg41b and Xgl-65b are heterozygotes, Xgl-561, Xg51 and Xgl-536 are homozygotes.

Two of the N. Levantine samples failed to yield any results so these samples were omitted and number of N. Levantine populations for microsatellite analysis dropped to 40. Results of all microsatellite loci are given in appendix B.

3.7 Results of the Statistical Analysis

3.7.1 mtDNA Data

3.7.1.1 Nucleotide Substitution Models

Best nucleotide substitution models of MD and MAD were selected by using MEGA v6 (Tamura et al., 2013) according to the both BIC (Schwarz, 1978) and AIC (Akaike, 1974). based on entire sequences and based on clades separately. Results are summarized in table 3.7.1.1.1.

Table 3.7.1.1.1: Best nucleotide substitution models of A) MD based on entire sequences, B) MD based on CI, C) MD based on CII, D) MAD based on entire sequences, E) MAD based on CI, F) MAD based on CII, selected according to the BIC (Schwarz, 1978) and AIC (Akaike, 1974). Gamma parameter (G) values were given in parenthesis when relevant.

	MD	
	AIC	**BIC**
A) Entire Sequences	T92+G(0.32)	GTR +G(0.32)
B) CI	T92+G(0.54)	GTR+ G(0.48)
C) CII	T92	TN93+G(0.26)
	MAD	
	AIC	**BIC**
D) Entire Sequences	K2+G(0.45)	GTR +G(0.45)
E) CI	K2	GTR +G(0.55)
F) CII	K2	TN93+G(0.27)

Nucleotide substitution models selected based on both BIC and AIC were used for F_{ST} and AMOVA analyses. Since results were very similar, only results of analyses performed based on BIC nucleotide substitution models were given in this study.

3.7.1.2 Clade and Sub-Clade Identification by Neighbor Joining (NJ) Trees

Clades and sub-clades of modern samples and ancient samples were determined based on neighbor joining (NJ) trees constructed by employing sequences from Alvarado Bremer et al.'s (2005a) and Viñas et al.'s (2010) studies as given in section 2.6.1.1, together with modern and ancient sequences of the present study, by using MEGA v6 (Tamura et al., 2013), with T92 (Tamura, 1992) +G (0.32) for MD and K2 (Kimura, 1980) +G (0.45) for MAD as explained in section 3.7.1.1. These NJ trees are given in figure 3.7.1.2.1.

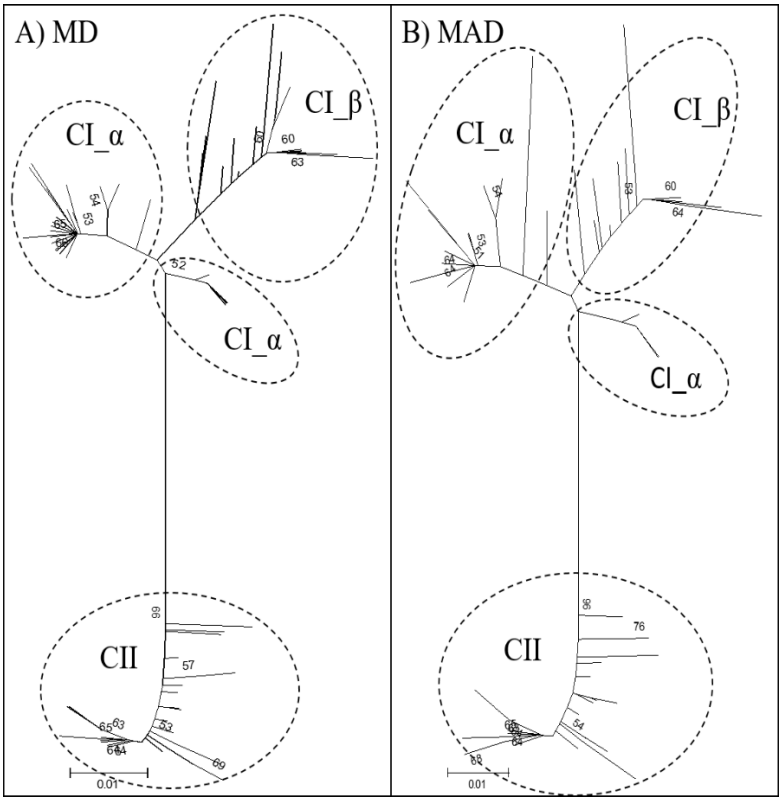

Figure 3.7.1.2.1: NJ trees constructed with A) MD B) MAD. Clade clusters based on the sequences of the Alvarado Bremer et al.'s (2005a) and Viñas et al.'s (2010) are shown in circles. Numbers at the nodes are bootstrap values. Bootstrap values lower than 50% are not shown.

According to the NJ tree of MD, all samples were clustered either with CI_α, CI_β or CII_θMed sub-clade of clade CII. Fourteen Aegean 2 samples were clustered with CI_α, 3 of them were clustered with CI_β and 9 of them were clustered with CII. Twenty N. Levantine samples were clustered with CI_α, 8 of them were clustered with CI_β and 14 of them were clustered with CII. Also, CI_α found to be represented in two separate clusters. According to the NJ tree of MAD, all samples were clustered either with CI_α, CI_β or CII_θMed sub-clade of clade CII. Three of the samples were identified as CI_α, one of them was identified as CI_β and the other two were identified as CII. Clades and sub-clades of samples are given in appendix C.

3.7.1.3 Median Joining (MJ) Network

Two MJ networks were created in order to visualize haplotype relationships between samples of MD and MAD respectively by using Network v5 (fluxus-engineering.com). MJ of MD is represented in figure 3.7.1.3.1 and MJ of MAD is represented in figure 3.7.1.3.2.

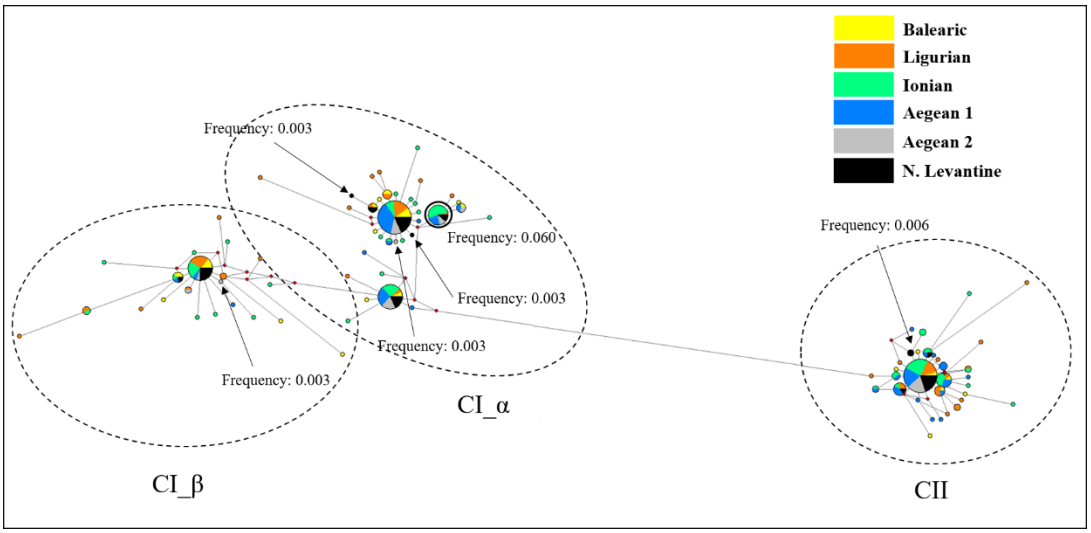

Figure 3.7.1.3.1: MJ network constructed using MD. Nodes representing the haplotypes are proportional to sample sizes and red nodes indicate medians. Arrows show private haplotypes observed in the present study. Frequencies of these private haplotypes are given by the arrows. One haplotype that is mostly represented by populations in the Eastern Mediterranean (Ionian, Aegean 1, Aegean 2 and Northern Levantine) is shown in a circle. Frequency of the haplotype is given near the node.

Overall, 88 haplotypes were defined from MD. CI was represented with three main nodes (two for CI_α and one for CI_β) and CII was represented with one main node. Based on MD, 5 private haplotypes of Aegean 2 and N. Levantine were defined for the first time. Among two private haplotypes of Aegean 2, one of them was a CI_α sequence and the other one was a CI_β sequence. Both haplotypes were represented with one sample and separated from their respective main nodes by one mutation. Two of the 3 private haplotypes of N. Levantine belong to CI_α and each of them were represented with one sample. One of them was separated from its respective main node by one mutation and the other one was separated from its respective main node by two mutations. Last private haplotype of N. Levantine belongs to CII. It was represented by two samples and separated from its respective main node by one mutation.

The CI_α haplotype is observed (indicated with a black circle in figure 3.6.1.3.1) with relatively high frequency (0.060 within entire dataset, 0.095 within CI) in 17

samples from the Eastern Mediterranean (Ionian, Aegean 1, Aegean 2 and Northern Levantine) populatıons and one sample from Ligurian population. However, Ligurian sample might be different from other Eastern sequences because it has two nucleotides marked as unknown. If Ligurian haplotype is different than the haplotype of this node it can be said that it is the private node of Eastern Mediterranean.

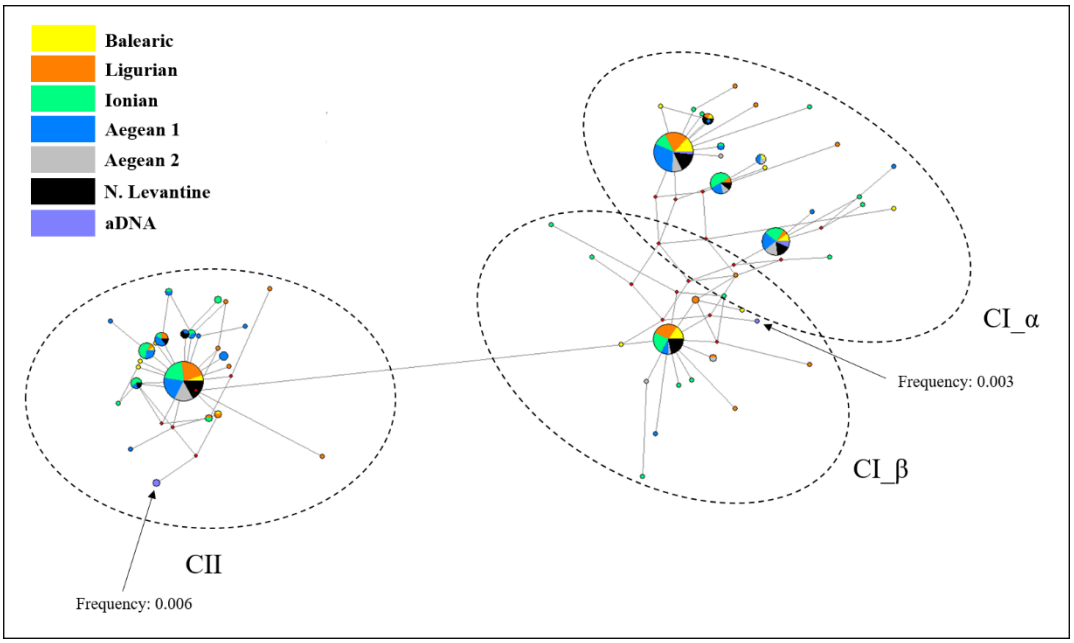

Figure 3.7.1.3.2: MJ network constructed using samples of MAD. Nodes representing the haplotypes are proportional to sample sizes and red nodes indicate medians. Arrows show private aDNA haplotypes observed in the present study. Frequencies (in the MAD) of these private aDNA haplotypes are given near arrows.

Overall, 67 haplotypes were defined from MAD. aDNA population was represented by 4 haplotypes and 2 of them were private. One of the private haplotypes belongs to CI_β. It is represented by one sequence and separated from its respective main node by four mutations. The other one belongs to CII. It is represented by two sequences and separated from its respected main node by three mutations.

Number haplotypes number of private haplotypes and the ratio of samples that have private haplotype with respect to total number of sequences are given in table 3.7.1.3.1.

Table 3.7.1.3.1: Number of haplotypes, number of private haplotypes and frequency of private haplotypes in Mediterranean A) based on entire sequences, B) based on CI C) based on CII (Htp: Haplotype, Freq: Frequency).

	# of Htp's	**# of Private Htp's**	**Freq. of Private Htp's**
A) Entire Sequences			
Balearic	21	11	0.04
Ligurian	33	21	0.08
Ionian	35	21	0.08
Aegean 1	25	11	0.04
Aegean 2	9	2	0.01
N. Levantine	12	3	0.01
B) CI			
Balearic	14	7	0.04
Ligurian	20	13	0.07
Ionian	24	17	0.09
Aegean 1	11	4	0.02
Aegean 2	8	2	0.01
N. Levantine	8	2	0.01
C) CII			
Balearic	7	4	0.04
Ligurian	13	8	0.08
Ionian	11	4	0.04
Aegean 1	14	7	0.08
Aegean 2	1	0	0.00
N. Levantine	4	1	0.02

The highest number of private alleles were found in Ionian and Ligurian for entire sequences and for CI and in Aegean 1 for CII, indicating possible isolation of these populations from the other populations. In total, CI (45) had higher number of private haplotypes than CII (24).

3.7.1.4 Sub-Clade Frequency Distribution of the Swordfish Samples

Since figure 3.7.1.3.1 indicated a substantial differentiation in CI as Cl_α and Cl_β, whenever possible, clades are examined over the sub-clade level. Sub-clade frequencies of 6 modern populations and 1 ancient population are displayed as pie charts on a Mediterranean map as represented in figure 3.7.1.4.1.

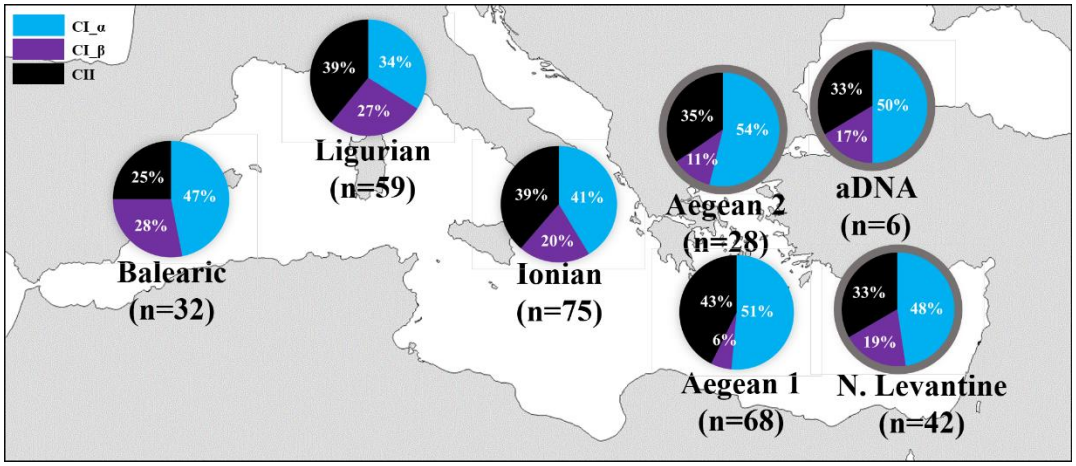

Figure 3.7.1.4.1: Sub-clade frequencies of modern and ancient populations represented as pie charts on a Mediterranean map. Pie charts that represent populations of this study are indicated by grey circles. Other populations are from Alvarado Bremer et al.'s (2005a) and Viñas et al.'s (2010) study.

According to the frequency distributions, CI_β frequency is relatively high in populations at the western side of Mediterranean (28% and 27% for Balearic and Ligurian respectively) than the eastern side of Mediterranean (6% for Aegean 1, 11% for Aegean 2 and 19% for N. Levantine). Significance of frequency differences in sub-clades were tested by using X^2 goodness of fit tests. Results are given in table 3.7.1.4.1.

Table 3.7.1.4.1: Pairwise X^2 values of goodness of fit test between populations based on sub-clade frequencies. Significant results are highlighted with grey.

	Balearic	**Ligurian**	**Ionian**	**Aegean 1**	**Aegean 2**	**N. Levantine**
Balearic	-					
Ligurian	2.11	-				
Ionian	2.03	1.20	-			
Aegean 1	10.20**	11.40**	6.28*	-		
Aegean 2	2.50	3.85	1.54	1.13	-	
N. Levantine	1.08	2.05	0.46	4.78	0.69	-
aDNA	0.40	0.67	0.17	1.07	0.12	0.02

*0.05>p**0.01>p***0.001>p

Results of goodness of fit test showed Aegean 1 has sub-clade frequency that is significantly different from Balearic, Ligurian and Ionian.

3.7.1.5 Principle Component Analyses (PCA)

Principle component analyses (PCA) were first performed based on genetic distances between individuals and mean genetic distances between populations of MD and MAD by using GenAlEx v6.4 (Peakall and Smouse, 2006), based on entire sequences and based on clades separately. PCA results based on genetic distances between individuals are given in figure 3.7.1.5.1 and PCA results based on mean genetic distances between populations are given in figure 3.7.1.5.2.

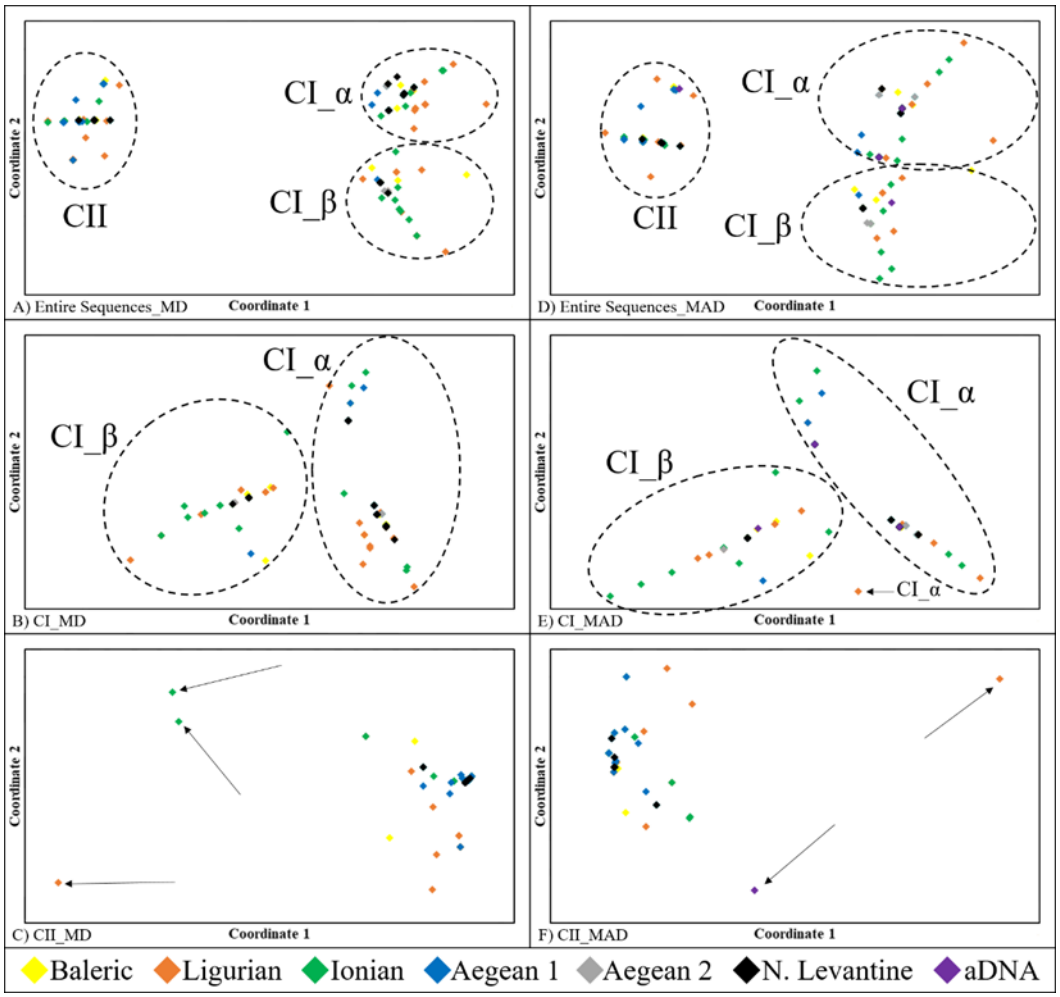

Figure 3.7.1.5.1: PCA analyses of genetic distances between individuals of A) MD based on entire sequences, B) MD based on CI, C) MD based on CII, D) MADD based on entire sequences, B) MAD based on CI, C) MAD based on CII. Outliers are indicated by arrows.

In figure 3.7.1.5.1.1A, first coordinate explains 85.32% and second coordinate explains 9.82% of the total variation. In figure 3.7.1.5.1.1B, first coordinate explains 64.11% and second coordinate explains 17.07% of the total variation. In figure 3.7.1.5.1.1C, first coordinate explains 47.68% and second coordinate explains 15.31% of the total variation. In figure 3.7.1.5.1.1D, first coordinate explains 66.58% and second coordinate explains 33.20% of the total variation. In figure 3.7.1.5.1.1E, first coordinate explains 64.74% and second coordinate explains 35.26% of the total

variation. In figure 3.7.1.5.1.1F, first coordinate explains 98.89% and second coordinate explains 0.88% of the total variation.

Individuals in the PCA of entire sequences were clustered based on their sub-clades and individuals in PCA of CI_α were clustered into two separate groups with an outlier that belongs to haplotype private to Ligurian. Meanwhile individuals in PCA of CI_β and CII were mostly clustered together except 2 CI_β outliers and 3 CII outliers who belong to haplotypes private to Ligurian and Ionian populations. Similar to the PCA results of MD, individuals of MAD in the PCA of entire sequences were clustered based on their sub-clades. Individuals in PCA of CI were clustered into two separate groups based on subclades CI_α and CI_β, except a CI_α outliner that belongs to haplotype private to Ligurian. Meanwhile individuals in PCA of CII were mostly clustered together except 2 outliers belong to haplotype private to in Ligurian and aDNA populations respectively.

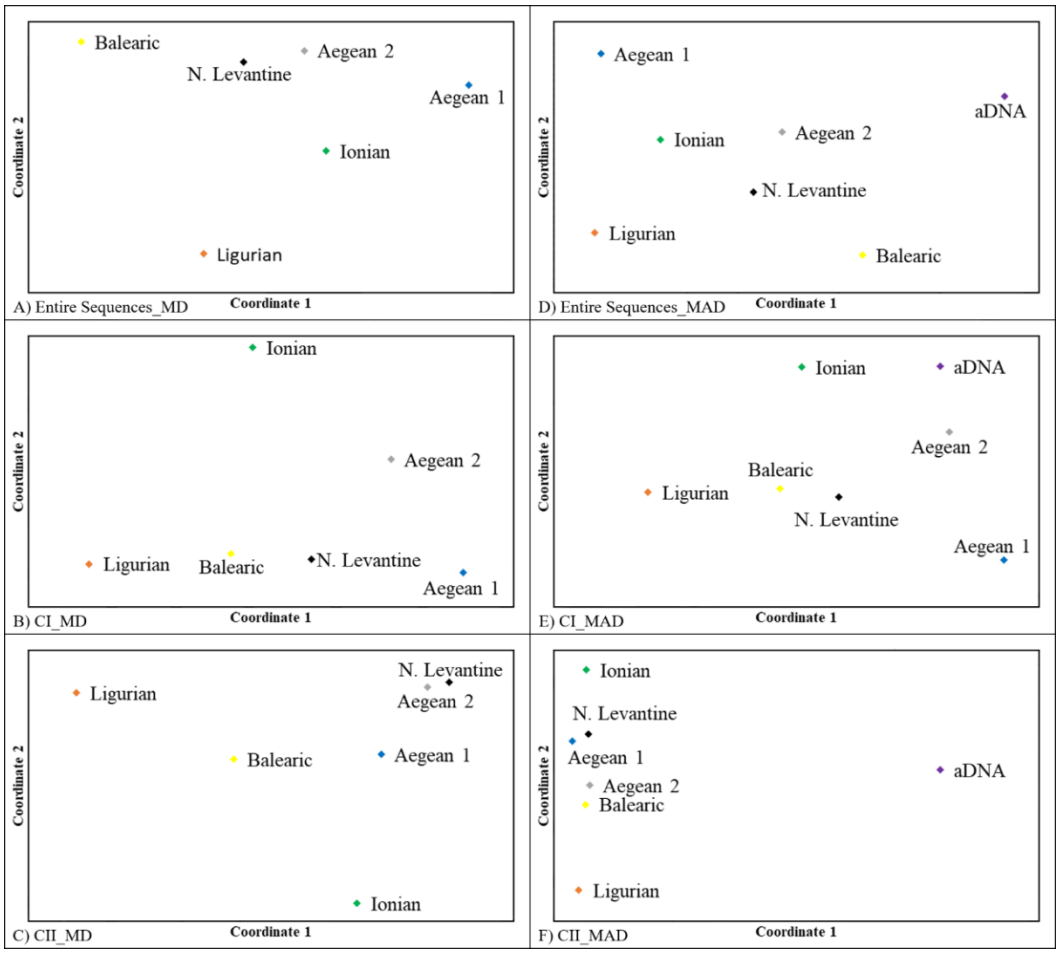

Figure 3.7.1.5.2: PCA analyses of mean genetic distances between populations of A) MD based on entire sequences, B) MD based on CI, C) MD based on CII, D) MADD based on entire sequences, B) MAD based on CI, C) MAD based on CII.

In figure 3.7.1.5.2.1A, first coordinate explains 68.56% and second coordinate explains 31.44% of the total variation. In figure 3.7.1.5.2.1B, first coordinate explains 83.78% and second coordinate explains 16.22% of the total variation. In figure 3.7.1.5.2.1C, first coordinate explains 84.70% and second coordinate explains 14.82% of the total variation. In figure 3.7.1.5.2.1D, first coordinate explains 66.58% and second coordinate explains 33.20% of the total variation. In figure 3.7.1.5.2.1E first coordinate explains 64.74% and second coordinate explains 35.26% of the total variation. In figure 3.7.1.5.2.1C first coordinate explains 98.89% and second coordinate explains 0.88% of the total variation.

According to the PCA of entire sequences of MD, Aegean 2 and N. Levantine were placed close to each other meanwhile rest of the populations were placed separately. In the PCA of CII, populations were placed relatively distant and Ionian was placed further away from the rest. Lastly, based on PCA of CII, Aegean 2 and N. Levantine were placed closer to each other, similar to PCA with entire sequences, but this time Aegean 1 was closer to them from the rest of the populations.

In PCA's based on entire sequences and CII of MAD, aDNA population was placed farther away than the rest of the populations. In PCA, based on CI, aDNA population was placed relatively closer to Ionian and Aegean 2.

3.7.1.6 Pairwise F_{ST}

Pairwise F_{ST} values between populations in the MD and MAD were calculated by using Arlequin v3.5 (Excoffier and Lischer, 2010), based on entire sequences and based on sequences of CI and CII. Results of MD are given in table 3.7.1.6.1 and results of MAD are given in table 3.7.1.6.2.

Table 3.7.1.6.1: Pairwise F_{ST} values between populations of MD calculated with A) entire sequences, B) CI, D) CII. Significant results are highlighted with grey.

A) Entire Sequences	**Balearic**	**Ligurian**	**Ionian**	**Aegean 1**	**Aegean 2**
Balearic					
Ligurian	0.008				
Ionian	0.010	-0.008			
Aegean 1	0.033	0.010	0.000		
Aegean 2	-0.013	-0.008	-0.019	-0.014	
N. Levantine	-0.013	-0.010	-0.011	-0.001	-0.027
B) CI	**Balearic**	**Ligurian**	**Ionian**	**Aegean 1**	**Aegean 2**
Balearic					
Ligurian	0.016				
Ionian	0.013	0.009			
Aegean 1	0.056*	0.122**	0.060**		
Aegean 2	0.006	0.052	-0.011	-0.010	
N. Levantine	0.029	0.003	-0.011	0.026	-0.022
C) CII	**Balearic**	**Ligurian**	**Ionian**	**Aegean 1**	**Aegean 2**
Balearic					
Ligurian	0.003				
Ionian	0.003	0.023			
Aegean 1	0.015	0.015	0.007		
Aegean 2	0.013**	-0.027	0.007	-0.013	
N. Levantine	0.044	0.000	0.010	0.002	-0.002

*0.05>p **0.01>p ***0.001>p

No significant differentiations between any of the populations were detected in the MD based on the analysis of entire sequences. According to the F_{ST} results of clades, Aegean1 was significantly different from Balearic, Ligurian and Ionian based on CI. In addition, Aegean 2 was significantly different from Balearic based on CII.

Table 3.7.1.6.2: Pairwise F_{ST} values between populations in the MAD calculated with A) entire sequences, B) CI, C) CII. Significant results are highlighted with grey.

A) Entire Sequences	Balearic	Ligurian	Ionian	Aegean 1	Aegean 2	N. Levantine
Balearic						
Ligurian	0.004					
Ionian	0.008	-0.007				
Aegean 1	0.028	0.008	0.001			
Aegean 2	-0.015	-0.011	-0.019	-0.014		
N. Levantine	-0.014	-0.013	-0.010	-0.001	-0.026	
aDNA	0.016	0.041	0.019	0.033	0.009	0.016
B) CI	**Balearic**	**Ligurian**	**Ionian**	**Aegean 1**	**Aegean 2**	**N. Levantine**
Balearic						
Ligurian	0.018					
Ionian	0.011	0.013				
Aegean 1	0.046*	0.098**	0.052*			
Aegean 2	0.015	0.042	-0.016	-0.010		
N. Levantine	0.033	-0.003	-0.008	0.017	-0.023	
aDNA	0.013	0.076	0.028	0.080*	0.062	0.008
C) CII	**Balearic**	**Ligurian**	**Ionian**	**Aegean 1**	**Aegean 2**	**N. Levantine**
Balearic						
Ligurian	0.000					
Ionian	0.007	0.022				
Aegean 1	0.004	0.013	0.006			
Aegean 2	0.013**	0.026	0.017	-0.009		
N. Levantine	0.037	0.001	0.014	0.005	-0.002	
aDNA	0.698*	0.677**	0.663**	0.693**	1.000*	0.845**

*0.05>p **0.01>p ***0.001>p

According to the F_{ST} results of MAD, no significant differentiation could be seen between aDNA and any of the modern populations based on the analysis of entire sequences. According to the F_{ST} results of clades, aDNA was significantly different from Aegean 1 based on CI analysis and significantly different from all modern populations based on CII analysis. Additionally, Aegean 1 was different Balearic Ligurian and Ionian based on CI analysis and Balearic was different from Aegean 2

based on CII analysis when modern populations in the context of MAD was considered.

3.7.1.7 AMOVA

Several AMOVA analyses were performed on MD. First, all populations were assigned to one gene pool and then populations were assigned to two gene pools as Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) All AMOVA's were calculated by both Tamura Nei (Tamura and Nei, 1993) model and pairwise difference model by using Arlequin v3.5 (Excoffier and Lischer, 2010). AMOVA tests were constructed based on entire sequences and clades separately. Results of the AMOVA tests were given in table 3.7.1.7.1.

Table 3.7.1.7.1: Variance component (VC), percent of variation (%V) and ϕ_{ST} fixation index (FI) values of AMOVA tests of MD for among regions and within regions calculated based on A) entire sequences B) CI and C) CII. Significant results are highlighted with grey.

All Populations (One Gene Pool)						
A) Entire Sequences	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	-0.014	-0.20	-0.002 p=0.511	0.009	0.13	0.001 p=0.370
Within Regions	6.810	100.20		6.469	99.87	
B) CI	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.060	2.11	0.021 p=0.058	0.082	2.78	0.028* p=0.025
Within Regions	2.804	97.89		2.848	97.22	
C) CII	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.370	0.87	0.009 p=0.179	0.018	2.25	0.023* p=0.018
Within Regions	0.650	99.13		0.799	97.75	
Western-Eastern (Two Gene Pools)						
D) Entire Sequences	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.021	0.31	0.003 p=0.229	0.060	0.91	0.009 p=0.108
Within Regions	6.790	99.69		6.450	99.09	
E) CI	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.083	2.85	0.028* p=0.023	0.124	4.14	0.041** p=0.005
Within Regions	2.817	97.15		2.862	95.86	
F) CII	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.008	1.19	0.012 p=0.083	0.036	4.35	0.043*** p=0.000
Within Regions	0.651	98.81		0.799	95.65	

*0.05>p **0.01>p ***0.001>p

According to the AMOVA test based on Tamura-Nei (Tamura and Nei, 1993) model, when entire sequences were considered, differentiation neither between populations nor between Eastern and Western Mediterranean were significant. Only significant differentiation was in CI between Eastern and Western Mediterranean populations. When AMOVA was carried out based on pairwise differences, at least one population was different from the others for CI and CII, furthermore Eastern and

Western Mediterranean populations were significantly differentiated both in CI and CII.

AMOVA tests were repeated by excluding Aegean 1 from datasets and results are given in table 3.7.1.7.2.

Table 3.7.1.7.2: Variance component (VC), percent of variation (%V) and ϕ_{ST} fixation index (FI) values of AMOVA tests of MD without Aegean 1, for among regions and within regions calculated based on A) entire sequences, B) CI, C) CII. Significant results are highlighted with grey.

All Populations (One Gene Pool)						
A) Entire Sequences	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	-0.051	-0.76	-0.008	-0.025	-0.39	-0.004
Within Regions	6.824	100.76	p= 0.727	6.523	100.39	p= 0.566
B) CI	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	-0.011	-0.35	-0.004	0.012	0.37	0.004
Within Regions	3.018	100.35	p=0.524	3.068	99.63	p=0.326
C) CII	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.006	0.96	0.010	0.024	2.91	0.029*
Within Regions	0.633	99.04	p= 0.215	0.792	97.09	p=0.016
Western-Eastern (Two Gene Pools)						
D) Entire Sequences	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.021	0.31	0.003	0.060	0.91	0.010
Within Regions	6.838	99.69	p=0.229	6.451	99.09	p=0.102
E) CI	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.032	1.07	0.011	0.064	3.04	0.021
Within Regions	2.994	98.93	p=0.140	3.046	97.93	p=0.054
F) CII	**Tamura-Nei**			**Pairwise Difference**		
	VC	**%V**	**FI**	**VC**	**%V**	**FI**
Among Regions	0.007	1.04	0.010	0.038	4.57	0.043***
Within Regions	0.635	98.96	p=0.126	0.792	95.43	p=0.000

*0.05>p **0.01>p ***0.001>p

When Aegean 1 was excluded from the AMOVA analysis previously observed differentiation in CI could not be observed neither based on Tamura-Nei (Tamura and Nei, 1993) model, nor with pairwise differences. Only significant differentiations remained were in CII between the populations and between Western and Eastern populations based on pairwise differences. Indicating differentiation between east and west are not very strong, significance of the differentiations depends on the populations and models involved in the AMOVA tests.

3.7.1.8 Diversity Indices

Nucleotide diversity (π) and haplotype diversity (H) of each population and pooled Western (Balearic, Ligurian) and Eastern (Ionian, Aegean1, Aegean 2, N. Levantine) populations, were calculated for MD and MAD by using DnaSP v5 (Librado and Rozas, 2009), based on entire sequences and based on clades separately. Results of each population in MD are given in figure 3.7.1.8.1 and Western/Eastern populations in MD compared with the diversity of pooled Mediterranean populations were given in figure 3.7.1.8.2. Results of each population in MAD are given in figure 3.7.1.8.3 and Western/Eastern populations in MAD compared with aDNA are given in figure 3.7.1.8.4.

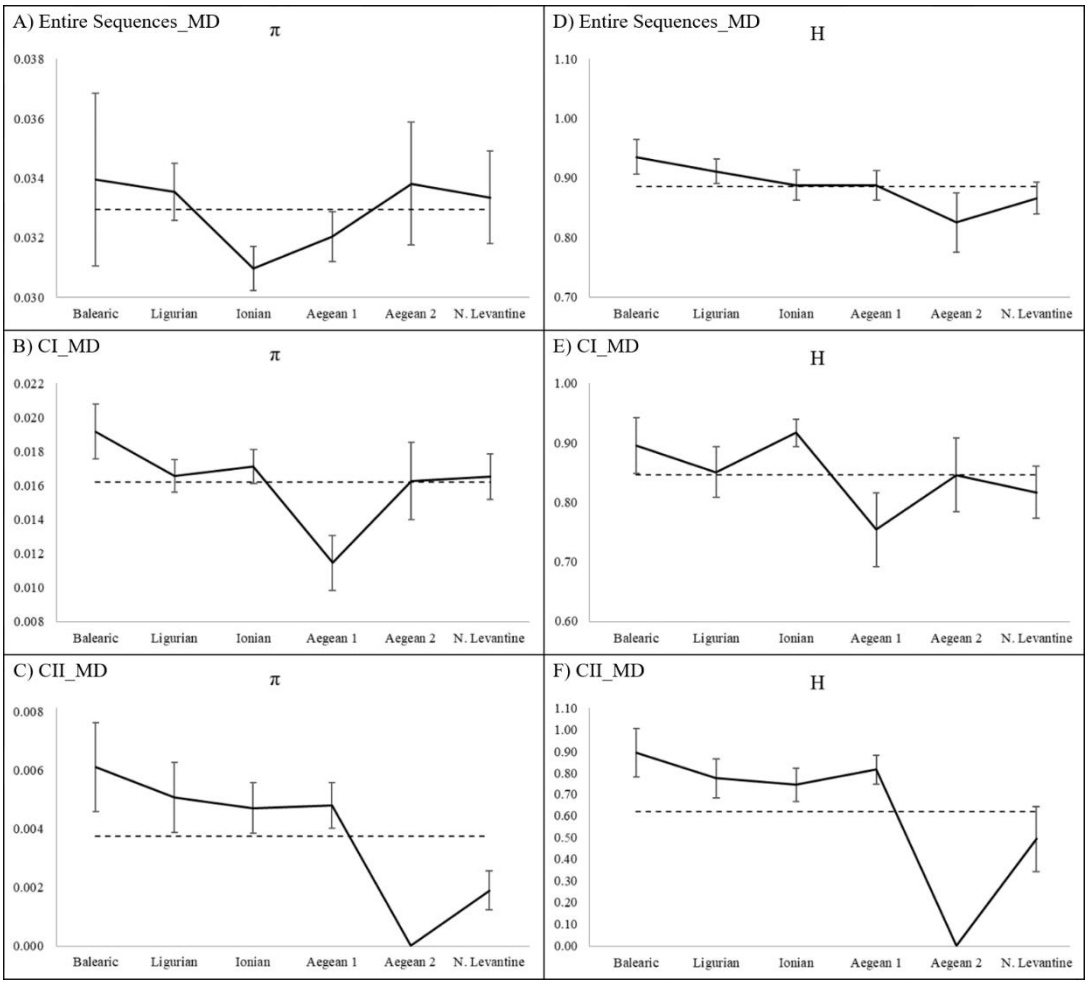

Figure 3.7.1.8.1: Diversity indices of each population in MD A) π of entire sequences, B) H of entire sequences, C) π of CI, D) H of CI, E) π of CII, F) H of CII. Vertical bars represent standard deviations of the diversity values and dashed lines represent means of the diversity values for the Mediterranean population as a whole.

According to the results of MD, Balearic, Ligurian and Ionian have higher diversities except in π based on entire sequences, in which Ionian have the lowest diversity. CII diversity of Aegean 2 is "0" because there is only one haplotype in Aegean 2 CII (n=9).

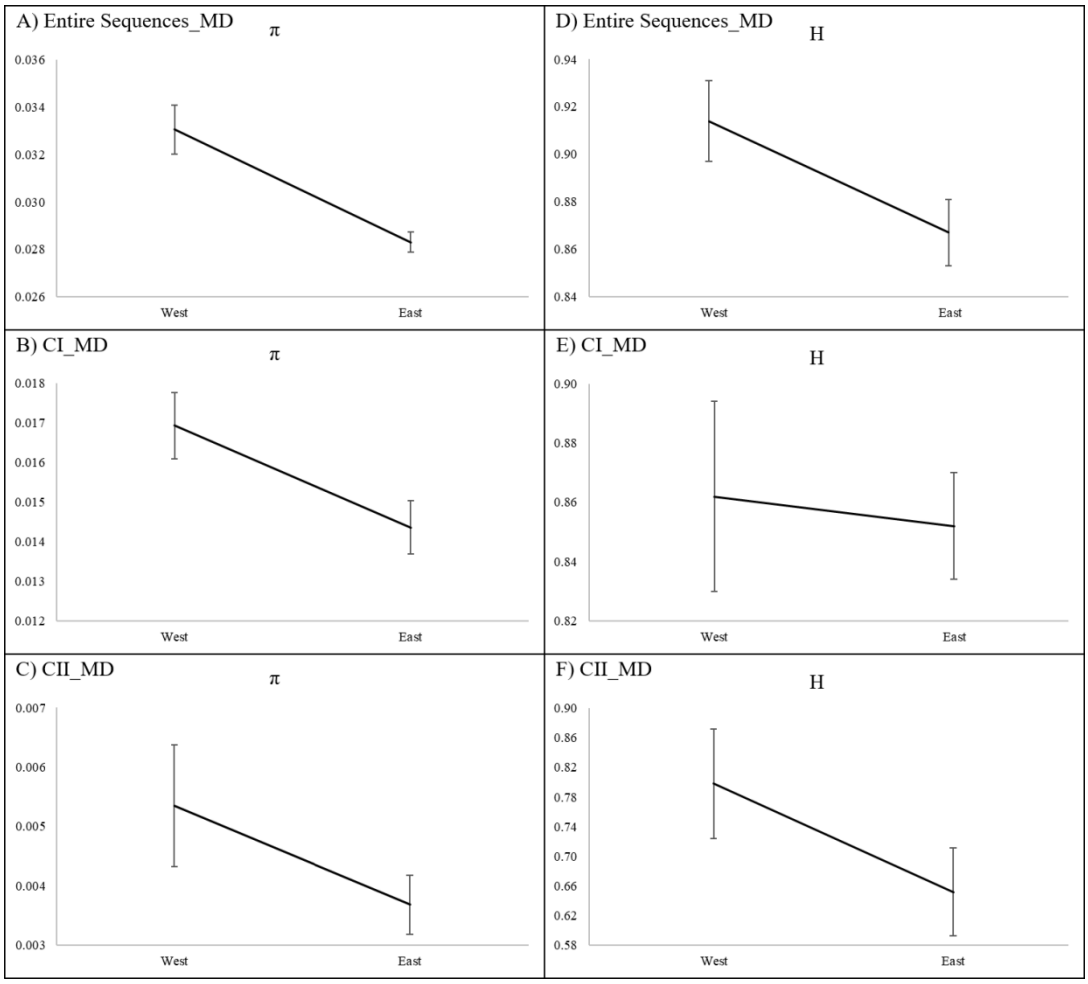

Figure 3.7.1.8.2: Diversity indices of Western and Eastern populations in MD. A) π of entire sequences, B) H of entire sequences, C) π of CI, D) H of CI, E) π of CII, F) H of CII. Vertical bars represent standard deviations of the diversity values.

When means of Western and Eastern populations were considered, in every diversity indices Western Mediterranean (Balearic, Ligurian) population has higher diversity than Eastern Mediterranean (Ionian, Aegean 1, Aegean 2 and N. Levantine) population. These differences are significant except *H* of CI and π of CII.

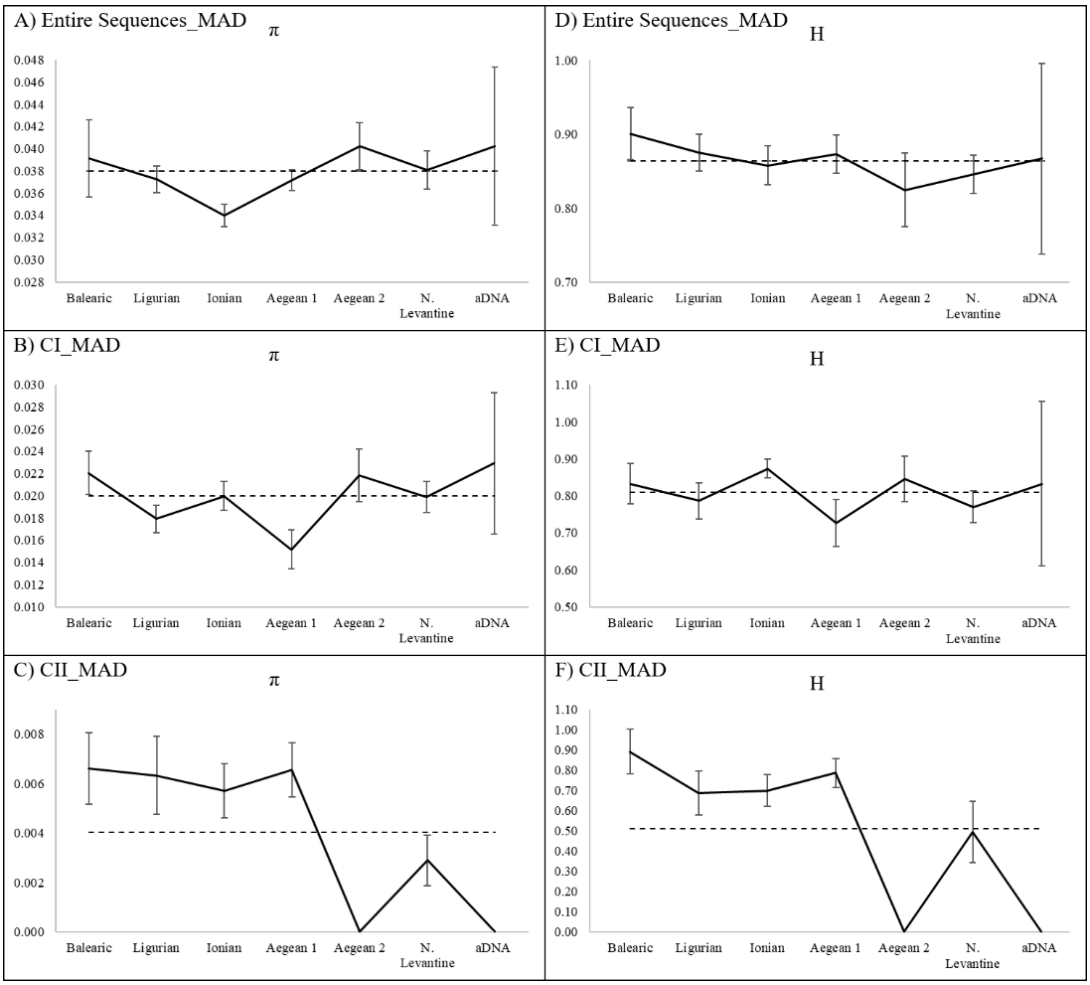

Figure 3.7.1.8.3: Diversity indices of each population in MAD represented graphically. A) π of entire sequences, B) H of entire sequences, C) π of CI, D) H of CI, E) π of CII, F) H of CII. Vertical bars represent standard deviations of the diversity values and dashed lines represent means of the diversity values.

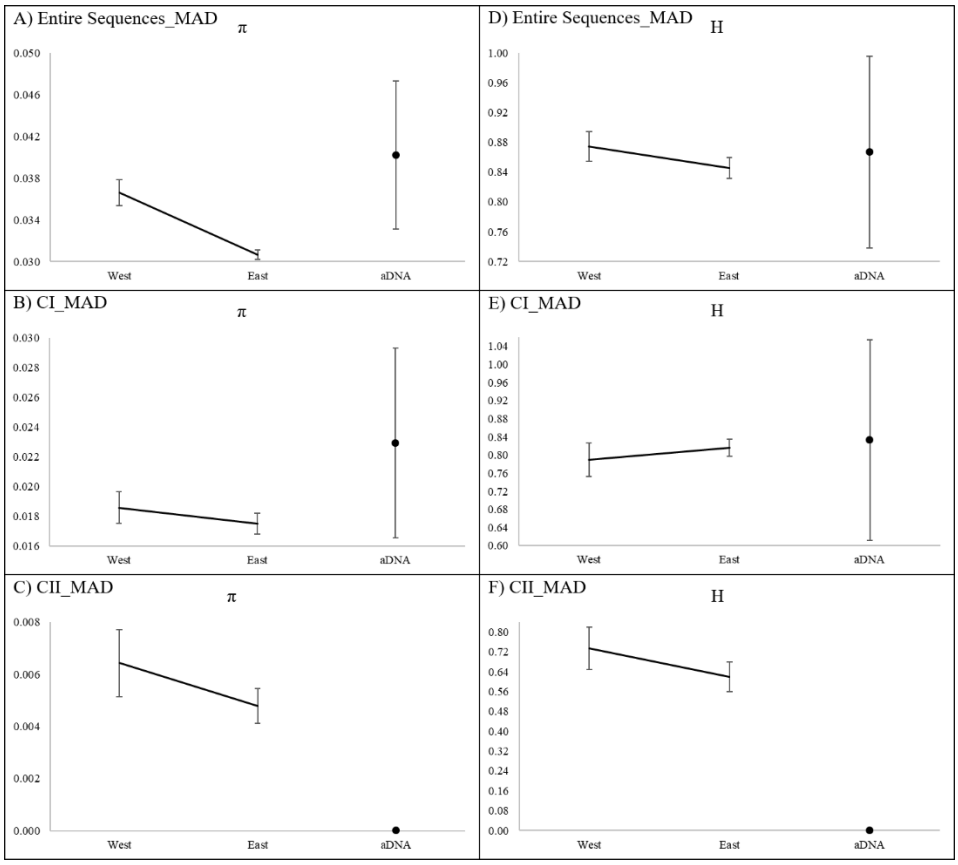

Figure 3.7.1.8.4: Diversity indices Western and Eastern populations in MAD compared with diversity of aDNA population. A) π of entire sequences, B) H of entire sequences, C) π of CI, D) H of CI, E) π of CII, F) H of CII. Vertical bars represent standard deviations of the diversity values.

Nucleotide and haplotype diversity of aDNA based on entire sequences and CI found to be higher than Eastern and Western populations with a wide confidence interval. Thus, none of the pairwise differences were significantly different from aDNA population. CII diversity of aDNA is “0” because there is only one haplotype in aDNA CII (n=2).

3.7.1.9 Demographic Analyses

3.7.1.9.1 Mismatch Distributions

Possible expansions of clades were evaluated by mismatch distributions of MD based on pooled Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) populations. Observed and expected pairwise differences under the sudden expansion model, raggedness index (r) and demographic expansion values (θ_0, θ_1 and τ) of mismatch distributions were calculated by using Arlequin v3.5 (Excoffier and Lischer, 2010) for each clade. Mismatch distributions were given in figure 3.7.1.9.1.1 and demographic expansion values (θ_0, θ_1 and τ) of mismatch distributions were given in table 3.7.1.9.1.1.

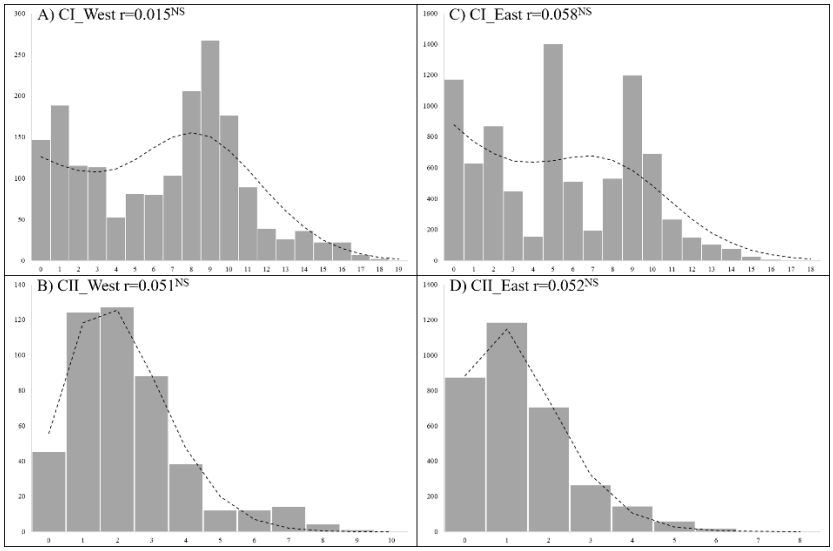

Figure 3.7.1.9.1.1: Mismatch distribution of A) CI of pooled Western populations, B) CII of pooled Western populations, C) CI of pooled Eastern populations, D) CII of pooled Eastern populations. Grey bars represent number of observed pairwise differences and dashed lines represent expected distribution of pairwise differences under sudden expansion model. Raggedness indices (r) are given at the top of the graphs.

Table 3.7.1.9.1.1: Results of θ_0, θ_1 and τ of A) CI of pooled Western populations, B) CII of pooled Western populations, C) CI of pooled Eastern populations, D) CII of pooled Eastern populations.

	θ_0	θ_1	τ
A) CI_Western	0.002	13.033	9.625
B) CII_Western	0.000	9999.000	2.096
C) CI_Eastern	0.000	8.534	9.166
D) CII_Eastern	0.000	9999.000	1.273

According to the results of demographic expansion values CI and CII expanded in the west slightly earlier than the east. Magnitude of CI expansion in the west is also slightly higher than the east, meanwhile magnitude of CII expansion is the same for both of the populations.

3.7.1.9.2 Neutrality

The expansions of clades for Western and Eastern populations were also evaluated with Tajima's D (Tajima, 1989), Fu's F_S (Fu, 1997) and R2 (Ramos-Onsins and Rozas, 2002) neutrality tests, by using DnaSP v5 (Librado and Rozas, 2009) for each clade. Results are given in table 3.7.1.9.2.1.

Table 3.7.1.9.2.1: Results of Tajima's D, Fu's F_S and R2 neutrality tests of A) CI of pooled Western populations, B) CII of pooled Western populations, C) CI of pooled Eastern populations, D) CII of pooled Eastern populations. Significant results are highlighted with grey.

	Tajima's D	**R2**	**Fu's F_S**
A) CI_Western	-0.573	0.0818	-7.223
B) CI_Eastern	-1.394	0.0476	-12.528
C) CII_Western	-2.102**	0.0431***	-12.563***
D) CII_Eastern	-1.959**	0.0320**	-14.697***

*0.05>p **0.01>p ***0.001>p

In CI, Fu's FS and Tajima's D results of CII are negative but not significant and R2 results are also not significant in both Eastern and Western populations, suggesting constant population size in CI. Meanwhile Fu's F_S and Tajima's D results of CII are significantly negative ($p<0.01$, $p<0.001$) and R2 results of CII were significant ($p<0.01$, $p<0.001$) in both Eastern and Western populations, suggesting expansions of CII.

3.7.1.10 Long Term Effective Population Size

Long term effective population size (Ne) parameter θ of each modern population was estimated by using Migrate-n (Beerli and Felsenstein, 2001) for both MD and MAD. Means of the resulted Bayesian distributions were used to calculate a long term Ne based on μ values of $1x10^{-6}$ and $1x10^{-8}$ per nucleotide per generations covering estimated μ for bluefin tuna (Suzuki and Chow, 2015) and by using $\mu=2.45x10^{-7}$ per nucleotide per generation, the value estimated for skipjack tuna and yellowfin tuna (Ely et al., 2005). Generation time of the swordfish is assumed to be 5 years (Palko et al., 1981). Table 3.7.1.10.1 represents Ne results of MD and table 3.7.1.10.2 represents Ne results of MAD.

Table 3.7.1.10.1: Estimated results of mean and 95% credibility indices of θ distributions of populations in the MD along with long term Ne calculated for different μ (per nucleotide per generation) (CreI=Credibility Indices).

	θ	**θ-CreI Lower**	**θ-CreI Upper**	**Ne $\mu=10^{-6}$**	**Ne $\mu=10^{-8}$**	**Ne $\mu=2.45x10^{-7}$**
Balearic	0.014	0.003	0.025	7180	718000	29306
Ligurian	0.015	0.006	0.025	7700	770000	31429
Ionian	0.012	0.002	0.024	6005	600500	24510
Aegean 1	0.005	0.000	0.018	2375	237500	9694
Aegean 2	0.006	0.000	0.021	3225	322500	13163
N. Levantine	0.007	0.000	0.023	3685	368500	15041

According to the results based on modern database (MD), highest Ne was for Ligurian and the lowest Ne was for Aegean 1. Generally, Ne's of the populations at the Western Mediterranean (Balearic, Ligurian) were higher than the Eastern Mediterranean (Ionian, Aegean 1, Aegean 2 and N. Levantine).

Table 3.7.1.10.2: Estimated results of mean and 95% credibility indices of θ distributions of populations in the MAD along with long term Ne calculated for different μ (per nucleotide per generation) (CreI=Credibility Indices).

	θ	**θ-CreI Lower**	**θ-CreI Upper**	**Ne $\mu=10^{-6}$**	**Ne $\mu=10^{-8}$**	**Ne $\mu=2.45x10^{-7}$**
Balearic	0.013	0.002	0.025	6735	673500	27490
Ligurian	0.013	0.001	0.025	6585	658500	26878
Ionian	0.012	0.002	0.024	5785	578500	23612
Aegean 1	0.001	0.000	0.003	565	56500	2306
Aegean 2	0.012	0.001	0.024	6195	619500	25286
N. Levantine	0.008	0.000	0.023	4105	410500	16755
aDNA	0.013	0.001	0.025	6425	642500	26224

According to the results, Ne of aDNA was higher than Eastern populations (Aegean 2 and N. Levantine) and closer to those of Western populations (Balearic and Ligurian).

Long term effective population size (Ne) parameter θ of pooled Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) populations were also estimated by using Migrate-n (Beerli and Felsenstein, 2001) based on entire sequences and based on clades separately, for both MD and MAD. Means of the resulted Bayesian distributions were used to calculate a long term Ne based on μ of $1x10^{-6}$ and $1x10^{-8}$ per nucleotide per generations covering estimated μ for bluefin tuna (Suzuki and Chow, 2015) and by using $\mu=2.45x10^{-7}$ per nucleotide per generation, the value estimated for skipjack tuna and yellowfin tuna (Ely et al., 2005). Generation time of the swordfish is assumed to be 5 years (Palko et al., 1981). Table 3.7.1.10.3 represents Ne results of MD and table 3.7.1.10.4 represents Ne results of MAD.

Table 3.7.1.10.3: Estimated results of mean and 95% credibility indices of θ distributions of pooled Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) populations in the in the MD along with long term Ne calculated for different μ (per nucleotide per generation) (CreI=Credibility Indices).

	θ	**θ-CreI Lower**	**θ-CreI Upper**	**Ne $\mu=10^{-6}$**	**Ne $\mu=10^{-8}$**	**Ne $\mu=2.45x10^{-7}$**
A) Entire Sequences						
West	0.016	0.006	0.026	8125	812500	33163
East	0.007	0.003	0.011	3375	337500	13776
B) CI						
West	0.019	0.010	0.026	9525	952500	38878
East	0.001	0.000	0.004	665	66500	2714
C) CII						
West	0.011	0.000	0.024	5445	544500	22224
East	0.003	0.000	0.006	1280	128000	5224

Based on all Θ results calculated with entire sequences, CI and CII, pooled Western populations (Balearic, Ligurian) has higher effective population size estimates than pooled Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) populations.

Table 3.7.1.10.4: Estimated results of mean and 95% credibility indices of θ distributions of pooled Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) populations in the MAD along with long term Ne calculated for different μ (per nucleotide per generation) (CreI=Credibility Indices).

	θ	**θ-CreI Lower**	**θ-CreI Upper**	**Ne $\mu=10^{-6}$**	**Ne $\mu=10^{-8}$**	**Ne $\mu=2.45x10^{-7}$**
A) Entire Sequences						
West	0.016	0.005	0.025	8125	812500	32327
East	0.007	0.002	0.022	3375	337500	19327
aDNA	0.013	0.001	0.025	6425	642500	26224
B) CI						
West	0.016	0.005	0.025	7845	784500	32020
East	0.002	0.000	0.005	1085	108500	4429
aDNA	0.015	0.002	0.025	7360	736000	30041
C) CII						
West	0.010	0.000	0.023	5055	505500	20633
East	0.003	0.000	0.010	1375	137500	5612
aDNA	0.011	0.000	0.023	5270	527000	21510

Based on all Θ results calculated with entire sequences, CI and CII, aDNA had higher effective population size estimates than pooled Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine), slightly lower and in case of CII higher, effective population size estimates than Western (Balearic, Ligurian) populations.

3.7.1.11 Migration

Pairwise migration rates between each of the modern populations were estimated by using Migrate-n (Beerli and Felsenstein, 2001). Means of the resulted Bayesian

distributions are visualized as percent rate in to the population and given in figure 3.7.1.11.1.

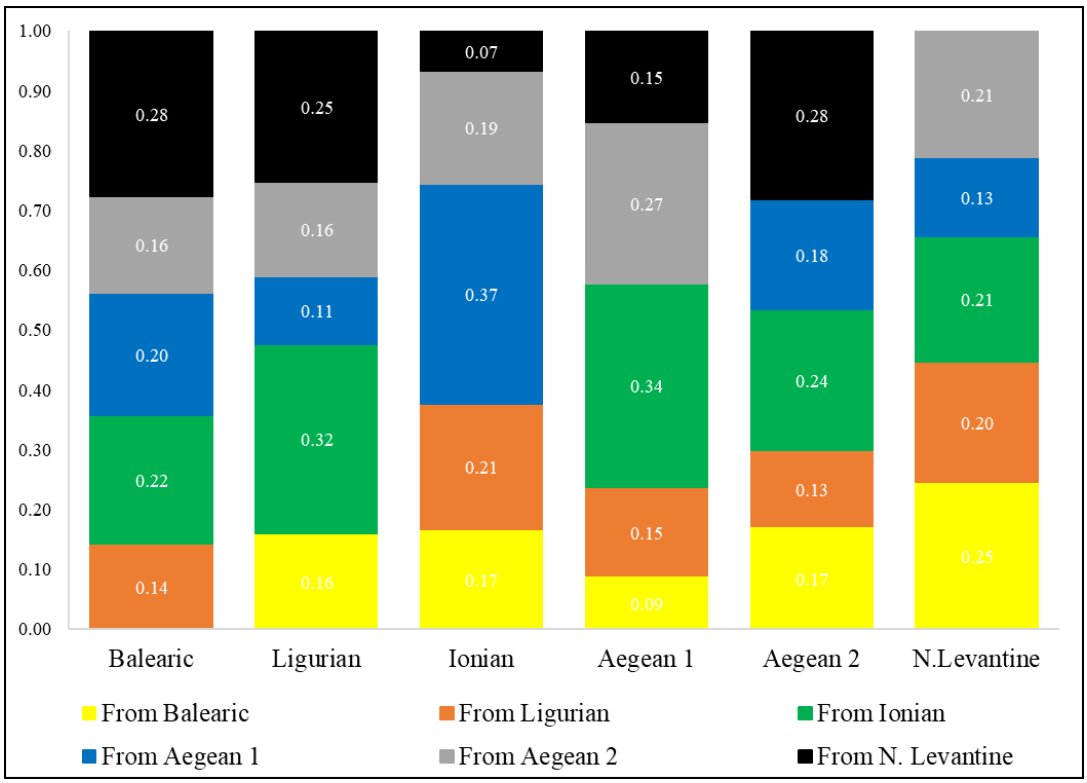

Figure 3.7.1.11.1: Rate of migration (in percentage) into each population from other considered populations.

Pairwise migration rates between the modern populations indicate that:

- Migrations were not dominated by one or few populations (per population).
- Migrations to the populations were generally the highest from one of their neighbors as for the populations of Ligurian, Ionian, Aegean 1 and Aegean 2.
- However, at the west and east edges of the Mediterranean, the previous pattern was not observed, on the contrary, highest migration to Balearic was not from

Ligurian (14%) but from N. Levantine (28%) and the highest migration to N. Levantine was from Balearic (25%).

Migration rates between pooled Western (Balearic, Ligurian) and Eastern (Ionian, Aegean 1, Aegean 2, N. Levantine) populations were also estimated by using Migrate-n (Beerli and Felsenstein, 2001) based on entire sequences and based on clades separately. Results are given in figure 3.7.1.11.2.

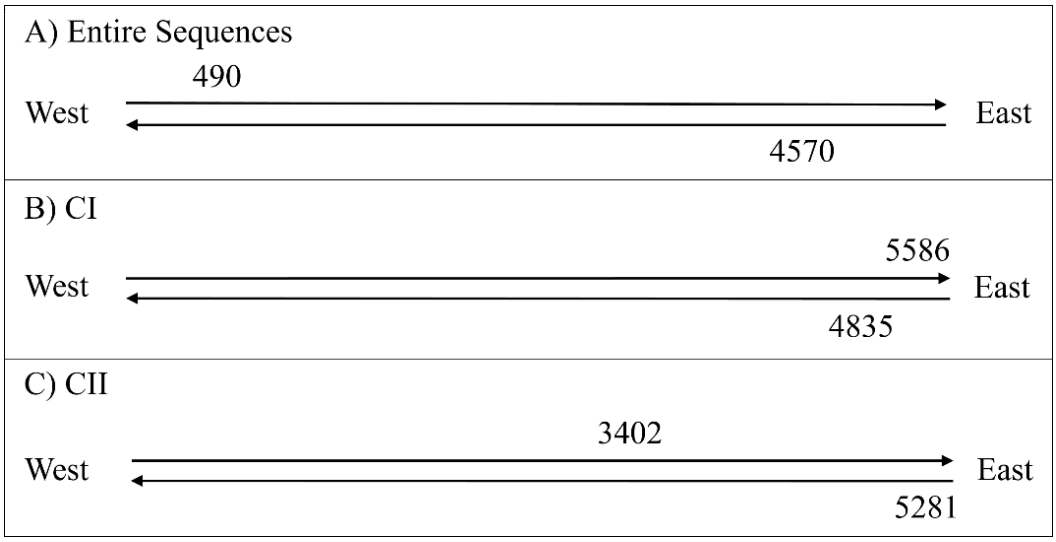

Figure 3.7.1.11.2: Rate of migrations into E and Western populations based on A) entire sequences, B) CI, C) CII.

According to the results of entire sequences and CII migration rate from east to west is higher than from west to east. On the contrary in CII, migration from west to east is higher than from east to west.

3.7.1.12 Continuity Tests

In order to test the null hypothesis of the "Observed F_{ST} values between ancient swordfish population and each of the geographically close modern populations

(Aegean 1, Aegean 2 and N. Levantine) could arise only by genetic drift", observed F_{ST} values were compared with F_{ST} values of 1000 coalescent simulations generated by fastsimcoal2 (Excoffier et al., 2013) with the assumption of modern population's Ne of 50 to 100 (based on the results of contemporary Ne calculated by microsatellites) and ancient NE of 25000 (based on the results of long term Ne calculated by mtDNA of aDNA with $\mu=2.45x10^{-7}$), generation time of 5 years (Palko et al., 1981) and of mutation rates 10^{-6} to 10^{-8} per site per generation covering estimated μ for bluefin tuna (Suzuki and Chow, 2015). Populations were samples serially trough time under the exponential population decline and negative exponential growth rate was calculated as the natural logarithm of the ratio of the population sizes of aDNA and modern populations. All tests were repeated three times by assuming dates of the ancient samples were 4^{th} or 7^{th} century, and finally as 10th century. Continuity test results for the assumption of "ancient population was representing a sample of swordfish that lived in the 7^{th} century" were given in figure 3.7.1.12.1. Results of the other continuity tests were given in appendix D.

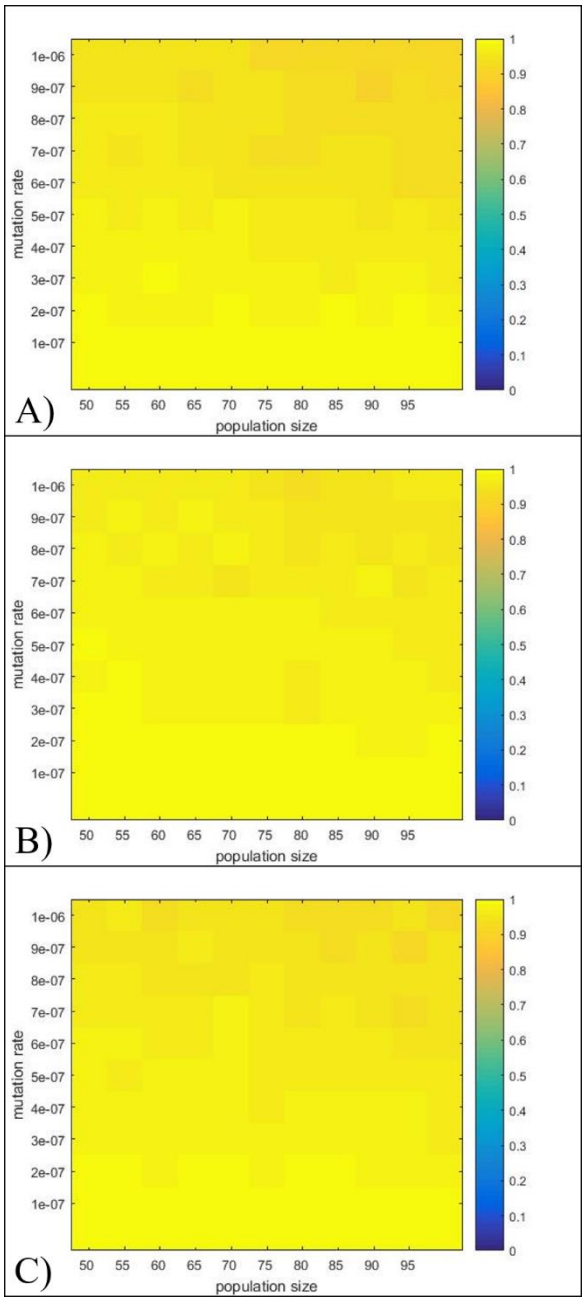

Figure 3.7.1.12.1: The heat map in the form of 11 X 11grids for each set of parameter combinations. Each grid represents the 1000 coalescent simulations to test population continuity under an exponential decline model based on parameter combinations A) starting with 7th aDNA and ending with modern Aegean 1, B) starting with 7th aDNA and ending with modern Aegean 2, B) starting with 7th aDNA and ending with modern N. Levantine. Ne of modern populations were represented on the x-axes and mutation rates on they-axes. The colors scale near heat maps show the proportion of simulated F_{ST} values greater than that observed values in each grid.

Since simulated F_{ST} values were greater than the observed values in all three tests (under the assumption of random drift), observed F_{ST}'s between Aegean1/aDNA (F_{ST}=0.049, non-significant), Aegean 2/aDNA (F_{ST}=0.000, non-significant) and Northern Levantine /aDNA (F_{ST}=0.026, non-significant) could have arose by genetic drift, hence continuity cannot be rejected when date of the aDNA samples were assumed as 7th century. Similar results were obtained when dates of the ancient samples were assumed as 4th and 10th century as can be seen in appendix D.

3.7.2 Microsatellite Results

3.7.2.1 Reliability of the Data

3.7.2.1.1 Null Allele Frequencies

Null allele frequencies of 8 microsatellite loci were calculated by FreeNA (Chapuis and Estoup, 2007) for both Aegean 2 and N. Levantine populations. Results are given in table 3.7.2.1.1.1.

Table 3.7.2.1.1.1: Null allele frequencies of 8 microsatellite loci calculated for Aegean 2 and N. Levantine populations.

Locus	Aegean 2	N. Levantine
XgSau98R1	0.000	0.040
Xg31	0.000	0.000
VBC201	0.000	0.001
Xgl-561	0.000	0.006
Xg41b	0.000	0.000
Xgl-65b	0.000	0.000
Xg51	0.007	0.024
Xgl-536	0.032	0.016

Null allele frequencies can be categorized as negligible for r<0.05, moderate for 0.05<r<0.20 or large for r>0.20 (Chapuis and Estoup, 2007). All of the 8 microsatellite loci seem to display negligible null allele frequencies.

3.7.2.1.2 Linkage Disequilibrium

In order to understand whether allele frequency of 8 microsatellite loci used in this study are independent or non-randomly associated, linkage disequilibrium coefficients of each pair of loci were calculated for both populations by using Genepop v4.1 (Rousset, 2008) and their significances are presented in table 3.7.2.1.2.1.

Table 3.7.2.1.2.1: P values for linkage disequilibrium coefficient for each pair of 8 microsatellite loci. Lower triangular matrix shows results for Aegean 2 and upper triangular of the matrix shows results for N. Levantine (Adjusted p values after Bonferroni correction are 0.05/28=0.00179, 0.01/28=0.00357, 0.001/28=0.00004).

	XgSau98R1	**Xg31**	**VBC201**	**Xgl-561**
XgSau98R1	-	0.475	0.232	0.478
Xg31	0.012	-	0.045	0.330
VBC201	0.111	0.146	-	0.855
Xgl-561	0.370	0.055	0.111	-
Xg41b	0.018	0.019	0.490	0.018
Xgl-65b	0.025	0.032	0.105	0.223
Xg51	1.000	0.116	1.000	1.000
Xgl-536	0.885	0.458	0.024	0.031
	Xg41b	**Xgl-65b**	**Xg51**	**Xgl-536**
XgSau98R1	0.039	0.510	0.301	0.805
Xg31	0.599	0.122	0.135	0.073
VBC201	0.991	0.722	0.741	0.062
Xgl-561	0.035	0.408	0.806	0.040
Xg41b	-	0.119	0.529	0.262
Xgl-65b	0.092	-	0.609	0.628
Xg51	1.000	1.000	-	0.491
Xgl-536	0.212	0.696	1.000	-

None of the results were significant suggesting all loci used in this study were in linkage equilibrium.

3.7.2.2 Genetic Diversity Indices

3.7.2.2.1 Number of Alleles (A) and Allelic Richness (Ar)

Genetic diversities of Aegean 2 and N. Levantine were first evaluated by calculating number of alleles (A) and allelic richness (Ar) of 8 microsatellite loci for both populations, by using FSTAT (Goudet, 2001). Results were given in table 3.7.2.2.1.1.

Table 3.7.2.2.1.1: Number of alleles (A) and allelic richness (Ar) values for 8 microsatellite loci of Aegean 2 and N. Levantine populations (n=Sample Size).

	Aegean 2 (n=26)		**N. Levantine (n=40)**	
	A	**Ar**	**A**	**Ar**
XgSau98R1	9	8.846	11	10.364
Xg31	7	6.959	7	6.205
VBC201	6	6.000	5	4.940
Xgl-561	6	5.842	6	5.977
Xg41b	11	10.800	13	10.528
Xgl-65b	7	7.000	7	6.839
Xg51	16	15.827	17	15.048
Xgl-536	7	6.765	10	8.355

In both of the populations, the highest A and the highest Ar were found at Xg51 (in Aegean 2 A=16, Ar=15.827 and in N. Levantine A=17, Ar=15.048). VBC201 had the lowest A in both Aegean 2 (6) and N. Levantine (5). In Aegean 2, Xgl-561 also had the lowest A (6) along with VBC201. The lowest Ar was found at Xgl-561 (5.842) in Aegean 2 and at VBC201 (4.940) in N. Levantine.

Existence of significant differences between Aegean 2 and N. Levantine populations based on A and Ar values were tested by Mann-Whitney rank tests. U value of Mann-Whitney rank tests was 28.5 ($p>0.05$) based on A and 30 ($p>0.05$) based on Ar. Thus, Aegean 2 and N. Levantine populations were not significantly different from each other with respect to A and Ar on the basis of 8 microsatellites loci studied in the present study.

3.7.2.2.2 Heterozygosity

Observed (Ho) and expected (He) heterozygosities of 8 microsatellite loci and significance of the deviations from Hardy-Weinberg equilibrium (pHW) were calculated by Arlequin v3.5 (Excoffier and Lischer, 2010) for both populations. Results were given in table 3.7.2.2.2.1.

Table 3.7.2.2.2.1: Expected heterozygosity (Ho), observed heterozygosity (He) and p values of the deviations from Hardy-Weinberg equilibrium (pHW) of each locus, for Aegean 2 and N. Levantine (Adjusted p values after Bonferroni correction were 0.05/8=0.00625, 0.01/8=0.00125, 0.001/8=0.000125, n=Sample Size).

Locus	Aegean 2 (n=26)			N. Levantine (n=40)		
	Ho	He	pHW	Ho	He	pHW
XgSau98R1	0.923	0.838	0.984	0.795	0.859	0.401
Xg31	0.760	0.766	0.014	0.667	0.691	0.673
VBC201	0.875	0.790	0.543	0.750	0.754	0.415
Xgl-561	0.577	0.544	1.000	0.575	0.705	0.118
Xg41b	0.960	0.852	0.032	0.825	0.827	0.014
Xgl-65b	0.885	0.829	0.202	0.750	0.782	0.074
Xg51	0.769	0.934	0.093	0.900	0.916	0.167
Xgl-536	0.654	0.697	0.121	0.744	0.793	0.550

Xg41b had the highest Ho (0.960) in Aegean 2 and Xg51 had the highest Ho (0.900) in N. Levantine. In both populations, the lowest Ho was at Xgl-561 (Ho=0.577 and

0.575 for Aegean 2 and N. Levantine respectively). There were no significant differences between Ho and He values of 8 loci hence both populations are in Hardy-Weinberg equilibrium.

Possible significant differences between Aegean 2 and N. Levantine populations based on Ho values were tested by Mann-Whitney rank tests. U was 22 and was not significant ($p>0.05$).

3.7.2.3 Population Differentiation

3.7.2.3.1 Pairwise F_{ST} Value

Possible differentiation between Aegean 2 and N. Levantine based on 8 microsatellite loci were first evaluated by testing the significance of pairwise F_{ST} value. F_{ST} value was calculated by using Arlequin v3.5 (Excoffier and Lischer, 2010) and it was 0.007 with $p=0.053$, indicating no significant differentiation.

3.7.2.3.2 PCA Results

Genetic relationship between individuals were visualized by a PCA constructed by GenAlEx v6.4 (Peakall and Smouse, 2006). Result was given in figure 3.7.2.3.2.1.

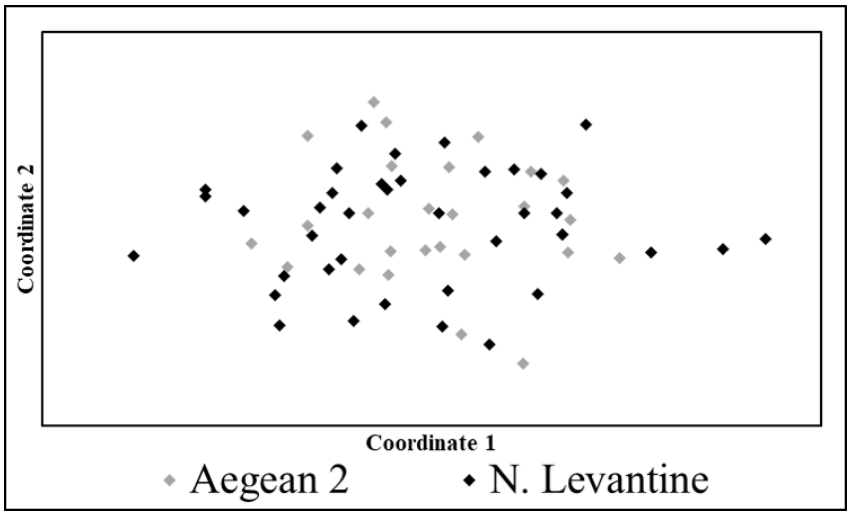

Figure 3.7.2.3.2.1: PCA analysis of genetic distances between individuals of Aegean 2 and N. Levantine populations.

In the Figure 3.7.2.3.2.1 coordinate 1 explains 22.93% of the variation, coordinate 2 explains 19.96% percent of the variation and coordinate 3 (not shown) explains 16.80% of the variation. Most of the individuals of the two populations (Aegean 2 and N. Levantine) were clustered together therefore; individuals of these two populations seem to be the samples of the same population.

3.7.2.3.3 Results of the STRUCTURE Analysis

Level of admixture in the Aegean 2 and N. Levantine were analyzed by using STRUCTURE v2 (Pritchard et al., 2000) for K=2-6. Most probable K was estimated by both Evanno, et al.'s (2005) and Rosenberg et al.'s (2002) methods and results of the most probable K were given in figure 3.7.2.3.3.1.

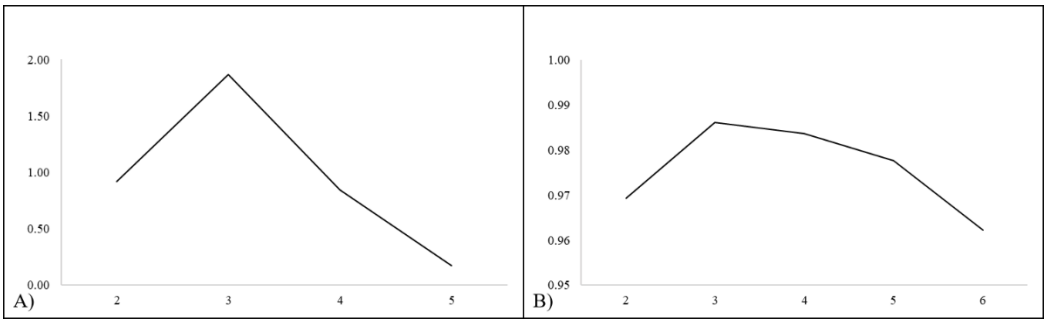

Figure 3.7.2.3.3.1: A) Delta K against K graph constructed based on Evanno, et al.'s (2005) method. B) The similarity coefficient against K graph constructed based on Rosenberg et al.'s (2002) method.

Most probable K was found as 3 by both methods. Results of the STRUCTURE were given in figure 3.7.2.3.3.2.

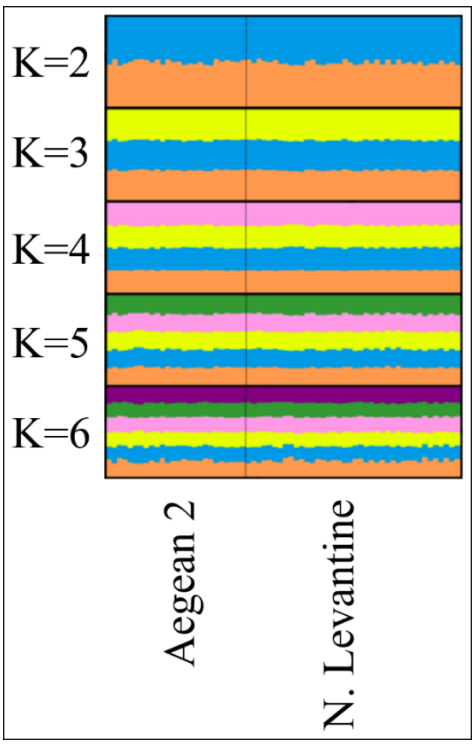

Figure 3.7.2.3.3.2: The visualization of the STRUCTURE output for A) K=2, B) K=3, C) K=4, D) K=5, E) K=6.

Based on STRUCTURE results, Aegean 2 and N. Levantine populations were not differentiated neither for K=3 nor for other K values.

3.7.2.4 Effective Population Sizes (Ne)

3.7.2.4.1 Long Term Ne

Long term Ne parameter θ of Aegean 2 and N. Levantine were estimated with Migrate-n (Beerli and Felsenstein, 2001) based on microsatellites. Means of the resulted Bayesian distributions were used to calculate a long term Ne estimate based on μ of $1x10^{-2}$ and $1x10^{-6}$ per nucleotide per generation covering μ used for bigeye tuna, (Gonzalez et al., 2008) . Table 3.7.2.4.1.1 represents results of the analyses.

Table 3.7.2.4.1.1: Estimated results of mean and 95% confidence intervals of θ distributions along with long term Ne calculated for the μ of $1x10^{-2}$and $1x10^{-6}$ per nucleotide per generation based on microsatellite data.

	θ	**θ-ConI (Lower)**	**θ-ConI (Upper)**	**Ne ($\mu=1x10^{-2}$)**	**Ne ($\mu=1x10^{-6}$)**
Aegean 2	2.190	0.233	4.400	55	547500
N. Levantine	4.240	3.067	5.267	106	1060000

According to the results, N. Levantine had higher Ne than Aegean 2 and Ne results were very low when mutation rate was assumed to be high.

3.7.2.4.2 Short Term Ne

Short term Ne of Aegean 2 and N. Levantine were estimated by using NeEstimator v2 (Do et al., 2014) where for the estimations linkage disequilibrium (LD), heterozygosity excess (HE) and molecular coancestry (MC) were employed. Mean

and 95% confidence intervals of the resulted distributions were summarized in table 3.7.2.4.2.1.

Table 3.7.2.4.2.1: Means and 95% confidence intervals of Ne distributions calculated with LD, HE and MC methods.

LD Method		
	Mean	**Confidence Intervals**
Aegean 2	56	31-170
N. Levantine	134	69-477
HE Method		
	Mean	**Confidence Intervals**
Aegean 2	Infinite	Infinite
N. Levantine	Infinite	Infinite
MC Method		
	Mean	**Confidence Intervals**
Aegean 2	13	4-29
N. Levantine	48	0-241

Short term Ne results calculated with LD and MC methods showed that both Aegean 1 and Northern Levantine had Ne values were lower than 150 and Aegean 2 has nearly 1/3 Ne of the N. Levantine. Meanwhile, short term Ne distribution of Aegean2 and N. Levantine calculated by HE method showed “infinite” indicating results had very large confidence intervals.

3.7.2.4.3 MSVAR Results

Current and ancestral Ne values, together with possible population expansions and/or declines and the approximate date of these possible demographic events were estimated by the Bayesian based MSVAR (Storz and Beaumont, 2002) for Aegean 2

and N. Levantine populations. Obtained graphs were given in figure 3.7.2.4.3.1 and summarized in table 3.7.2.5.3.1.

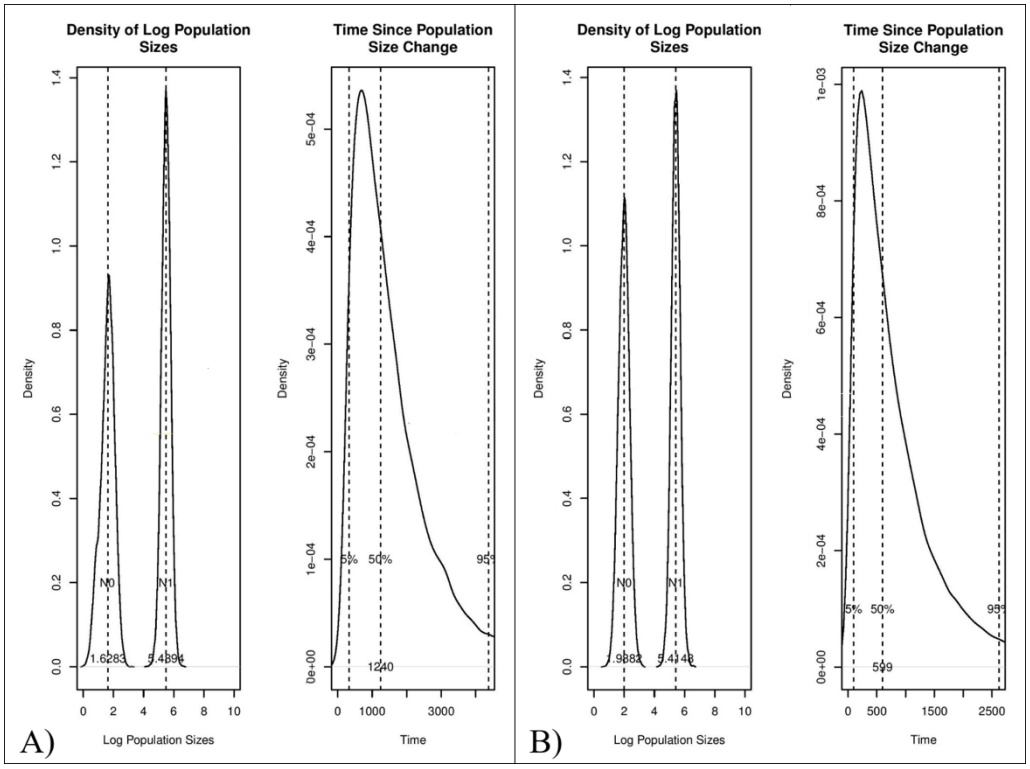

Figure 3.7.2.4.3.1: MSVAR results of the A) Aegean 2, B) N. Levantine, populations based on 8 microsatellite loci. First graph shows distribution of ancestral (N1) and current (N0) Ne in logarithmic scale. Second graph shows time since Ne change.

Table 3.7.2.4.3.1: Estimated results of mean and 95% confidence intervals of Ne distributions calculated with MSVAR A) for current Ne B) for ancestral Ne (CreI=Credibility Indices).

A) Current Ne			
	Mean	**ConI (Lower)**	**ConI (Upper)**
Aegean 2	43	1	1909
N. Levantine	98	4	2037
B) Ancestral Ne			
	Mean	**ConI (Lower)**	**ConI (Upper)**
Aegean 2	309030	14215	5191222
N. Levantine	263027	16621	4617508

When the result of Aegean 2 population was examined, mean of the current Ne distribution was log 1.63 (43). There was a vast amount of population decrease from the mean of the ancestral Ne distribution, which is log 5.49 (309030). Finally, time since the population size start to decrease was estimated approximately as 1240 years ago.

When the result of N. Levantine population was examined, mean of the current Ne distribution was log 1.99 (98). There was again dramatic population decrease from the mean of the ancestral Ne distribution, which was log 5.42 (263027). Finally, time since the population size started to decrease was estimated approximately as 599 years ago.

CHAPTER 4

DISCUSSION

In this study partial mtDNA CR of 42 swordfish samples from Mediterranean coasts of Turkey and 26 samples from Northern Aegean Sea were sequenced, and their 8 microsatellite loci were genotyped. Moreover, partial mtDNA CR of 6 swordfish bones that were unearthed from Marmaray-Yenikapı excavations were sequenced. Previously, several swordfish populations from the Mediterranean (Balearic Sea, Ligurian Sea, Tunisian Coast, Ionian Sea and the Southern Aegean Sea) were examined on the basis of mtDNA CR sequences (Viñas et al., 2010). In the present study Northern Levantine and Northern Aegean populations, the most eastern and north eastern populations of Mediterranean sword fish, together with the samples of an ancient population are studied to better understand the past and present genetic diversity distribution of Mediterranean swordfish.

All partial mtDNA CR sequences obtained in the present study were wanted to be compared with the sequences from rest of the Mediterranean swordfish (Balearic Sea, Ligurian Sea, Tunisian Coast Ionian Sea and the Southern Aegean Sea). However, sequences of the present study were not fully compatible with those of the previous ones. Therefore, maximum possible length for the sequences was maintained by eliminating some individuals from the previous data set. As a result of this adjustment Tunisian Coast population size dropped to n=8 and this population was removed from the further analysis. Total number of sequences of the previous study (Viñas et al., 2010) that could not be employed in the present study was 17 out of 251 sequences. Thus in the present study, in the context of modern populations, 6 swordfish populations were examined. Results of the mtDNA (maternally inherited DNA) analyses were used to evaluate genetic diversity, degree of genetic

differentiation, effective population sizes and migration rates of the Mediterranean swordfish. aDNA data, although very limited, was used to compare past genetic diversity with those of the present in order to make inferences about a) possible existence of a distinct ancient population, b) possible changes in swordfish genetic composition over the time. Microsatellite data were used to assess genetic diversity of swordfish populations living around the coast of Turkey, to calculate their contemporary and historical effective population sizes based on nuclear markers.

4.1 Mitochondrial DNA Results

4.1.1 Genetic Differentiation within Mediterranean

In Viñas et al.'s (2010) study, a cline of the diversities (π and H) from west to east of Mediterranean populations were observed when clades were separately analyzed and a significant differentiation was observed between Western (Balearic Sea, Ligurian Sea, Tunisian Coast) and Eastern (Ionian Sea and the Southern Aegean Sea) populations by means of AMOVA test. The possible existence of discrete spawning areas, and possible fidelity to these areas because of the homing behavior, of swordfish in the east and west of Mediterranean were proposed to cause this observed differentiation.

Furthermore, Western Mediterranean have lower water transparency, lower salinity and it is less oligotrophic than Eastern Mediterranean (Krom et al., 2005). Here, surface current run anti clock wise and more complicated with eddies and jets (Hamad et al., 2005), which can cause larval retention Viñas et al. (2010) proposed that these differences in oceanographic conditions between eastern and western basin may be another reason of observed differentiation between Aegean and populations in the western Mediterranean (Balearic, Ligurian and Tunisian).

Significance of the observation was: if there were two subpopulations in the Mediterranean, then conservation strategy should be planned accordingly. In other

words, instead of one fishing quota (as it is at the present), may be two different fishing quotas should be implemented for the Mediterranean basin.

In the present study, the expanded data was examined to check if the existences of "Eastern" and "Western" populations are confirmed among the modern swordfish populations and if there is a distinct population among the examined swordfish populations.

The visual descriptions of the haplotypes on NJ tree (Figure 3.7.1.2.1), MJ network (Figure 3.7.1.3.1) and PCA based on sequences (Figure 3.7.1.5.1) as well as populations (Figure 3.7.1.5.2) indicated that swordfish populations do not seemed to be differentiated based on their geographical locations, even if there is a differentiation it is not detected by the visual examination. In all swordfish populations, private haplotypes (for the entire sequences 21-2; CI 17-2; CII 7-0 per population, Table 3.7.1.3.1) are separated from their respective main nodes by only small number of mutations (1-5), and their frequencies were small (0.09-0.00) including the private haplotypes described in this study (5). CI exhibited higher frequency of private alleles (45) than CII (24) in total.

CI and CII frequencies were similar over the 6 Mediterranean populations and 1 ancient population. But when sub-clade frequencies were compared (Figure 3.7.1.4.1, Table 3.7.1.4.1) it was seen that CI_β frequency of Aegean 1 (6%) was significantly ($p<0.05$ - $p<0.01$) lower than those of Balearic (28%), Ligurian (27%) and Ionian (20%). However, sub-clade frequencies of other Eastern populations (Ionian, Aegean 2 and N. Levantine) were not significantly different than those of western populations (Balearic and Ligurian). Thus the observation for Aegean 1 (significantly low CI_β) was not a rule for the Eastern populations.

No significant differentiation can be observed based on F_{ST} of the entire sequences (Table 3.7.1.6.1). CI of the Aegean 1 found to be significantly different form Balearic, Ligurian and Ionian, parallel to the differences in the sub-clade frequency based on MD.

According to the results of migration rates per population of MD, it is seen that members of all populations are migrating towards each other (Figure 3.7.1.11.1). Thus none of the Mediterranean swordfish populations seems to be isolated.

When the total diversity observed within Mediterranean Sea was partitioned as "Eastern" (Ionian, Aegean 1, Aegean 2 and Northern Levantine) and "Western" (Balearic and Ligurian) by AMOVA, significant (p=0.023) differentiation for CI was observed (Table 3.7.1.7.1). In this analysis, Tamura 3 parameter model (Tamura, 1992) based on BIC was found to be suitable for the MD, but since this model cannot be implemented to the Arlequin (Excoffier and Lischer, 2010) more inclusive Tamura Nei model (Tamura and Nei, 1993) was used instead. Under the similar conditions CII did not reveal a significant (p=0.083) result, but it was close to the level of significance (p=0.05). Thus CII result was not fully supporting the findings of Viñas et al.'s (2010). However, when AMOVA tests were repeated by using pairwise distances which is the default in the AMOVA test in Arlequin (Excoffier and Lischer, 2010) highly significant differentiation in both of the clades (p=0.005, p=0.000 in CI and CII respectively) were observed in parallel to Viñas et al.'s (2010) results. Incidentally, parallel to the test results, p values were also similar in this latter version of the AMOVA test (Table 3.7.1.7.1).

Since Aegean 1 the only population that was significantly different from Balearic, Ligurian and Ionian according to the F_{ST} results of CI, AMOVA tests were repeated by excluding Aegean 1 from the analyses. This time no significant difference was seen neither in CI (p=0.140) nor in CII (p=0.126) suggesting significant CI difference between east and west is mostly due to Aegean 1 (Table 3.7.1.7.2).

Eastern and Western Mediterranean can be separated from Sicily and Messina based on their oceanographic conditions (Krom et al., 2005). Because of that Ionian was assumed as part of the Eastern population even though it is significantly different from Aegean 1.

It can be argued that Eastern and Western populations in Mediterranean are not isolated completely, but as evidenced from AMOVA analysis there is a minor sub-division in Mediterranean (Table 3.7.1.7.2). Its presence can be detected based on the models used and or populations employed. Existence of a highly frequent (0.06) haplotype exclusively among the Eastern populations thus "private node of the Eastern population" can be considered as a further evidence for the existence of minor sub-division.

As a conclusion, presence of, at least minor subdivision, as "Eastern" and "Western" is also observed in the present study. This sub-division mostly caused by Aegean 1 (but Aegean 1 is similar to Aegean 2 and N. Levantine in each of the mtDNA based comparisons except in CII diversities). Presence of two spawning areas and oceanographic conditions could be the reason(s) of this subdivision as was proposed by Viñas et al. (2010).

Another interesting observation was about the levels of genetic diversity (π, H) of CI (π=0.0147, H=0.860**)** and CII (π=0.0041, H=0.683), in Mediterranean. CI diversity, especially π is 3 times higher than that of CII (Figure 3.7.1.5.1). Results of the present study confirms the suggested demographic histories of the clades, CI (τ=9.625-9.166) expanded in Mediterranean Sea earlier than the CII (τ= 2.096-1.273) which was originated and expanded from Atlantic Ocean. However, contrary to the findings of Viñas et al., (2010) study newly added populations from the east (N. Levantine and Aegean 2) in the present study suggested that expansion may not be in the east first (τ in the west= 2.096, τ in the east=1.273) and expansion may not be larger in the west (Θ ranges are the same in east and west, 0-9999) (Table 3.7.1.9.1.1).

Why do we see expansions in CI in mismatch distributions (Figure 3.7.1.9.1) but not in neutrality tests (Table 3.7.1.9.1.1)? Perhaps because it harbors high diversity (let's remember CI_α and CI_β) also manifested as multimodal (but not significant)

distribution as observed by mismatch distributions and this diversity interferes with the sensitivity of the neutrality tests.

4.1.2 Variabilities of the Clades in the Eastern and Western Mediterranean, Migrations, Ne's

Genetic diversities (π and H) are lower in the east (π=0.0283, H=0.867) compared to west (π=0.0291, H=0.874). Nutrient content of Mediterranean Sea tends to decline from west to east and general productivity is lowest in the east of Mediterranean with some local exceptions due to the inflow of nutrients from rivers and from the Black Sea (Margalef, 1985). One of the reasons of high diversity in the west can be explained by the existence of nutrient rich waters in the west inflowing from Atlantic to the west Mediterranean (Margalef, 1985). Nutrient rich area can maintain a larger population and larger genetic diversity. Furthermore, it may attract individuals from the east which will further increase the diversity in the west. When migration rates (calculated by Bayesian approach) was used for the entire data set, appreciable level of asymmetry in the form of high migration rate to the west (from east to west=4570, from west to east=490) was observed. However, in terms of CII, the difference in the two directions was minor (from east to west=5281, from west to east=3402) and in terms of CI the direction was opposite i.e. slightly more to the east (from east to west=4835, from west to east=5586) These results do not fit to the observations of Viñas et al. (2010) study (where both clades and entire sequences revealed high migration rates to the west) (Figure 3.7.11.2). They used maximum likelihood (ML) estimation approach. In the present study Bayesian method was used and according to the results of simulated dataset of Beerli's (2006) study, Bayesian method have higher accuracy and coverage than the ML.

In parallel to the diversities, long term effective population sizes (in terms of Θ) of Balearic (0.014) and Ligurian (0.015) were higher than those of Ionian (0.012), Aegean 1 (0.005), Aegean 2 (0.006), and N. Levantine (0.007) (Table 3.7.1.10.1). Lowest Θ was found at Aegean 1 population. When long term effective population

sizes for the west and east in terms of Θ was estimated west had higher effective population size (all sequences=0.016, CI=0.019, CII=0.011) than east (all sequences=0.007, CI=0.001, CII=0.003) as expected (Table 3.7.1.10.3). Since the exact mtDNA CR mutation rates are not known numerical estimates of Ne using different mutation rates ($1x10^{-6}$- $2.45x10^{-7}$-$1x10^{-8}$) were done. West exhibited high Ne values (all sequences=8125 -33163 -812500, CI=9525 -38878 -952500, CII=5445 -22224 -544500) compared to those of east (all sequences=3375 -13776 -337500, CI=665 -2714 -66500, CII=1280 -5224 -128000).

4.1.3 Ancient DNA Results

In the present study 5 pairs of primers were designed to study the partial mtDNA CR region which was compatible with the available data from modern samples of Mediterranean swordfish and they were successfully used to genotype 6 ancient swordfish samples for the first time in the literature.

Topology of NJ tree (Figure 3.7.1.2.1) and MJ network constructed with MAD (Figure 3.7.1.3.2) is the same with the topology of NJ tree and MJ network constructed with MD (Figure 3.7.1.3.1) even though sequences of modern samples were shortened in accordance to the sequence lengths of aDNA (192 bp). All aDNA samples are clustered with one of the sub-clades. Total of 4 haplotypes were defined for the 6 aDNA. One of those haplotypes is clustered with CII, a private haplotype of the ancient population represented by 2 ancient samples and not observed among 234 individuals of modern swordfish samples and it is 3 mutations away from one of the main nodes of the modern samples. Two ancient samples which have this same private haplotype are assigned to different dates so possibility of them belonging to same individual is low.

Regarding F_{ST} analysis with the entire sequences: ancient population was not significantly different from other populations. Meanwhile ancient population was significantly different from Aegean 1 based on CI and was significantly different

from every modern population based on CII because CII of aDNA consist of a single private haplotype (Table 3.7.1.6.2).

When PCA based on mean genetic distances between populations of MAD was examined, aDNA was located relatively further away than modern populations based on PCA of entire sequences and CII, but it was located close to the modern populations based on CI (Figure 3.7.1.5.2). Results suggested that aDNA was different from modern populations but this difference was mostly because of CII sequences. With the simulation tests between aDNA and Aegean 1, Aegean 2, N. Levantine, assuming three different time periods for aDNA samples, in all of the tests continuity could not be rejected (Figure 3.7.1.12.1). However, this observation must be retested with higher number of aDNA samples, since the lack of significant difference can be partly because of small sample sizes (n=6). Similarly, number of CI and CII samples in aDNA (n=4 for CI and n=2 for CII) are low, so tests based on CI and CII of MAD, has to be considered cautiously.

If we assume the continuity between ancient and modern fish populations then one may argue that aDNA population was not discrete or isolated population of the ancestral populations of Aegean 1, Aegean 2 and N. Levantine.

According to the long term effective population sizes (Θ) of MAD, although 95% credibility indices of populations were largely overlapping, aDNA had higher long term mean Ne (Θ=0.013) than Ionian (Θ=0.012) Aegean 1 (Θ=0.001) Aegean 2 (Θ=0.012) and Northern Levantine (Θ=0.008) and it was same as the two western populations: Balearic (Θ=0.013) and Ligurian (Θ=0.013) (Table 3.7.1.10.2). Overall, aDNA have higher Ne (Θ=0.013, Ne=6425 -26224 -642500), than Eastern (Θ=0.007, Ne=3375 -19327- 337500) and lower than Western (Θ=0.016, Ne=8125 -732327- 812500) populations.

4.2 Microsatellite Results

4.2.1 Reliability of Data

None of the analyzed loci have significantly high frequency of null alleles (Table 3.7.2.1.1.1) and all microsatellite loci used in this study were found to be in linkage equilibrium (Table 3.7.2.1.2.1) as previously reported (Kasapidis et al., 2009). Also no significant excess or deficiency of heterozygosity was detected (Table 3.7.2.2.2.1). Therefore, possibility of having genotyping error for microsatellite data is low and thus, the data set is reliable.

4.2.2 Genetic Diversity

Differences between number of alleles, allelic richness (Table 3.7.2.2.1.1) and expected heterozygosity values (Table 3.7.2.2.2.1) of Aegean and N. Levantine were not significantly different based on Mann-Whitney U rank test. Therefore, no significant differences in genetic diversity was observed among these populations. Number of alleles and observed heterozygosities of Aegean 2 and N. Levantine populations were also compared with Northwest Atlantic and West Mediterranean data presented in Kasapidis et al.'s (2009) study. Results are given in table 4.2.2.1.

Table 4.2.2.1: Number of alleles (A) and observed heterozygosities (Ho) of Northwest Atlantic (NW Atl) (Kasapidis et al., 2009), West Mediterranean (West Med) (Kasapidis et al., 2009), Aegean 2 and N. Levantine based on each locus (n=sample size).

Locus	NW Atl (n=43)		West Med (n=62)		Aegean (n=26)		N. Levantine (n=40)	
	A	Ho	A	Ho	A	Ho	A	Ho
XgSau98R1	15	0.907	11	0.871	9	0.923	11	0.795
Xg31	8	0.814	7	0.694	7	0.760	7	0.667
VBC201	9	0.744	6	0.855	6	0.875	5	0.750
Xgl-561	10	0.791	6	0.516	6	0.577	6	0.575
Xg41b	29	0.953	14	0.935	11	0.960	13	0.825
Xgl-65b	12	0.907	7	0.790	7	0.885	7	0.750
Xg51	26	0.953	15	0.887	16	0.769	17	0.900
Xgl-536	17	0.907	8	0.806	7	0.654	10	0.744

Number of alleles and observed heterozygosity values were similar in the Western Mediterranean and the Eastern Mediterranean (Aegean 2 and N. Levantine) but number of alleles and heterozygosity values of the Northwest Atlantic are higher than those of Mediterranean with the exception of heterozygosity value of VBC201. Primers of VBC201 designed based on a clone of Gilthead Seabream (*Sparus aurata*) (Kasapidis et al., 2009). Since another species was used for the design of primers it might have caused an ascertainment bias. These comparisons indicated that Eastern and Western Mediterranean populations are quite similar compared to Atlantic swordfish population in terms of number of alleles and their Ho values. However, alleles and their frequencies cannot be compared because genotyping of the same microsatellite locus in different laboratories /studies might be different.

4.2.3 Genetic Differentiation

Based on 8 microsatellite loci, F_{ST} revealed no significant differentiation (F_{ST}=0.007, p=0.053) between Northern Levantine and Aegean populations. Both PCA based on genetic distances between samples (Figure 3.7.2.3.2.1) and STRUCTURE (Figure 3.7.2.3.3.1) results also failed to show significant difference between these two

populations. Thus, it can be assumed that these two populations are not genetically differentiated based on the available microsatellite data just as was observed on the basis of mtDNA based results.

4.2.4 Long and Short Term Effective Population Sizes

Microsatellite data of the Aegean II and N. Levantine populations enabled us to provide another independent (other than mtDNA based) estimate of the long term effective population size (Ne) by Migrate-n of the Eastern swordfish populations in Mediterranean Sea.

Long term Ne indicator Θ (Table 3.7.2.4.1.1) of N. Levantine (Θ=4.240) found to be higher than that of Aegean 2 (Θ=2.190), which is parallel to the mtDNA based Θ estimations of MD and MAD.

Means of contemporary Ne distribution calculated by LD (Aegean 2=56, N. Levantine=134) and MC (Aegean 2=13, N. Levantine=48) methods were under 150, for both Aegean 2 and N. Levantine (Table 3.7.2.4.2.1) Short term Ne cannot be calculated by HE method because all results were “infinite”, indicating confidence intervals of the distribution is very large. HE method can give such a result if Ne is higher than 20 and the sample size less than 200 (Luikart et al., 2010) which is the case in here.

Results of the mean of contemporary Ne distribution calculated by MSVAR (Aegean 2=43, N. Levantine=98) are similar to the results of mean of Ne distribution of LD (Aegean 2=56, N. Levantine=134) and higher than those of MC (Aegean 2=13, N. Levantine=48). In addition, means of long term Ne distributions obtained by MSVAR analyses were very high (Aegean 2=309030, N. Levantine=263027) (Figure 3.7.2.4.3.1, Table 3.7.2.4.3.1). MSVAR indicated that effective sizes of both populations declined over time, Aegean 2 (1240 years ago) might have started declining earlier than the N. Levantine (599 years ago) (Figure 3.7.2.4.3.1). Because MSVAR method is shown to be efficient for detecting population declines or

expansions if they are not too weak or too recent, in this respect, it is shown to outperform two other methods; the M-ratio test (Garza and Williamson, 2001) and Bottleneck (Piry et al., 1999) that are also commonly used for determining ancient population size changes (Girod et al., 2011).

One of the assumptions of MSVAR is population under consideration is a closed population (effect of migration is omitted) (Storz and Beaumont, 2002). However, this assumption is not valid for swordfish populations. Yet, MSVAR was previously used to determine the Ne of species like, migratory whitefish (McCairns et al., 2012) and reed warblers (Procházka et al., 2008), none of which are composed of closed populations. Thus, these studies encouraged us to use MSVAR method.

Different estimation methods (MC, LD, HE, MSVAR) revealed different Ne sizes. The smallest Ne estimate is for Aegean 2 with MC method (Ne=13), whereas the maximum estimate is for N. Levantine with LD method (Ne=134).

As a conclusion it can be said that N. Levantine and Aegean 2 populations, with different estimation methods, indicated that current Ne values are less than 150 and sometimes estimates are as small as 13 (for Aegean 2). Furthermore, there is more than 4-fold decrease between the ancestral population and current populations of swordfish in the Eastern Mediterranean. This sharp decline is quite recent nearly 1240 years / 600 years ago.

4.3 Swordfish Around the Coasts of Turkey

Prior to 1970, the swordfish fishery in the Sea of Marmara was important. The famous Turkish traveler Evliya Çelebi in 17th century mentions, in his Book of Travels, that swordfish were caught during their migrations from the Sea of Marmara to the Black Sea in the Dalian of Beykoz in the Strait of Istanbul (Kahraman and Dağlı, 2003). It is reported that swordfish eggs were found in Marmara by the ichthyoplankton surveys which were carried out in the spring and summer months of 1954, 1955 and 1964 (Demir et al., 1956). In the similar research conducted on the

gonads of swordfish between August and January, it was shown that spawning occurs from spring until the beginning of summer (Artüz, 1963). These observations strongly suggested that there was a possible spawning area for the swordfish in the Sea of Marmara. The number of swordfish caught from the Sea of Marmara showed an increasing trend between 1909-1952, followed by a quick decline between 1956 and 1969 (Alıçlı et al., 2012). In the past decade, swordfish have not been caught in the Sea of Marmara indicating that Marmara Sea population is extinct and it could be caused by the effects of overfishing and/or aquatic pollution eventually resulting in habitat loss for swordfish.

The question of "Whether the extinct Marmara population was a distinct/ highly differentiated stock from the other populations of Mediterranean or not?" can be answered, at least roughly, with the help of aDNA samples of the present study. If ancient DNA samples of the present study might be the representatives of currently extinct population that used to live in Marmara Sea since the differences from the modern populations (pairwise F_{ST}'s in table 3.7.1.6.2 and PCA's in figure 3.7.1.5.2) can be accounted by the drift as shown by the continuity tests, it can be concluded that, on the basis of available data, Marmara population was not distinct from the Aegean or N. Levantine populations. Furthermore, consistently higher Θ in aDNA compared to Eastern populations (although not significant) might be indicating the loss of genetic diversity since the time of aDNA population until modern days.

The closest neighbors of Aegean 2 and N. Levantine is Aegean 1 population. Because of the lack of microsatellite data in Aegean 1 only mtDNA based comparisons can be made. Since there is no significant difference in clade frequencies and sequences it can be assumed that Aegean 1 is also part of the population where N. Levantine and Aegean 2 are.

4.4 Implications for Management Strategies

Understanding the genetic diversity and genetic structure as well as demographic status of natural populations is essential for sustainable management and

conservation of species. In order to be able to plan educated conservation strategies it is necessary to know whether there is a substructure within the gene pool or if a gene pool can be considered as panmictic. It is also important to assess changes in population sizes. It is known that, declining populations with small effective population sizes become more prone to effects of drift, lose their genetic diversity, have reduced fitness and accumulate deleterious mutations, consequently lose their evolutionary potential and thus they are under high risk of extinction. Effective population sizes and time of population declines can be estimated using genetic data which may also resolve population structure, especially valuable in species with continuous distribution over a vast range of habitat without geographical barriers such as marine fishes. Indeed, genetic data have been quite useful in disentangling population dynamics of marine species with high mobility. Even though oceans are relatively homogenous and lack most physical barriers, intra and inter-oceanic differences have been observed in many highly migratory fishes, such as; bigeye tuna (Gonzalez et al., 2008; Wu et al., 2014), Atlantic bluefin tuna (Carlsson et al., 2004; Riccioni et al., 2010; 2013) and albacore (Nakadate et al., 2005; Montes et al., 2012; Laconcha et al., 2015), blue marlin (Finnerty and Block, 1992; McDowell et al., 2007), whitefish (McCairns et al., 2012) and sailfish (Lu et al., 2015).

An extensive evaluation of the Western, Central and Eastern Mediterranean and Black Sea fisheries for the duration between 1970-2010 concluded that fisheries resources of these basins are at risk due to overexploitation, especially Eastern Mediterranean and Black Sea fisheries being in the worst status (Tsikliras et al., 2015). In the Eastern Mediterranean and Black Sea, the cumulative percentage of overexploited and collapsed stocks were reported as 50 % (Tsikliras et al., 2015) and this number calls for an immediate need for detailed stock assessment across, if not all, for most species, in order to be able to define fully exploited and overexploited species and also concert integrated management/conservation plans involving many species at the same time.

International Commission for the Conservation of Atlantic Tunas (ICCAT) is currently monitoring the conservation and management of swordfish living in the Atlantic Ocean and the Mediterranean Sea. Swordfish in these areas have been managed as three separate stocks; North Atlantic, South Atlantic and Mediterranean, as discussed before. Although, currently swordfish is classified as "least concern" globally, (Collette et al., 2011) regional evaluation of the Mediterranean swordfish classified this stock as "Near Threatened" and in danger of overfishing (Abdul Malak et al., 2011). Swordfish, an apex predator with only a few predators, is the only species of it genus (Palko et al., 1981). It is both ecologically and economically important for Mediterranean region (Collette et al., 2011). Dramatic decrease or increase in its population size may cause cascade of events in trophic network affecting many other species.

Since Mediterranean swordfish is considered as one uniform stock, single conservation and management plans are implemented to cover the entire Mediterranean. Data presented in this study contributed to the understanding of spatial and temporal genetic diversity of swordfish in Mediterranean by providing local data from the Eastern peripheries. Existence of previously suggested Western and Eastern Mediterranean swordfish populations is supported by the present study. Population parameter Θ indicated that Eastern populations as a whole have lower Ne values compared to Western populations. Furthermore, although limited, aDNA data exhibits the decline in Ne of Eastern population. Microsatellite data from N. Levant and Aegean 2 helped us to estimate current Ne values for the Eastern Mediterranean population. These estimates may be more reliable than the mtDNA based estimates, because mtDNA being haploid, single gene and inherited maternally cannot give accurate information about the genome. If short term Ne is already less than or equal to 50 and if the 4-fold decline is more recent (600 years) viability of the Eastern swordfish population (harboring the swordfish of Turkish coasts) is at great risk. Results of this study suggests that, Ne estimations must be repeated and if Eastern population is in greater risk, then ICCAT rules must be modified accordingly, quotas

must be set separately based on different demographic characteristics of Eastern and Western swordfish populations.

CHAPTER 5

CONCLUSION

First time in this study, swordfish samples from the north of the Aegean Sea and Mediterranean coasts of Turkey were studied genetically. Moreover, 5 pairs of primers were designed and successfully used to sequence the partial mtDNA CR of ancient swordfish and 6 ancient swordfish samples are genotyped for the first time in the literature. Conclusions of the present study can be listed as follows:

- Significant differentiation but not complete isolation was observed among Western Mediterranean (Balearic, Ligurian) and Eastern Mediterranean (Ionian, Aegean 1, Aegean 2 and N. Levantine) swordfish populations. Significance of the differentiation was mostly attributable to Aegean 1 population.

- Eastern Mediterranean has lower diversity than that of Western Mediterranean.

- Long term effective population size of Eastern Mediterranean is lower than the long term effective population sizes of Western Mediterranean.

- Even though aDNA population is slightly different from modern populations, continuity between aDNA and Aegean 1, Aegean 2 and N. Levantine cannot be rejected.

- Long term effective population size and genetic diversity of aDNA is higher than eastern and lower than western populations indicating genetic diversity decline since the time of aDNA population, but because of the low samples in aDNA populations (n=6) these results may not be very reliable.

- Aegean 2 and N. Levantine are not genetically significantly different based on the available microsatellite data.

- Number of alleles and observed heterozygosity values were similar in the Western Mediterranean and the Eastern Mediterranean (Aegean 2 and N. Levantine) but lower than Northwest Atlantic.

- Short term Ne values of N. Levantine and Aegean 2 populations are less than 150 may be as low as 13 and it suffered from4-fold decrease between the ancestral population and current populations of swordfish in the Eastern Mediterranean. This sharp decline is quite recent: 1240 years / 600 years ago.

- In contrary to the existing single management plan for Mediterranean swordfish, at least two management plans must be implemented for Eastern and Western populations. In particular, Eastern population harboring the swordfish of Turkish coasts needs an urgent conservation plan.

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APPENDIX A

Ingredients of chemical solutions used in the extraction of aDNA based on Rohland et al.'s (2010) method as follows:

Extraction Solution:

0.5 M EDTA

10 mg/ml Proteinase K

Binding Buffer:

5 M GuSCN

3 M Sodium Acetate

Washing Buffer (pH 8.0):

%50 etanol

125 mM NaCl

10 mM Tris

1 mM EDTA

TE buffer

10 mM Tris

1 mM EDTA

100 µl Tris (1M)

20 µl EDTA (0,5M)

APPENDIX B

Table 1: Results of the 8 microsatellite loci of A) Aegean 2, B) N. Levantine

A) Aegean 2				
	XgSau98R1	**Xg31**	**VBC201**	**Xgl-561**
7	169171	212214	251260	125141
8	183185	220224	248254	141149
30	169177	214214	251260	141141
31	177185	212218	245251	141141
93	161171	218220	248260	125141
94	161175	214214	000000	141149
95	161175	214214	248254	141149
96	161169	214218	251257	125125
97	161177	212214	251257	141141
98	161161	214216	000000	137141
99	163171	212214	248251	141141
100	163175	214214	257257	141149
101	161175	214214	248251	125141
102	175177	212214	254254	125141
103	171185	224226	251260	139141
104	171175	214224	251260	125141
105	161177	212214	254260	141143
106	175177	212220	251254	137141
107	177185	212218	245251	141141
108	171177	214214	251260	141141
109	161185	220224	248254	141149
110	161167	000000	251251	141141
111	161163	218220	251254	141141
112	161177	218220	251254	141141
113	161175	218220	248251	125149
114	161161	214216	254260	141141
	Xg41b	**Xgl-65b**	**Xg51**	**Xgl-536**
7	190202	267271	135153	242268
8	204206	261269	143177	250250
30	198206	271273	143147	242242
31	190198	269275	117123	242250
93	176190	271275	117117	250262

Table 1 cont.

94	188190	261265	117129	248274
95	188190	261265	129129	248248
96	190200	267271	125137	250250
97	190198	265267	123125	242248
98	176176	267271	117153	242252
99	190198	261269	135147	242248
100	176206	265271	123133	242248
101	176202	271271	135161	242248
102	198206	267271	123149	248248
103	190192	271271	117135	242242
104	190202	267271	143151	242248
105	190202	261275	137151	242268
106	198206	267269	139139	242248
107	190198	269275	135151	242248
108	182188	271273	141141	242242
109	184198	261267	153153	248248
110	198206	261267	133139	242250
111	176190	267267	117141	242248
112	176202	261273	133141	242248
113	190202	271273	117117	242242
114	000000	267271	153161	242250
B) N. Levantine				
	XgSau98R1	**Xg31**	**VBC201**	**Xgl-561**
1	175183	000000	254257	141149
2	161175	214220	251260	125143
3	161161	214214	248254	139141
4	165167	214214	251254	137141
5	161169	214218	254254	141141
6	161175	218220	254254	125141
9	173185	212226	251251	125141
32	167175	214214	251254	141141
33	175177	214214	251260	141141
34	161177	212212	251260	125125
35	161171	212212	248248	141141
37	177177	214214	248260	125143
40	163163	214220	248251	137141
41	163171	212214	251260	139141
53	169171	212214	248260	141141
54	000000	000000	000000	000000
55	173175	212224	251251	139149
56	177177	214214	260260	139149
57	167171	212214	248260	141149

Table 1 cont.

58	161161	214214	254260	125137
61	175177	210212	248248	141141
62	175175	212212	251260	141141
63	173177	212220	251254	141141
64	161185	214224	251254	141141
65	169175	212218	254257	141143
66	161173	214220	251254	149149
72	169177	212212	248254	141141
73	161175	214224	251254	125139
74	161169	212214	251251	143143
75	161175	214224	251254	125139
76	161173	212214	251251	141149
83	000000	000000	000000	000000
84	169185	214214	254260	141141
85	000000	212214	248257	137149
86	163171	218220	254254	141149
87	165175	212214	248251	141141
88	161169	214214	251260	139143
89	175175	212214	251254	141143
90	161161	214220	251254	139141
91	177185	214224	254260	141141
92	161175	212220	251254	143143
115	161177	212214	251260	141143
	Xg41b	**Xgl-65b**	**Xg51**	**Xgl-536**
1	174182	267269	133161	250268
2	190206	271275	151151	242252
3	206218	269271	123133	250250
4	184218	269271	117147	242248
5	190198	271271	147153	242248
6	198218	269269	135155	242250
9	174190	261273	125141	262274
32	190198	269275	133135	242242
33	190198	271275	135147	242242
34	198218	261271	133135	268268
35	198198	271271	135149	248268
37	206206	267275	133147	250250
40	190202	269275	123137	250250
41	202206	271271	117135	248296
53	190198	261273	117123	268296
54	000000	000000	000000	000000
55	198198	261275	147147	250268

Table 1 cont.

56	190206	271275	147177	242248
57	194202	271271	133151	248248
58	202210	267269	147147	248250
61	190198	265273	129129	246250
62	192208	271271	125141	242250
63	198218	271271	125147	242248
64	206218	267275	123149	242250
65	198198	271275	151155	248248
66	190208	267271	123147	242248
72	188190	273275	133147	000000
73	190206	261275	123129	242248
74	190206	271271	139143	242262
75	190206	261275	123129	242248
76	202206	261275	143151	242268
83	000000	000000	000000	000000
84	202206	267271	117147	242250
85	198198	271271	133149	242268
86	192198	273275	117125	242250
87	198198	269271	117135	242242
88	198206	267273	135149	254268
89	198218	261271	137147	242242
90	202218	265271	135147	248262
91	190198	271271	117125	242248
92	198206	261271	117161	248250
115	198198	261275	117161	242296

APPENDIX C

Table 1: Clades and sub-clades of A) Aegean 2, B) N. Levantine c) aDNA αβ

A) Aegean 2					
7	CII	**98**	CI_α	**107**	CI_α
8	CI_α	**99**	CI_α	**108**	CII
30	CII	**100**	CI_α	**109**	CI_α
31	CI_α	**101**	CII	**110**	CI_α
93	CII	**102**	CII	**111**	CI_β
94	CI_α	**103**	CI_α	**112**	CII
95	CI_α	**104**	CII	**113**	CI_α
96	CI_β	**105**	CI_α	**114**	CI_α
97	CII	**106**	CI_β		
B) N. Levantine					
1	CI_α	**53**	CI_α	74	CI_β
2	CII	**54**	CII	**75**	CI_β
3	CII	**55**	CI_α	**76**	CI_α
4	CI_α	**56**	CII	**83**	CI_α
5	CI_α	**57**	CI_α	**84**	CI_α
6	CI_α	**58**	CI_α	**85**	CII
9	CII	**61**	CII	**86**	CII
32	CII	**62**	CI_β	**87**	CI_α
33	CI_α	**63**	CII	**88**	CI_α
34	CI_α	**64**	CII	**89**	CII
35	CI_β	**65**	CI_α	**90**	CI_α
37	CII	**66**	CII	**91**	CI_α
40	CI_β	**72**	CI_β	**92**	CI_β
41	CI_α	**73**	CI_β	**115**	CI_α
C) aDNA					
YK1	CI_α	**YK3**	CII	**YK5**	CII
YK2	CI_α	**YK4**	CI_β	**YK6**	CI_α

APPENDIX D

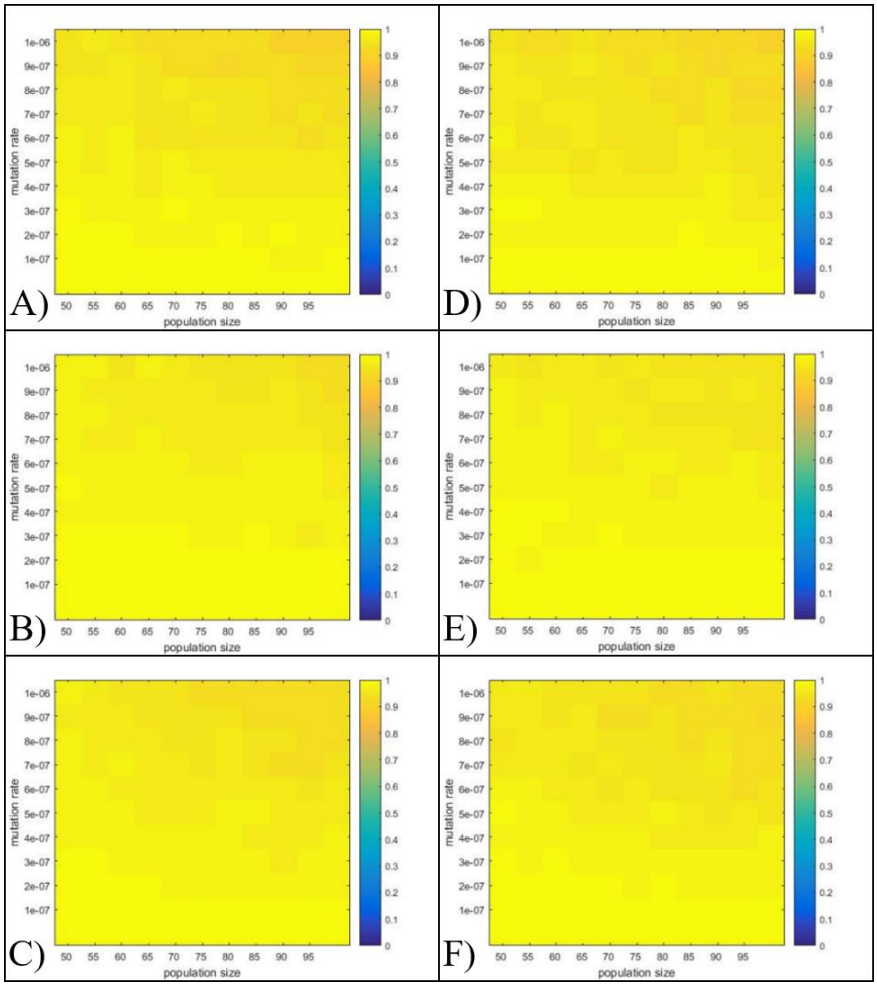

Figure 1: The heat map in the form of 11 X 11grids for each set of parameter combinations. Each grid represents the 1000 coalescent simulations to test population continuity under an exponential decline model based on parameter combinations A) starting with 4th aDNA and ending with modern Aegean 1, B) starting with 4t^{h} aDNA and ending with modern Aegean 2, B) starting with 4th aDNA and ending with modern N. Levantine, D) starting with 10th aDNA and ending with modern Aegean 1, E) starting with 10th aDNA and ending with modern Aegean 2, F) starting with 10th aDNA and ending with modern N. Levantine. Ne of modern populations were represented on the x-axes and mutation rates on they-axes. The colors scale near heat maps show the proportion of simulated F_{ST} values greater than that observed values in each grid.

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EDUCATION

PhD, Biology 2017
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Thesis: Genetic structure of modern and ancient swordfish populations from coasts of Turkey

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PUBLICATIONS

Peer Reviewed Articles:

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