INVESTIGATING THE ROLE OF THE TURKISH STRAITS SYSTEM AS A PHYLOGEOGRAPHICAL BREAK IN THE MEDITERRANEAN-BLACK SEA TRANSITION

by

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To my dad, Erdem Kalkan

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ABSTRACT

Understanding the allopatric isolation and evolutionary processes in the marine realm can be difficult due to high dispersal potential of pelagic larvae. In addition, the role of barriers in shaping gene flow patterns between the populations of marine species can be less pronounced than their terrestrial counterparts. Straits are such potential barriers to gene flow in the marine environment, resulting in the isolation of populations on either side. The Turkish Straits System, comprising two straits (the Dardanelles and the Bosphorus Strait) and the Sea of Marmara forms the only connection between the Black Sea and the Mediterranean, and is a unique ecosystem with a well-defined two-layered stratification and current regime. The role of Turkish Straits System on gene flow, as a barrier and/or corridor has been proposed, but not extensively tested using genetics. Here, using three regions of mitochondrial DNA (CO1, COIII and 16S) and five microsatellite markers I tried to understand the effect of the system on gene flow in populations of the Mediterranean Mussel, *Mytilus galloprovincialis* (Lamarck, 1819), the common European prawn, *Palaemon elegans* Rathke, 1837 and the marbled crab, *Pachygrapsus marmoratus* (Fabricius, 1787). With this study, individuals belonging to three species were collected from 42 sampling sites, encompassing the Black Sea, the Turkish Straits System and the Mediterranean Sea. The results of the mtDNA analyses of *Mytilus galloprovincialis* showed that the Black Sea populations were isolated and differentiated from those in the Aegean during the last ice age and subsequently were able to colonize the Sea of Marmara and Aegean twice, with larval transport via the surface currents of the Turkish Straits System. However, individuals from the Aegean population were not able to migrate into the Turkish Straits System and the Black Sea in the reverse direction due to the lower-layer currents of the system. Microsatellite analyses did not support the mtDNA differentiation observed among the Black Sea and the Aegean mussel populations, suggesting that they did not correspond to different species. Two different haplogroups were detected in *Palaemon elegans* as a result of the mtDNA analyses, though with a lower degree of differentiation than previously recorded in the literature. The results of CO1 analysis for *Pachygrapsus marmoratus* also indicated a weak restriction of gene flow from the Mediterranean to the Black Sea. For all three species, the Turkish Straits System played a

semi-permeable barrier role to gene flow and dispersal. This semi-permeable characteristic of the Turkish Straits System, simultaneously acting as a barrier and corridor to gene flow is relatively uncommon, and has been documented in the Turkish Straits System for the first time, using genetics methods.

ÖZET

Pelajik larvaların yüksek dağılım potansiyellerinden dolayı, denizel ortamda allopatrik izolasyonu ve evrimsel süreçleri anlamak çok kolay değildir. Denizel türlerin popülasyonları arasında gen akışını şekillendirmede bariyerlerin rolü de karadaki kadar belirgin değildir. Boğazlar her iki taraflarında kalan popülasyonların birbirleriyle iletişimlerine engel olduklarından denizel ortamda gen akışı için potansiyel bariyerlerdir. İki boğazı (İstanbul ve Çanakkale Boğazları) ve Marmara Denizi'ni kapsayan Türk Boğazlar Sistemi, Karadeniz'in Akdeniz'le bağlantısını sağlayan tek su yoludur ve iki tabakalı akıntı sisteminden dolayı kendine has bir ekosistem oluşturur. Türk Boğazlar Sistemi'nin gen akışındaki bariyer ve/veya koridor olarak rolünden bugüne kadar bahsedilmiş ancak genetik yöntemler kullanılarak kapsamlı şekilde test edilmemiştir. Burada mitokondrial DNA'nın üç bölgesini (CO1, COIII ve 16S) ve beş mikrosatelit lokusunu kullanarak Türk Boğazlar Sistemi'nin kara midye, *Mytilus galloprovincialis* (Lamarck, 1819), teke karidesi, *Palaemon elegans* Rathke, 1837 ve mermer yengeci, *Pachygrapsus marmoratus* (Fabricius, 1787) türlerinin popülasyonları arasında gen akışını nasıl etkilediğini anlamaya çalıştım. Bu çalışma ile bu üç türe ait bireyler Karadeniz, Türk Boğazlar Sistemi ve Akdeniz'i kapsayan toplam 42 noktadan örneklendi. *Mytilus galloprovincialis* mtDNA analiz sonuçları, son buzul çağı süresince Karadeniz midye popülasyonunun Ege Denizi'ndekinden izole olduğunu ve daha sonrasında Türk Boğazlar Sistemi'nin üst akıntısı ile larvaların taşınmasıyla Marmara ve Ege Denizi'ni iki kere kolonize ettiklerini göstermiştir. Ancak Ege popülasyonundan bireyler sistemin alt akıntı rejiminden dolayı ters yönde Türk Boğazlar Sistemi ve Karadeniz'e geçiş yapamamışlardır. Mikrosatelit analiz sonuçları Karadeniz ve Ege midye popülasyonları arasında mtDNA'da gözlemlenen farkı desteklemeyerek, bu iki grubun iki farklı tür olmadıklarını göstermiştir. mtDNA analizleri sonucunda *Palaemon elegans* türünde daha önce literatürde tespit edilenin aksine daha az bir farkla birbirinden ayrılmış iki farklı haplogrup tespit edilmiştir. *Pachygrapsus marmoratus* türü içinse CO1 analiz sonuçları Akdeniz ve Karadeniz'deki iki popülasyon arasında gen akımında zayıf bir sınırlama olduğunu göstermiştir. Türk Boğazlar Sistemi üç tür için de gen akışı ve dağılım açısından yarı-geçirgen bir bariyer rolü oynamıştır. Türk Boğazlar Sistemi'nin gen akışı için hem bir

bariyer hem de koridor olmasına neden olan yarı-geçirgen özelliği genetik metodlar kullanılarak bu çalışma ile ilk defa tespit edilmiştir.

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LIST OF SYMBOLS/ABBREVIATIONS

Symbol	Explanation	Units used
AMOVA	Analysis of molecular variance	
bp	Base pair	
CO1	Cytochrome c oxidase subunit 1	
COIII	Cytochrome c oxidase subunit III	
dNTP	Deoxyribonucleotide triphosphates	
D	Tajima's D	
DUI	Doubly uniparental inheritance	
EF-1α	Elongation factor 1 α	
Fs	Fu's Fs	
h	Haplotype diversity	
He	Expected heterozygosity	
Ho	Observed heterozygosity	
IBD	Isolation by distance	
k	Mean number of pairwise differences	
kbp	Kilo base pair	
µl	Microliter	
min	Minute	
mM	Milimolar	10^{0}mol/m^{3}
µM	Micromolar	10^{-3}mol/m^{3}
mtDNA	Mitochondrial DNA	
Mya	Million years ago	
n	Number of samples	
Na	Number of alleles	
Nh	Number of haplotypes	
Np	Number of polymorphic sites	
Ne(mt)	Mitochondrial effective population size	
PCR	Polymerase chain reaction	

R_2	Ramos- Onsins and Rozas's R_2
rRNA	Ribosomal RNA
s	Second
SSR	Simple sequence repeat
tRNA	Transfer RNA
TSS	Turkish Straits System
ya	Years ago
yr	Year
π	Nucleotide diversity
16S rRNA	16S Ribosomal RNA

1. INTRODUCTION

1.1. Phylogeography

Phylogeography was described as "the field of study concerned with the principles and processes governing the geographical distributions of genealogical lineages especially those at the intraspecific level" by Avise and Vrijenhoek (1987). The field has proved dramatically successful in explaining how historical events, which happened million years ago, has affected animal and plant distributions (Beebee and Rowe, 2004), and operates on the basic premise that the most distant populations should accumulate more differences because they were isolated from each other for longer periods (Beebee and Rowe, 2004; Hewitt, 2001). The genetic differentiation among geographic scales is generally observed in the populations of all species and the level of the differentiation between the populations is related to the patterns of gene flow (Hewitt 2005, 2001; Ehrlich and Raven, 1969). Gene flow can be broadly defined as the movement of genes, mediated by individual organisms or their gametes, between subpopulations.

Phylogeographic boundaries are important because they delineate zones of major genetic change (Ehrlich and Raven, 1969). Physical barriers (current or ancient) can cause reproductive isolation between species in the marine environment, leading in some instances to allopatric speciation. In allopatric speciation, a population gets divided into two or more populations as a result of a formation of a geographic barrier which prevents gene flow between populations; subsequently these populations can diverge sufficiently to become distinct species (Carlon and Budd, 2002).

1.2. Mitochondrial DNA and Microsatellite DNA

The use of molecular tools, having the potential to address questions in phylogeography and population genetics allows combination of genetic information with that from geological, paleontological, and climatic records to reconstruct the population history of species (Avise, 1992). Microsatellites, known as simple sequence repeats or SSRs, are di-, tri-, or tetra- nucleotide tandem repeats in DNA sequences. They are very

polymorphic and show high levels of allelic variation, due to different numbers of repeats (Beebee and Rowe, 2004). Mutation rates in microsatellite loci are generally related with a number of features such as repeat length, repeat composition and flanking sequences outside the microsatellite locus (Masters et al., 2011). Microsatellites are very popular for phylogeographic research (e.g. Escorza-Trevino and Dizon, 2000; Koskinen, 2002; Duran, 2004) because phylogeographic information can be inferred from microsatellite data by the geographical clustering of populations in relation to allele frequency similarities (Hedrick, 1999; Lemaire et al., 2005).

Mitochondrial DNA (mtDNA), on the other hand, is found in the mitochondria of eukaryotic cells, is mostly haploid and maternally inherited (Giles et al., 1980; Avise and Vrijenhoek, 1987; Hoarau, 2004), and encode rRNA and tRNA molecules so it can perform its own protein synthesis. Animal mtDNAs are circular double-stranded molecules that are in the 15-17.5 kbp size range (Beebee and Rowe, 2004). On the other hand, nuclear DNA is usually diploid and bipaternally inherited. Because of these differences, mitochondrial effective population size [Ne(mt)] is four times smaller than that of nuclear loci (Hefti-Gautschi et al., 2009), therefore haplotype frequencies can drift rapidly, creating genetic differences among populations in relatively short timescales (Friesen et al., 1996). Also, drift might be too small to detect in nuclear DNA, and nuclear DNA will reach migration-drift equilibrium later than mitochondrial DNA (Birky-Jr et al., 1989). In addition, each uniparentally inherited haplotype has just one ancestor in the previous generation because it has also been widely assumed that mtDNA does not undergo recombination like nuclear DNA does (Avise, 1994; Beebee and Rowe, 2004).

1.3. Gene Flow and the Barriers in the Marine Environment

As mentioned above, allopatric isolation, especially through geographic barriers has been considered to comprise one of the main mechanisms of speciation (Ladoukakis et al., 2011; Evanno et al., 2005; Hoarau et al., 2004; Mayr and Ashlock, 1991). In addition to vicariance, environmental factors (e.g. currents, glaciation) are some of the main factors causing the genetic discontinuities across geographical ranges (Cunningham and Collins, 1998) and differentiation between and within species (Wares, 2002; Cunningham and Collins, 1998; Roy et al., 1996). Understanding these processes in the marine environment

is not easy because the barriers are less obvious, and marine species may show panmixia over wide geographical ranges, due to long-distance dispersal capabilities of their eggs, larvae or adults (Cunningham and Collins, 1998; Ward et al., 1994; Palumbi, 1994).

For species with planktotrophic larvae, the general idea is that of little genetic structure and high gene flow between populations of the species because they leave their gametes and larvae into the water column and the duration of the planktonic stage changes from one species to the other depending life cycle of the species. More usually, the planktonic stage of benthic marine invertebrates lasts from a few days to weeks (Avise, 1994). On the other hand, species with low dispersal potential (*i.e.* with lecitotrophic larvae) are expected to show more clear patterns of genetic structure. High levels of actual gene flow does not always correspond to high larval dispersal potential, however (Taylor and Hellberg, 2003), as other biologic, oceanographic and biogeographic factors can shape the population structure of the species, especially the marine invertebrates through space and time (Galarza et al., 2009; Taylor and Hellberg, 2003; Arndt and Smith, 1998; Palumbi, 1994).

There are many studies reporting marine species that show population differentiation beyond their dispersal capabilities (Riginos and Nachman, 2001; Palumbi, 1994). Straits (e.g. Cook, Tsugaru, Danish, Gibraltar) are such potential geographic barriers to gene flow in the oceans, and the distribution of genetic diversity around them can help to understand different aspects of vicariant speciation in the marine realm. Various studies investigating the effects of straits on gene flow all around the world point out to their importance as barriers. For instance, in the Pacific, the Tsugaru and the Cook Straits constitute a significant barrier for some fish species (Briggs and Bowen, 2012), intertidal endemic limpets (Goldstien et al., 2006) and a sea star (Ayers and Waters, 2005). In the North Atlantic, the Danish Straits act as a semi-permeable barrier to migration for Atlantic salmon (Nilsson et al., 2001) and European nine-spinned sticklebacks (Teacher et al., 2011).

Another important strait is Gibraltar, connecting the Atlantic and the Mediterranean. A comprehensive evaluation of the Strait of Gibraltar as a barrier to intraspecific gene flow has been undertaken by Patarnello et al. (2007). Reviewing 20 studies on 61 species, they

found that not only vicariant barriers (the Gibraltar Strait and the Almeria-Oran Front), but also paleoclimate fluctuation and life-history traits have had important effects on the phylogeographical patterns observed in the Atlantic-Mediterranean transition.

1.4. Genetic Differentiation around the Turkish Straits System – An Overview of the Literature

There are not many studies that focus on the role of the Turkish Straits System on the population differentiation of marine species. The studies that exist mostly have researched the extent of genetic differentiation between the Black Sea and the Mediterranean. Population surveys over a wide geographical range spanning the northeast Atlantic Ocean, the Mediterranean Sea (west and east) and the Black Sea were reported for three sea grass species (*Zostera marina*, *Z. noltii* and *Posidonia oceanic*; Coyer et al. 2004; Olsen et al. 2004; Meinesz et al. 2009), for seven invertebrates (*Mytilus galloprovincialis*; Ladoukakis et al. 2002; Kalkan et al. 2011; *Sagitta setosa*; Peijnenburg and Pierrot-Bults 2004; Peijnenburg et al. 2006; *Calanus helgolandicus*, *C. euxinus*, Papadopoulos et al. 2005; *Chthamalus stellatus* and *C. montagui*; Shemesh et al. 2009); for four fishes (*Engraulis encrasicolus*; Magoulas et al. 2006; Erdoğan et al. 2009; *Trachurus mediterraneus*; Turan et al. 2009a; *Trachurus trachurus*; Turan et al. 2009b; *Mugil cephalus*; Durand et al. 2013) and for a mammal (*Phocoena phocoena*; Viaud-Martínez et al. 2007).

Olsen et al. (2004) and Coyer et al. (2004) detected three well-resolved groups (north Europe, Mauritania and Black Sea/Azov Sea) in two species of *Zostera*. Meinesz et al. (2009) firstly recorded the presence of large *Posidonia oceanica* meadows in the Sea of Marmara and in the Dardanelles, and they suggested that the presently isolated *P. oceanica* beds in the Sea of Marmara were a relic population composed of genotypes adapted to brackish waters and growing clonally.

Peijnenburg and Pierrot-Bults (2004), in a mtDNA study of the holoplanktonic chaetognath *Sagitta setosa*, revealed strong phylogeographic structure suggesting that Northeast (NE) Atlantic, Mediterranean and Black Sea populations were genetically disjunct. Peijnenburg et al. (2006) used a higher sampling intensity and a combination of mitochondrial and four microsatellite markers to reveal population structuring between and

within basins. The analysis indicated significant differentiation in *S. setosa* populations confirming earlier results.

Shemesh et al. (2009) tried to revise the distribution of *Chthamalus montagui* and *C. stellatus* by using both mtDNA and nuclear DNA markers to confirm the genetic differences in the Eastern Atlantic, Mediterranean Sea and Black Sea. While they found significant genetic differentiation between the Mediterranean and East Atlantic populations of *C. montagui* for all DNA markers, only the nuclear EF-1α sequences of *C. stellatus* showed the isolation of the Atlantic population from the Mediterranean and Aegean ones.

Magoulas et al. (2006) focused on European anchovy, *Engraulis encrasicolus*, distributed in the Black Sea, throughout the Mediterranean and in the Atlantic off West Africa and Europe, to better understand the historical and contemporary components of anchovy population structure. This study together with Magoulas et al. (1996) covered most of the distribution of the anchovy in the Mediterranean and eastern North Atlantic and showed that European anchovy populations were genetically structured to a greater extent than seen in populations of other species of anchovies and small pelagic fishes. The population genetic structure of the European anchovy again was examined by Erdoğan et al. (2009) throughout the Black Sea, Sea of Marmara and the Aegean Sea as well. While the Aegean Sea and Marmara Sea samples were the most isolated from all others for morphometric and meristic characters, genetic analyses suggested low levels of differentiation in the eastern Black Sea samples.

Turan et al. (2009a) examined genetic variations between geographic populations of Atlantic horse mackerel (*Trachurus trachurus*) from the Black, Marmara, Aegean and north eastern Mediterranean Seas, and they suggested high differentiation of the Black Sea sample with respect to the other samples. In another similar study, the population differentiation between the populations of *Trachurus mediterraneus* (Mediterranean horse mackerel) in Turkish territorial waters was investigated by Turan et al. (2009b). The results provided the first genetic evidence for the existence of three distinct *T. mediterraneus* populations in the Black Sea and north-eastern Mediterranean Sea.

The population structure and evolutionary history of grey mullet, *Mugil cephalus*, were investigated in the northeast Atlantic Ocean, Mediterranean and Black Seas (Durand et al., 2013). They detected the highest level of genetic heterogeneity at nuclear markers at Almeria-Oran Front and the Bosphorus Strait, and revealed genetic heterogeneity in the eastern Mediterranean basin.

Another interesting study was performed on a marine mammal by Viaud-Martínez et al. (2007) on the northeast Atlantic, western and eastern basins of the Mediterranean Sea and the Black Seas. They tested the hypothesis that Black Sea porpoises differ from those in the Atlantic Ocean. Within the Black Sea, they found relatively low levels of genetic diversity and surprisingly the Black Sea population shared no haplotypes with the eastern Atlantic populations, suggesting that they have been separated for thousands of years. Consequently, they described a subspecies *Phocoena phocoena relicta* from the eastern region.

As can be seen above, in the above studies, the transition between the Black Sea and the Mediterranean, namely the Turkish Straits System has been understudied generally with low or no sampling effort. The sample sizes in most of these studies, especially in the Black Sea and the Turkish Straits System, have not been sufficient to allow reliable comparisons in a local or broad geographic context.

1.5. General Overview of the Study Area

My study area consists of the coasts of Anatolian Peninsula surrounded by eastern Mediterranean Sea in the south and southwest, the Turkish Straits System in the northwest and the Black Sea in the north. I discuss the basic properties of these seas in greater detail next.

The Black Sea, the world's largest inland sea, is connected to the Mediterranean Sea via the Turkish Straits System (the Bosphorus Strait, the Sea of Marmara and the Dardanelles) and to the Azov Sea via the shallow Kerch Strait (Bakan and Büyükgüngör, 2000). The Black Sea area is a deep basin (maximum depth of ~2200 m) with a large shelf (Stanev, 2005; Zaitsev and Mamaev, 1997). Low-saline surface water of river origin

overlies high-saline Mediterranean water (Murray et al., 1989). Ninety percent of the Black Sea is anoxic as a result of past geological events and contains high levels of hydrogen sulphide (Aksu et al., 2002; Zaitsev and Mamaev, 1997). The hydrogen sulphide layer lies some 100 to 200 m below the surface. The amount of evaporation, precipitation and river runoff are the main reasons affecting the salinity concentration of the surface waters but those variations are almost absent below 200 m. The average surface salinity is reported as 18.0-18.5‰ and it has been measured to be 1.0-1.5‰ lower or higher than this value depending on seasonal changes (Balkas et al., 1990).

The Mediterranean Sea is connected to the Atlantic Ocean through the Straits of Gibraltar on the west, and to the Black Sea through the Turkish Straits System (the Bosphorus Strait, the Sea of Marmara and the Dardanelles). While the Strait of Gibraltar divides the Atlantic Ocean and western Mediterranean Sea, the Straits of Sicily and Messina separate the eastern Mediterranean from the western Mediterranean. In addition, the Mediterranean Sea connects to the Red Sea through the man-made Suez Canal in the southeast (Patarnello et al., 2007). The maximum depth of the eastern and western basins of the Mediterranean Sea are reported to be approximately 3400 m and 4200 m, respectively (Hersey, 1965). Less dense water of the North Atlantic Ocean (36.15 ‰), the only oceanic water source, enters the Mediterranean Sea though the Straits of Gibraltar (Millot, 1999). Subsequently, the salinity tends to increase from west to east (38-38.5 ‰) due to the intense evaporation in the basin (Patarnello et al., 2007).

The Aegean Sea constitutes the northeasterly part of the eastern Mediterranean Sea. It is connected to the Black Sea with the Turkish Straits System (TSS). The connection of the Aegean Sea is with the Mediterranean Sea to the south, through the passages between Crete-Carpathos-Rhodos-Turkey (southeast) and Crete-Kithira-Peloponnesos (southwest) (Poulos et al., 1997).

The Sea of Marmara is an almost completely enclosed sea and its connection with the Black Sea and the Aegean Sea is through two straits (the Bosporus Strait and the Dardanelles). The average depth of the Bosporus Strait is 35m and it is 31 km long and 0.7-3.5 km wide. The Dardanelles is nearly 62 km long and 1.3-7 km wide. The average depth of the strait is 55 m (Poulos et al., 1997; Ergin et al., 1991).

The colder and less saline Black Sea water enters to the Aegean Sea though the Turkish Straits System (Figure 1.1). The system actually comprises a very unique ecosystem with each of its parts having different biological, physiographical and hydrological characteristics (Ozturk and Ozturk, 1996; Ozsoy et al., 1996; Besiktepe et al., 1994; Oguz et al., 1990). It has a two-layer stratification and the reason of opposing current flow is due to the density differences between the Aegean and Black Sea waters (Oguz and Sur, 1989). While the cooler (5-15°C) and brackish (17-20 ‰) Black Sea waters flow down to the Aegean on the upper layer (the northward flow), the warmer (15-20°C) and more saline (38-39 ‰) Mediterranean waters flow in the opposite direction on the lower layer (the southward flow) (Ozsoy et al., 1996; Oguz et al., 1990). These two layers are separated by a pycnocline, at an average depth of 25 m (Patarnello et al., 2007).

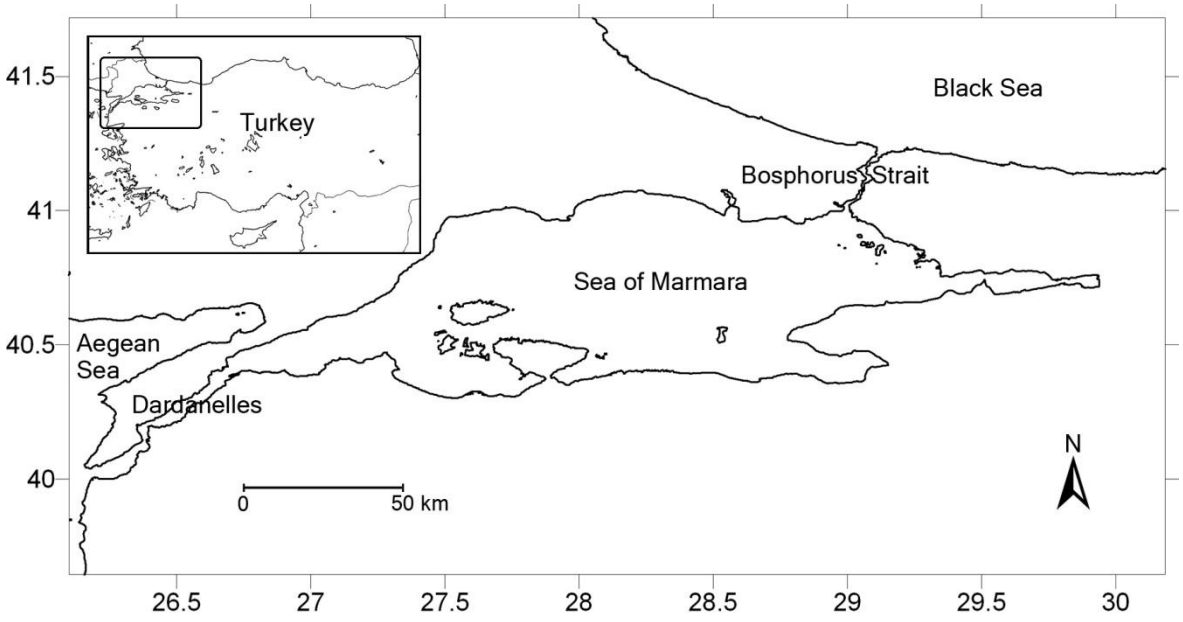


Figure 1.1. The map showing the Turkish Straits System.

1.6. Palaeogeography of the Turkish Straits System

The Turkish Straits System has importance in terms of the paleoceanographic evolution of the eastern Mediterranean (Aksu et al., 1995). During the last ~30,000 yr, the Sea of Marmara was isolated from both the Black Sea and the Aegean Sea at least two times. This isolation happened because the levels of the Aegean Sea and the Black Sea stayed below the level of the sills of the Bosphorus Strait and the Dardanelles. During the transition to the last glacial maximum, the water level of the eastern Mediterranean and the Black Sea decreased, and the Sea of Marmara was completely isolated from both Black Sea and the Aegean (~15,000-21,000 ya). Therefore, the Sea of Marmara and the Black Sea did not have a connection with the eastern Mediterranean.

In the post-glacial interval (~11,000-14,000 ya), the sea level of the Black Sea and the eastern Mediterranean started to rise because the humidity level progressively increased, which caused large rivers such as Don, Dnieper, Dniester, to raise the water level in the Black Sea. Subsequently, waters coming from the Black Sea breached the sill in the Bosphorus Strait and flowed through the Sea of Marmara and the Aegean (Aksu et al., 2002; Zaitsev and Mamaev, 1997). Therefore, approximately 10,000 ya the Black Sea turned to its current phase, and its connection with the Mediterranean was re-established through the Turkish Strait System approximately 7000 ya (the Bosphorus Strait, the Sea of Marmara and the Dardanelles) (Zaitsev & Mamaev 1997; Herman, 1988).

1.7. General Information about Selected Species

Three marine invertebrate species (*Mytilus galloprovincialis* Lamarck, 1819, *Palaemon elegans* Rathke, 1837 and *Pachygrapsus marmoratus* (Fabricius, 1787), were chosen for this study due to their wide distribution range. One important point was to be able to find them in three different water bodies of interest; Black Sea, TSS and the Mediterranean to be able to study genetic differences among the populations. Another reason for selecting these species was that they are relatively easy to collect in large numbers.

1.7.1. *Mytilus galloprovincialis* Lamarck, 1819

The Mediterranean mussel, *Mytilus galloprovincialis,* can be observed from the Black Sea and Mediterranean to the British Isles in Europe (Seed, 1992; Gosling, 1992; Koehn, 1991). It is essentially sessile, and fertilization of its eggs and larval development occur in the water column; therefore its dispersal occurs during its planktonic larval stage. According to Sigurdsson et al. (1976), the postlarvae can remain planktonic using long monofilament threads to increase viscous drag. However, empirical estimates related with dispersal distances of mussels showed that larvae can typically disperse 20-50 km (Gilg and Hilbish, 2003; McQuaid and Phillips, 2000; Hilbish and Koehn, 1985), which depends on the velocity of the currents.

While the mitochondrial genome in animals is maternally inherited, there is an exception in the mussel family Mytilidae (Zouros et al., 1994; Skibinski et al., 1994). This mechanism is called doubly uniparental inheritance (DUI), where there exist three types of mitochondrial genomes and the mitochondrial DNA can be transmitted both maternally and paternally. While one type of the mitochondrial DNA is transmitted maternally to offspring of both sexes (F genome), the other one is transmitted paternally to male offsprings only (M and C genomes) (Venetis et al., 2007; Ladoukakis et al., 2002).

One of the important studies in the Mediterranean Sea was performed by Ladoukakis et al. (2002). They investigated the mtDNA variation in the mussel *Mytilus galloprovincialis.* They collected specimens from the Black Sea, the Mediterranean Sea and the Spanish Atlantic coast and found differentiation between the populations of *M. galloprovincialis* from the Mediterranean (Adriatic, Ionian, and southern, middle and northern Aegean) and northern Black Sea (Sevastopol, Ukraine). Focusing on the differentiation between the northern Aegean and the Black Sea, and sampling around the Bosphorus Strait, Kalkan et al. (2011) used mitochondrial DNA and microsatellites to test whether the strait restricted gene flow locally, and was a barrier that caused the previously observed differentiation between northern Black Sea and northern Aegean. This work showed that the Bosphorus Strait did not present a hydrographic barrier limiting the gene flow between the Black Sea and the Sea of Marmara – ‘and raised the hypothesis that the critical point limiting the gene flow might be the Dardanelles’.

1.7.2. *Palaemon elegans* Rathke, 1837

The native distribution of *Palaemon elegans* is from the Atlantic Ocean (from Scotland and Norway to Mauritania including the Azores, Madeira and Canary Islands) to the entire Mediterranean Sea and the Black Sea (Lapinska, 2006; D'Udekem d'Acoz, 1999). *P. elegans* usually lives in the tidal zone and the species can be observed not only in hypersaline lagoons but also in brackish estuaries due to its high tolerance of variable environmental conditions such as a wide range of salinities, temperatures and oxygen (Reuschel et al., 2010; Berglund and Bengtsson, 1981; Berglund, 1980). It occurs in large numbers in ports and fishing harbours, and usually prefers sandy bottom substrate covered with macroalgae and seagrasses, and stony bottom substrate (Hayward and Ryland, 1995; Dalla Via, 1985). The complete larval development of this species takes places in the marine environment with 6-9 zoeal stages based on environmental factors (Fincham, 1977).

Reuschel et al. (2010) detected a genetic structure in *Palaemon elegans* using two mitochondrial genes; the 16srRNA and the more variable the cytochrome c oxidase subunit I (CO1). They defined three clearly distinct haplogroups: Type I (Atlantic and Alboran Sea), Type II (entirely Mediterranean and Type III (Mediterranean plus Baltic, Caspian and Black Seas). They did not detect any morphological differences between the different genetic groups and suggested that *P. elegans* represents a species complex. da Silva et al. (2011) also discovered a difference in one amino acid position between Northeast Atlantic Ocean and Baltic populations of *P. elegans*. They did not consider this difference as indicating species separation and suggested reexamination of specimens.

1.7.3. *Pachygrapsus marmoratus* (Fabricius, 1787)

The marbled crab, *Pachygrapsus marmoratus* (Fabricius, 1787), lives on the upper and middle levels of rocky shores. Its natural distribution includes the Black Sea, the Mediterranean Sea and the eastern Atlantic coasts of Europe and Africa (Dauvin, 2012; d'Udekem d'Acoz, 1999). It is omnivorous, and algae and small animals comprise its main diet (Cannicci et al., 2007). Adults are sedentary, occupying a specific area (Cannicci et al.,

1999). Its planktonic larval phase lasts about four weeks and the connection between the populations is potentially provided by larvae (Cuesta and Rodríguez, 2000).

Fratini et al. (2008) tried to understand the effect of heavy metal stress on the genetic diversity of natural populations of *Pachygrapsus marmoratus*. They collected tissue samples from polluted and unpolluted sites along the Tuscan coast (Mediterranean Sea). They found that the populations from polluted sites were genetically less variable when compared to populations from unpolluted sites. Their results supported the "genetic erosion" hypothesis for heavy metal exposure in natural environments.

Silva et al. (2009), using six variable microsatellite loci, studied the population genetic structure of the shore crab *Pachygrapsus marmoratus* along the Portuguese coast. They did not detect genetic differentiation among populations based on a geographic gradient. However, they found that one population (in Praia das Avencas, Portugal) was genetically more separated from all other populations due to coastal hydrological events. They suggested that the forces causing genetic differentiation may be acting on a local scale and that the larval pool is possibly not always mixed homogeneously.

Population genetic structure of *Pachygrapsus marmoratus* has been recently investigated throughout the western Mediterranean Sea and the eastern Atlantic Ocean by Fratini et al. (2011). They constructed population structure of *P. marmoratus* using mitochondrial and nuclear DNA markers, and while they detected weak genetic differentiation in the mitochondrial data, they found local genetic differentiation among populations in the microsatellite data set.

1.8. Main Objectives

The aim of this thesis is to understand if the Turkish Straits System (The Bosphorus Strait, the Sea of Marmara and the Dardanelles) acts as a barrier or corridor to gene flow, and to make inferences on the natural and evolutionary history of the selected marine invertebrates (the Mediterranean mussel, *Mytilus galloprovincialis* Lamarck, 1819, the common European prawn, *Palaemon elegans* Rathke, 1837 and the marbled crab, *Pachygrapsus marmoratus* (Fabricius, 1787) in the region. Its two-layered current regime

may be responsible for the dispersal of planktonic larvae; acting as a biological corridor, or may constitute a physical barrier, restricting the gene flow between the Black Sea and the Mediterranean.

A mentioned above, the phylogeography of only a few species (e.g. *Zostera marina*, *Sagitta setosa*, *Calanus helgolandicus*, *Engraulis encrasicolus*) have been examined with genetic markers around TSS, and none with a high geographic sampling intensity. This was mainly because of the focus of these studies having the extent of differentiation between the Black Sea and the Mediterranean in a broad sense, without a dense sampling methodology in and around the TSS. Hence the fine scale sampling intensity that we achieved in this study is unprecedented for the TSS, and considering the genetics studies on straits in general, provides a robust sampling framework for investigating their effect on genetic differentiation.

2. MATERIALS AND METHODS

In the study, individuals belonging to three species (*Mytilus galloprovincialis*, *Palaemon elegans* and *Pachygrapsus marmoratus*) were collected from 41 sampling sites, encompassing the Black Sea (12), the TSS (14), the Aegean Sea (11) and the Levantine Sea (four) (Figure 2.1, Appendix B).

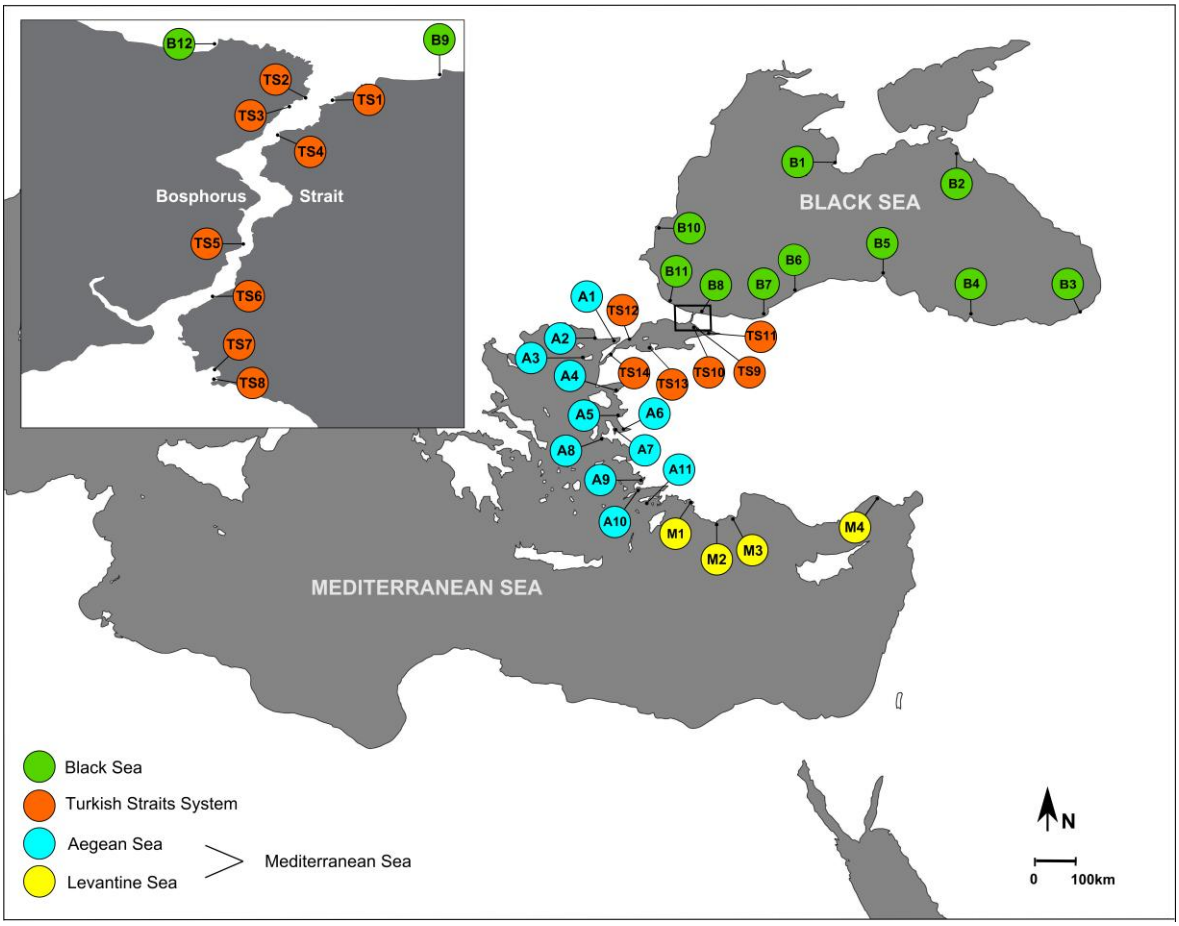


Figure 2.1. Map showing the sampling sites. The different seas are shown in different colors. The name and coordinates of the sampling sites are given in Appendix B.

Mytilus galloprovincialis individuals were collected from 26 sites, encompassing the Black Sea (11), the TSS (nine), the Aegean Sea (five) and the Levantine Sea (one) (Figure 2.2). *M. galloprovincialis* was not observed in sampling sites located at Levantine coast, with the exception of M1 (Fethiye). Mussel samples were collected by hand from the upper littoral zone at depths ranging from 0.5 to 1m.

Specimens of *Palaemon elegans* were collected from a total of 21 sites along the Turkish coasts. The sampling sites were as follows; the Black Sea (six), the TSS (four), the Aegean Sea (seven) and the Levantine Sea (four) (Figure 2.3). Samples were collected by SCUBA and free diving (manually or with the assistance of hand-nets) from the depths of 0.5 to 10m.

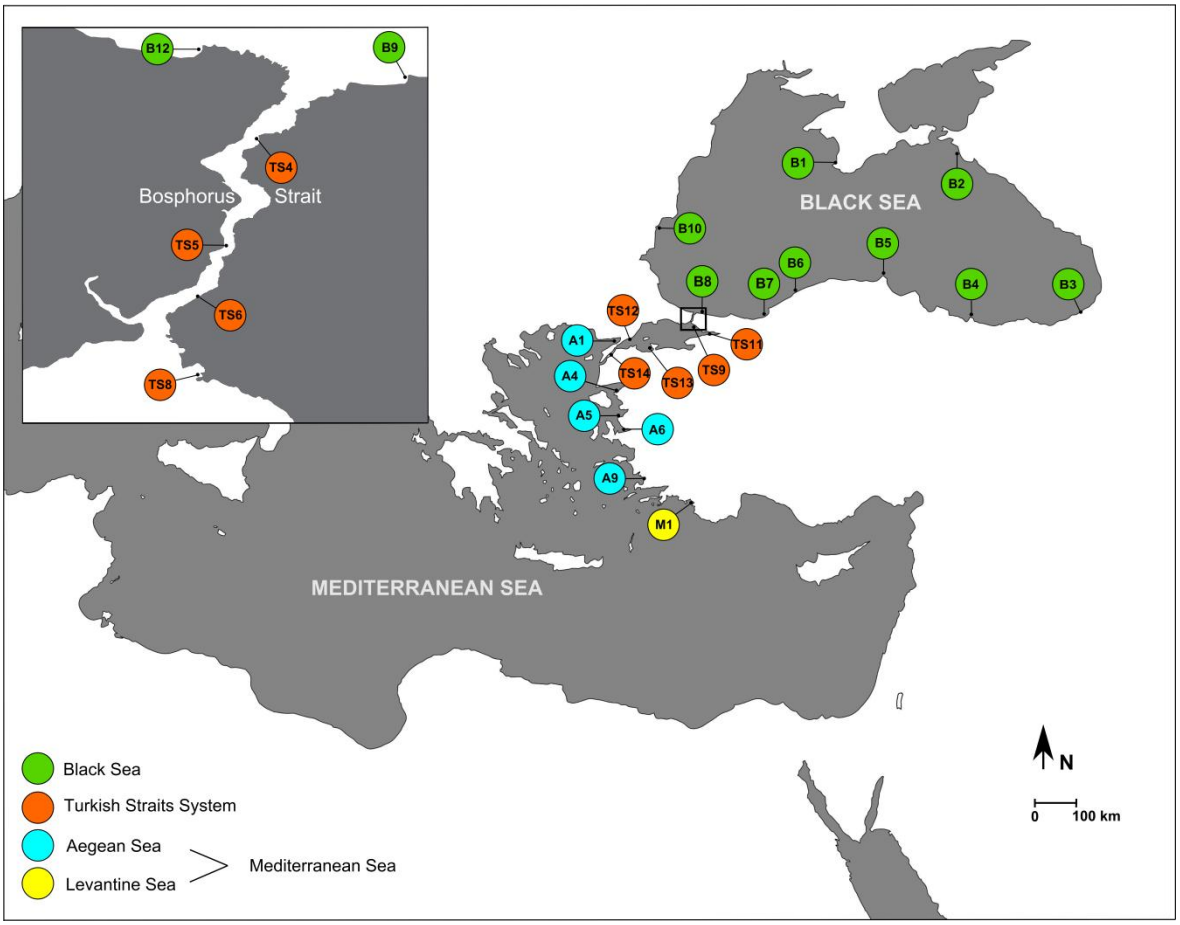


Figure 2.2. Geographic locations of sampling sites of *Mytilus galloprovincialis*.

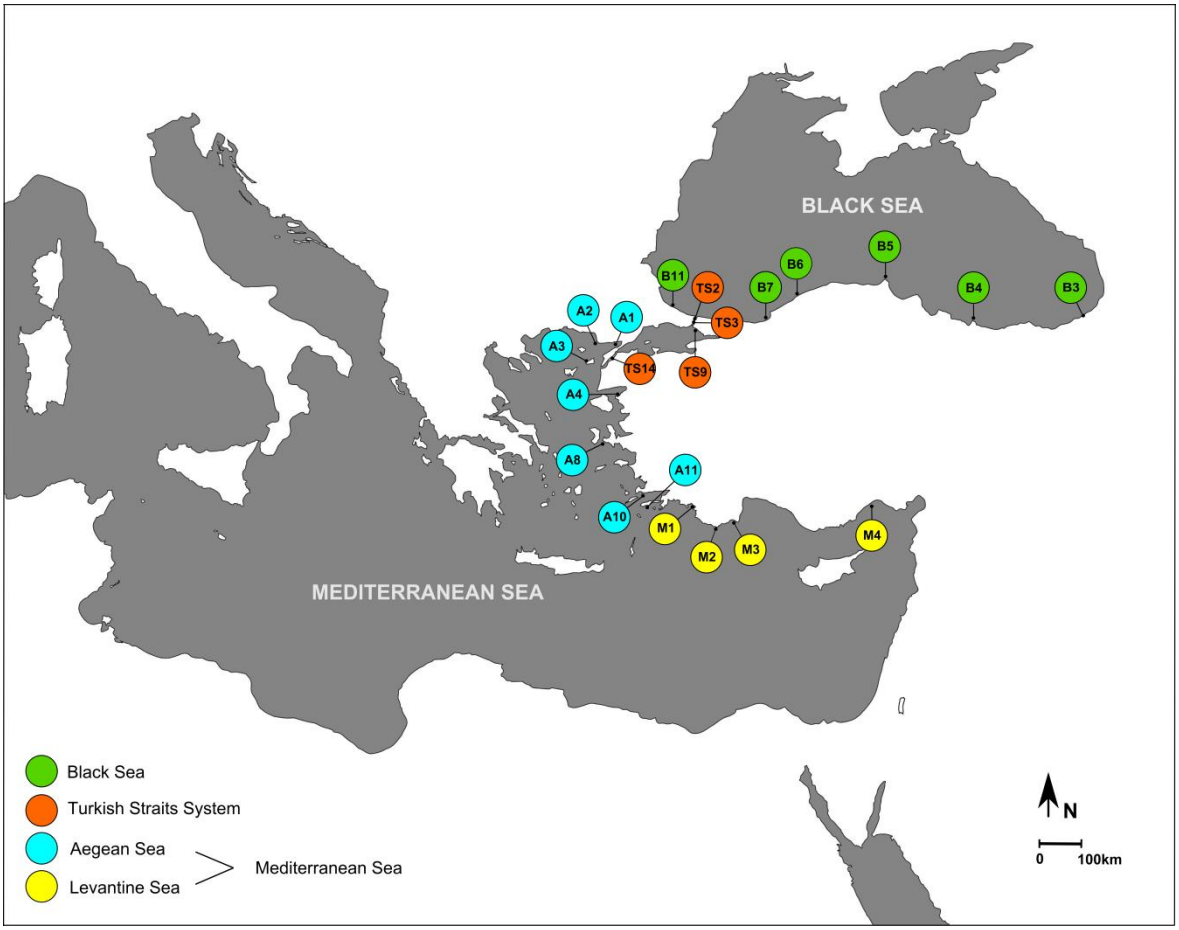


Figure 2.3. Geographic locations of sampling sites of *Palaemon elegans.*

Pachygrapsus marmoratus samples were obtained from the upper littoral zone, at a depth range of 0.5-5m, from 18 sampling sites. These were from the Black Sea (four), the TSS (three), the Aegean Sea (nine) and the Levantine Sea (two) (Figure 2.4). The samples were collected by free diving (manually).

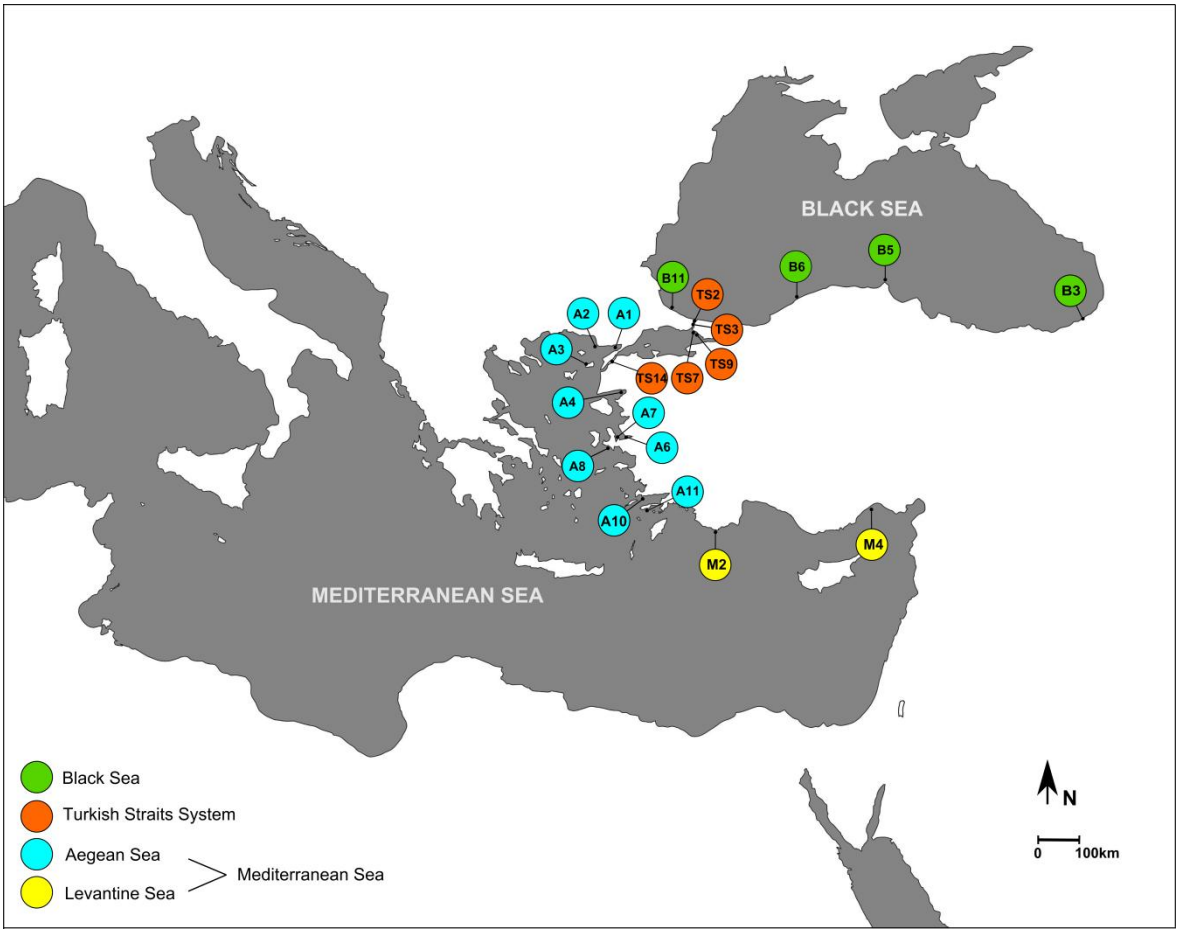


Figure 2.4. Geographic locations of sampling sites of *Pachygrapsus marmoratus.*

A total of 295 *Mytilus galloprovincialis*, 216 *Palaemon elegans* and 129 *Pachygrapsus marmoratus* individuals were collected over a period of five years (2007-2012), most often from rock pools, man-made rock jetties and within harbors. After collection, samples were preserved in 95% ethanol. In the laboratory, *P. elegans* and *P. marmoratus* individuals were identified under a stereomicroscope (Olympus SZ61) using the taxonomic keys of González-Ortegón and Cuesta (2006) and Poupin et al. (2005).

Gill tissue from *M. galloprovincialis*, the abdominal muscle tissue from *P. elegans* and pereiopod muscle tissue from *P. marmoratus* were used for DNA extraction (Figure 2.5). The total genomic DNA was extracted using Roche High Pure PCR Template Preperation Kit (Indianapolis, USA) following the instructions of the supplier and stored at -20°C. Prior to DNA extraction, each tissue was washed in distilled water to remove excess ethanol.

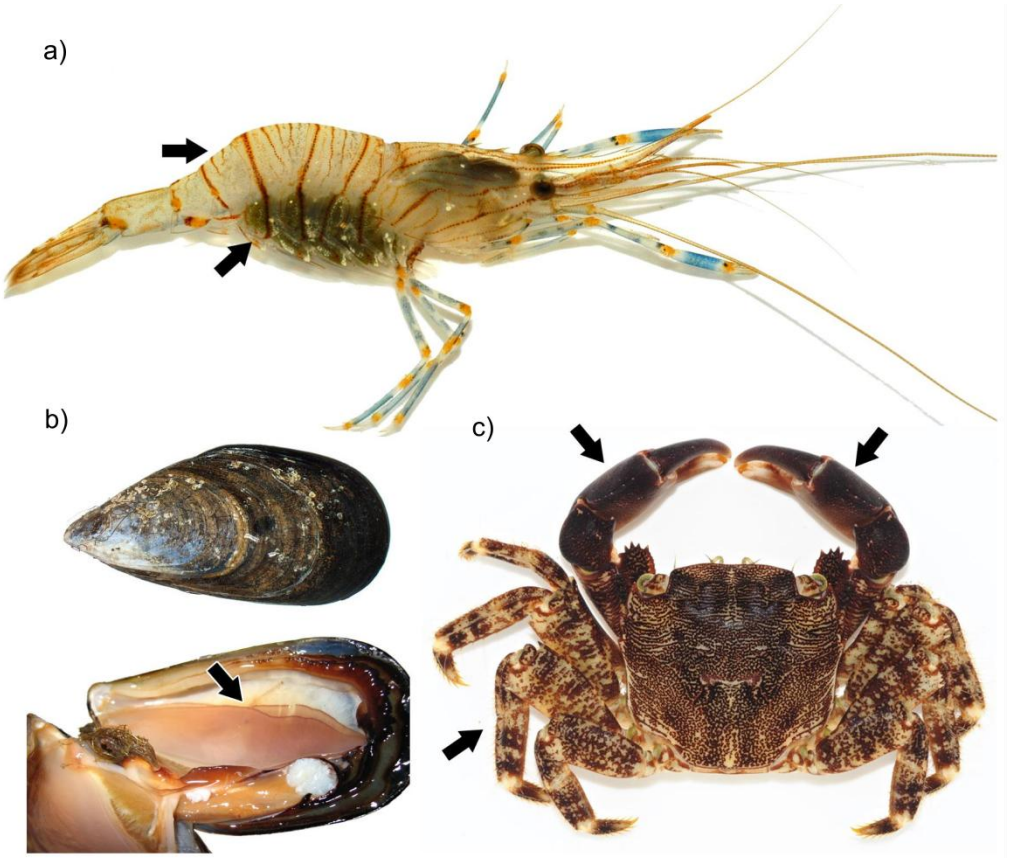


Figure 2.5. The selected species (a) *Palaemon elegans*, (b) *Mytilus galloprovincialis*, (c) P*achygrapsus marmoratus.* The arrows indicate the regions where the soft tissues were taken from (a) abdomen, (b) gill, (c) chelipeds and walking legs.

A partial fragment of the mitochondrial COIII gene (COIII mtDNA) was amplified with the primers 5'-TATGTACCAGGTCCAAGTCCGTG-3' and 5'-ATGCTCTTCTTGAATATAAGCGTAC–3' for *M. galloprovincialis*. For each PCR, 2 µl of genomic DNA was added to a 50 µl reaction mixture, containing 5 µl of 10x high fidelity buffer, 6 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (10 mM), 0.625 µl of each primer (20 µM), 34.5 µl of H_2O and 1.25 units of Taq DNA polymerase. The PCR thermal profile was 2 min at 94°C, 35 cycles of (1 min at 94°C, 45 sec at 54°C, 1 min at 72°C), and 7 min at 72°C.

Mitochondrial DNA from the large subunit rRNA (16S) gene and from the cytochrome oxidase subunit I (CO1) gene were amplified by means of polymerase chain reactions (PCR) for the species *P. elegans* and *P. marmoratus*. mtDNA amplification was performed with the primers 16L2 (5'-TGC CTG TTT ATC AAA AAC AT-3') (Schubart et al., 2002), 16L29 (5'-YGC CTG TTT ATC AAA AAC AT -3') (Schubart, 2009), 16H11 (5'-AGA TAG AAA CCR ACC TGG-3') (Schubart, 2009), 16H3 (5'-CCG GTT TGA ACT CAA ATC ATG T-3') (Reuschel and Schubart, 2006), LCO1490 (5'-GGT CAA CAA ATC ATA AAG ATA TTGG-3'), HC02198 (5'-TAA ACT TCAG GGT GAC CAA AAA ATCA-3') (Folmer et al., 1994), COL6Pe (5'-AAG ATA TTG GAA CTC TAT AT-3') and COH6Pe (5'-GTG SCC AAA GAA YCA AAA TA-3') (Reuschel et al., 2010).

The PCR of CO1 for *P. elegans* was carried out in a 50 µl reaction volume containing 5 µl of 10x high fidelity buffer, 6 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (10 mM), 0.75 µl of each primer (20 µM), and 0.5 units of Taq DNA polymerase. The PCR thermal profile was 2 min at 94°C, 40 cycles of (1 min at 94°C, 45 sec at 56°C, 1 min at 72°C), and 7 min at 72°C.

The PCR of 16S rRNA for *P. elegans* was carried out in a 50 µl reaction volume containing 5 µl of 10x high fidelity buffer, 5 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (10 mM), 0.5 µl of each primer (20 µM) and 0.5 units of Taq DNA polymerase. The PCR thermal profile was 2 min at 94°C, 35 cycles of (1 min at 94°C, 45 sec at 45°C, 1 min at 72°C), and 7 min at 72°C.

The PCR of CO1 for *P. marmoratus* was carried out in a 50 µl reaction volume containing 5 µl of 10x high fidelity buffer, 5 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (10 mM), 0.5 µl of each primer (20 µM) and 0.5 units of Taq DNA polymerase. The PCR amplification was performed with the following PCR conditions: 35 cycles with 45s at 94°C for denaturation, 1 min at 50°C for annealing, 1 min at 72°C for extension, followed by 10 min at 94°C for initial denaturation and 10 min at 72°C for final extension.

The PCR of 16S rRNA for *P. marmoratus* was carried out in a 50 µl reaction volume containing 5 µl of 10x high fidelity buffer, 5 µl of $MgCl_2$ (25 mM), 1 µl of dNTPs (10 mM), 0.5 µl of each primer (20 µM), and 0.5 units of Taq DNA polymerase. The PCR amplification was performed with the following PCR conditions: 35 cycles with 45s at 94°C for denaturation, 1 min at 50.3°C for annealing, 1 min at 72°C for extension, followed by 10 min at 94°C for initial denaturation and 10 min at 72°C for final extension.

Amplified products were purified using the Roche High Pure PCR Product Purification Kit (Indianapolis, USA), following the manufacturer's instructions and subsequently sequenced commercially (Macrogen, Holland).

Regarding the nuclear marker analysis, a total of 295 individuals were screened for polymorphism at five microsatellite loci specially isolated for *M. galloprovincialis* (Presa et al., 2002) (Table 2.1). Five microsatellite loci (Mgµ2, Mgµ3, Mgµ4, Mgµ6 and Mgµ181) were amplified in reactions with a total volume of 25µL containing 1µL genomic DNA, 2.5µL 10X high fidelity buffer, 2µL $MgCl_2$ (25mM), 0.25µL dNTPs (10mM each), 0.75µL of each primer (10µM) and 0.1 units of Taq polymerase following the protocol described in Presa et al. (2002). The PCR products were sent to Macrogen, Holland for sizing of the fragments. Alleles were subsequently processed with the software program Peak Scanner V.1.0 (Applied Biosystems) and scored manually.

Table 2.1. Characterization and primer sequences of the five microsatellite loci.

Name	**Repeat motif**	**Forward and reverse primer sequences 5'- 3'**	**Annealing Temperature (°C)**	**Size (bp)**
Mgμ2	(CT)n	GGGATCGTTCAATAAGTTC AAATTTTACTGAATAAATAAATCG	55	84–138
Mgμ3	(TG)n	AAACTAAAAACTTCATCTAATCCC AAGCAATCCAAAGTGAGAGG	58	143–151
Mgμ4	(TG)n	CCTTACTATGCGTCGTTCAA TGACCAACACTCCAAAAATC	55	91–129
Mgμ6	(ATT)n	GGGAAAGACTGCCTAACAAT CTCTTACATAGAAAATGGTTCG	57	214–236
Mgμ181	(CGTC)n	CTGCTTCAGGTTTTATGTCC TCTGACAAATTGGCTTTTAAT	55	181

2.1. Statistical Methods

2.1.1. Mitochondrial DNA

Sequencing results were manually edited with the software Sequencher v. 4.8, and aligned using the ClustalX program (Thompson et al., 1997). Seven COIII sequences of *Mytilus galloprovincialis*, 10 16S rRNA and 22 CO1 sequences of *Palaemon elegans*, and four 16S rRNA and 38 CO1 sequences of *Pachygrapsus marmoratus* were retrieved from GenBank and were included in the mtDNA data set. Accession numbers of the sequences retrieved from the GenBank are given in Appendix A.

Neighbor-joining and maximum likelihood trees were constructed from aligned sequences by using the software Mega v. 5.05 (Tamura et al., 2011). The neighbour-joining tree estimates the minimum evolution tree. The robustness of the topologies was tested by bootstrapping (10000 replicates) (Felsenstein, 1985). MEGA can implement likelihood methods for estimating evolutionary distances between sequence pairs as well as distance-based and character-based methods for inferring phylogenetic trees. It also chooses the best-fit model of DNA and protein substitution, estimating the extent of rate variation among sites, testing molecular clocks among species and paralogous genes, constructing nucleotides and amino acids in the ancestral sequences, and inferring phylogenetic trees (Tamura et al., 2011; Kumar et al., 2008). Maximum likelihood method operates directly on the sequences or on functions derived from the sequences rather than on pairwise distances. Therefore, this method avoids the loss of information that occurs when sequences are converted into distances. Maximum likelihood chooses the tree that of all trees is most likely to have produced the observed data. On the other hand, the neighbour-joining method uses the evolutionary distance data for constructing phylogenetic trees. It is a heuristic method for estimating the minimum evolution tree (Page and Holmes, 1998).

Genetic heterogeneity within sampling sites was estimated as haplotype diversity (h) (Nei and Tajima, 1981) and nucleotide diversity (π) (Nei, 1987) calculated with DnaSP (DNA Sequence Polymorphism) v. 5.0 (Librado and Rozas, 2009). DnaSP is a software addressed to molecular population geneticists and can compute several measures of DNA

sequence variation within and between populations in noncoding, in synonymous or in nonsynonymous sites; including gene flow, gene conversion (Betrán et al., 1997), recombination, and linkage disequilibrium parameters (Rozas, 2009). Haplotype diversity is a measure of the uniqueness of a particular haplotype in a given population. Nucleotide diversity (π) is the average proportion of nucleotide differences between all possible pairs of sequences in the sample and it is used to assess polymorphism at the DNA level (Hartl and Clark, 1997).

Evolutionary relationships among sequences and organisms are also represented by networks. Network version 4.6.1.0 (Bandelt et al., 1999) was used to construct a haplotype network with the median-joining method. Network generates evolutionary trees and networks from genetic, linguistic, and other data.

AMOVA (analysis of molecular variance) was performed to examine the extent of population genetic structure using Arlequin v. 3.11 (Excoffier et al., 2005). The amount of genetic structuring at different hierarchical levels was measured by quantifying the inter- and intragroup component of total variance by F-statistics, and tested by 1000 permutations. The same program was used to compute pairwise Φ_{st} values and test their significance by performing 10000 permutations among the individuals between populations. The AMOVA, a standardized measure of population differentiation, is methodologically very similar to the ANOVA-based estimation of Weir and Cockerham (1984)'s. AMOVA produces estimates of variance components and F-statistic analogs, reflecting the correlation of haplotypic diversity at different levels of hierarchical subdivision (Meirmans, 2006). It constitutes a coherent and flexible framework for the statistical analysis of molecular data (Excoffier et al., 2005). An estimator of Φ_{st} can be used for DNA sequences data based on mean number of pairwise differences between sequences taken either from the same subpopulation or from different subpopulations (Hudson et al., 1992). In an AMOVA, F_{ST}-analogues are computed as a ratio of variance components that are obtained from a matrix of squared Euclidean distances between pairs of individuals (Excoffier et al., 1992).

The neutrality tests, Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997) and Ramos-Onsins and Rozas' R_2 (Ramos-Onsins and Rozas, 2002) were also performed using DnaSP

v. 4.0 to determine whether the species sampled experienced selection or recent demographic expansions at each sampling site (Rozas et al., 2003). These tests are used to detect population growth. The changes that occurred in population size, such as a population growth, can leave a particular footprint that may eventually be detected in DNA sequence data. This theoretical framework prompted the development of statistical tests for detecting population expansion. The behavior of R_2 is better for small sample sizes, whereas Fs is better for bigger sample sizes (Ramos-Onsins and Rozas, 2002). While negative values of D and Fs indicate population growth, positive values show population decline (Beaumont, 2003).

2.1.2. Microsatellite DNA

MICRO-CHECKER (Van Oosterhout et al., 2004) was used to identify potential genotyping errors due to null allele dominance, large allele dropout and scoring errors due to stuttering. The number of alleles per locus (Na), observed heterozygosity (Ho) and expected heterozygosity (He) were calculated using the program GENALEX v.6.5 (Peakall and Smouse, 2012; Peakall and Smouse, 2006). The same program was also used to estimate the significance of deviations from Hardy-Weinberg equilibrium (HWE).

The variation in alleles is very important for the survival of the organism because it allows organisms to adapt to changes. Allele frequency is used to characterize the genetic diversity, or richness of the gene pool, in a population. Observed heterozygosity (Ho) and expected heterozygosity (He) are calculated to estimate the extent of genetic variability in the population. These values range from 0 to 1 (Hamilton, 2009). The Hardy-Weinberg equation is a mathematical expression that can be used to calculate the genetic variation of a population at equilibrium. The most important part of the Hardy-Weinberg theorem is that the amount of genetic variation in a population will remain constant from one generation to the next in the absence of disturbing factors (Page and Holmes, 1998).

A simple measure of the amount of recombination is the degree of linkage disequilibrium. Linkage equilibrium implies that alleles at different loci are randomly associated. If genes are not in random association, it is said to be in linkage disequilibrium (Page and Holmes, 1998; Hartl and Clark, 1997). Linkage disequilibrium was tested for

each locus-population combination using the web version of GENEPOP 4.0.10 (Rousset, 2008; Raymond and Rousset, 1995).

Arlequin v. 3.11 was used to carry out R_{ST}-based analyses of molecular variance (AMOVA) to assess the levels of population differentiation over all loci (Excoffier et al., 1992). The estimator R_{ST} is frequently used with microsatellites loci or simple sequence repeat (SSR) loci to account for high rates of stepwise mutation that can obscure population structure (Slatkin, 1995).

To test the signatures of population expansion in five microsatellite loci, the Microsoft Excel macro KGTESTS (Bilgin, 2007) was used to perform a within locus k test. The k test was used to assess the allele-length distribution, which is expected to be unimodal in an expanding population (Reich et al., 1999; Reich and Goldstein, 1998).

3. RESULTS & DISCUSSION

3.1. *Mytilus galloprovincialis* Lamarck, 1819

3.1.1. Mitochondrial Diversity

The COIII fragment was successfully amplified in 242 *Mytilus galloprovincialis* individuals from 26 sampling locations along the Turkish coasts (Table 3.1). A fragment of 543 bp was used for all analyses. In 242 individuals, a total of 57 unique haplotypes were found (Appendix C). When the sequences of these 60 haplotypes were compared with the sequences of the C and M genomes retrieved from the GenBank, nine haplotypes (24 individuals) were grouped with the C-genome. Majority of these individuals (22/24) were from the Black Sea. No M-genome haplotypes were detected in this study. The C-genome, first defined by Venetis et al. (2007), is characterized by an unusually long major control region that is transmitted through sperm of males. C-genome haplotypes were previously detected in multiple individuals by Kalkan et al. (2011). In this study, C-genome sequences were excluded from the main data set for the population genetic analysis; only the remaining F-genome haplotypes were used for these purposes. In these 48 haplotypes we found a total of 65 polymorphic sites, among which 25 sites were singleton variable with two variants and 38 were parsimony informative with two variants. The number of haplotypes per site ranged from one (Gideros, Akcakoca, Sile, Riva and Murefte) to 12 (Gulluk). The most common haplotype (Hapt 01) was found in all Black Sea and TSS sampling sites (in about 58% of individuals sampled) but was not found in Aegean sampling sites except in Saros (one individual) and Izmir (one individual).

Table 3.1. The number of *M. galloprovincialis*, *P. elegans* and *P. marmoratus* specimens used for genetic comparisons of the COIII, the CO1 and the 16S genes and the five microsatellite loci.

		M. galloprovincialis						*P. elegans*		*P. marmoratus*	
	Codes	COIII	Mgμ2	Mgμ3	Mgμ4	Mgμ6	Mgμ181	CO1	16S	CO1	16S
Black Sea	B1	5	22	21	23	23	20	-	-	-	-
	B2	4	20	18	20	20	20	-	-	-	-
	B3	7	18	20	17	20	19	7	14	1	2
	B4	3	21	21	17	15	21	4	11	-	-
	B5	12	16	15	14	14	16	6	3	1	1
	B6	6	23	21	23	18	22	20	12	2	2
	B7	2	14	16	15	15	15	20	14	2	-
	B8	2	15	15	14	18	20	-	-	-	-
	B9	11	14	18	16	17	18	-	-	-	-
	B10	2	21	20	22	20	22	-	-	-	-
	B11	-	18	19	16	-	-	-	2	1	3
	B12	5	18	19	16	18	18	-	-	-	-
Turkish Straits System	TS1	-	-	-	-	-	-	1	1	-	-
	TS2	-	-	-	-	-	-	7	2	19	17
	TS3	-	-	-	-	-	-	11	12	5	6
	TS4	13	17	18	15	18	17	-	-	-	-
	TS5	11	21	21	21	14	21	-	-	-	-
	TS6	16	22	22	20	21	21	-	-	-	-
	TS7	-	-	-	-	-	-	-	-	2	-
	TS8	17	21	21	19	20	20	-	-	-	-
	TS9	14	23	24	22	21	23	16	16	-	-
	TS10	-	-	-	-	-	-	-	-	3	3
	TS11	12	24	23	23	24	23	-	-	-	-
	TS12	13	20	20	20	18	13	-	-	-	-
	TS13	10	18	19	19	14	19	-	-	-	-
	TS14	16	12	13	10	10	13	5	8	4	3
Aegean Sea	A1	21	20	20	18	16	20	-	4	1	1
	A2	-	-	-	-	-	-	26	21	3	12
	A3	-	-	-	-	-	-	23	13	12	21
	A4	8	18	17	15	17	18	5	2	1	-
	A5	6	17	20	20	19	20	-	-	-	-
	A6	13	17	18	18	17	17	-	-	1	-

Table 3.1. The number of *M. galloprovincialis*, *P. elegans* and *P. marmoratus* specimens used for genetic comparisons of the COIII, the CO1 and the 16S genes and five microsatellite loci (cont.)

		M. galloprovincialis						*P.elegans*		*P. marmoratus*	
	Codes	COIII	Mgμ2	Mgμ3	Mgμ4	Mgμ6	Mgμ181	CO1	16S	CO1	16S
Aegean Sea	A7	-	-	-	-	-	-	-		16	17
	A8	-	-	-	-	-	-	1	-	2	1
	A9	14	25	25	25	25	22	-	-	-	-
	A10	-	-	-	-	-	-	2	3	20	10
	A11	-	-	-	-	-	-	5	12	8	7
Mediterranean Sea	M1	4	20	22	18	21	21	8	6	-	-
	M2	-	-	-	-	-	-	16	11	18	15
	M3	-	-	-	-	-	-	1	16	-	-
	M4	-	-	-	-	-	-	11	14	4	4

The haplotype network (Figure 3.1) showed three main clades (clade A, which was further divided into sub-clades A1 and A2, clade B, and clade C), and each clade was characterized by a star-like genealogy. Clade B was separated from clade A and clade C by 26 and 14 mutations, respectively. In addition, sub-clade A2 was separated from the main A subclade (A1) by three mutations and it was dominated by individuals from the Aegean (9 out of 13). Sub-clade A1 included the most common haplotype (Hapt 01), connected to numerous less-frequent haplotypes by one to 10 mutations. While this clade was dominated by the Black Sea and TSS individuals (175 of 182) and was found partially in the Aegean (four sites and seven individuals), clade B was detected in all sampling sites in the Aegean and only two individuals in the TSS (Bosphorus Strait, TS3 - Kuzguncuk station). In addition, considering the Aegean ~28% (16 out of 56) of the individuals were grouped in clade A (Figure 3.2). On the other hand, clade B had a frequency of ~72% (40 out of 56) in the Aegean. Clade C constituted the sequences that grouped with C-genome and it was dominated by the Black Sea individuals (22 of 24), as mentioned previously. Finally six individuals, which separated from the clade A by at least 11 mutations, are also shown in the network. Five of these were sampled from the Aegean Sea (A1, A3 and A5), and one was from the TSS (TSS8). These individuals were grouped with the Atlantic *M. galloprovincialis* haplotypes (GenBank accession numbers given in Appendix A).

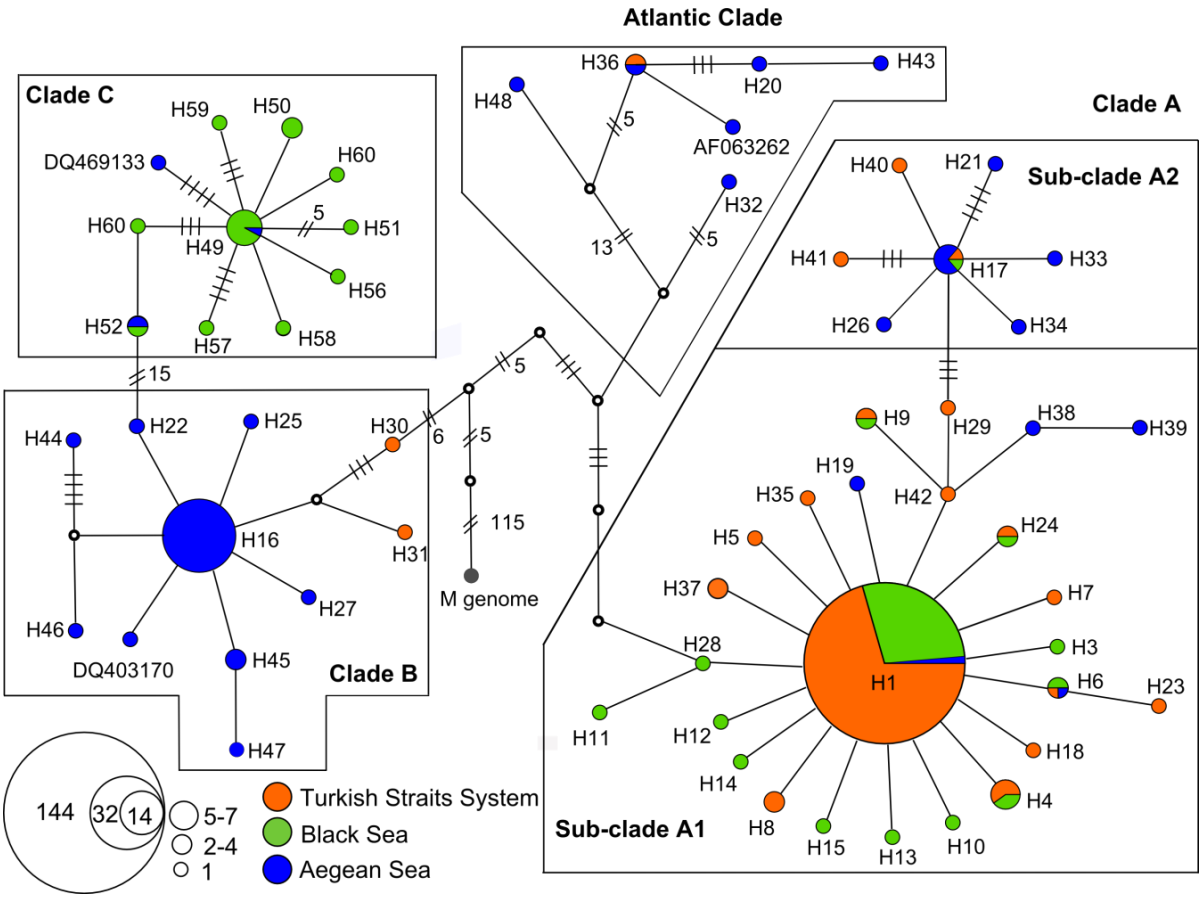


Figure 3.1. The haplotype network based on COIII sequences from *Mytilus galloprovincialis*. The partitions inside a circle represent the frequency of the haplotype for each population and its diameter is proportional to the frequency of the haplotype. Small white circles represent missing haplotypes and each line represents a single mutational change.

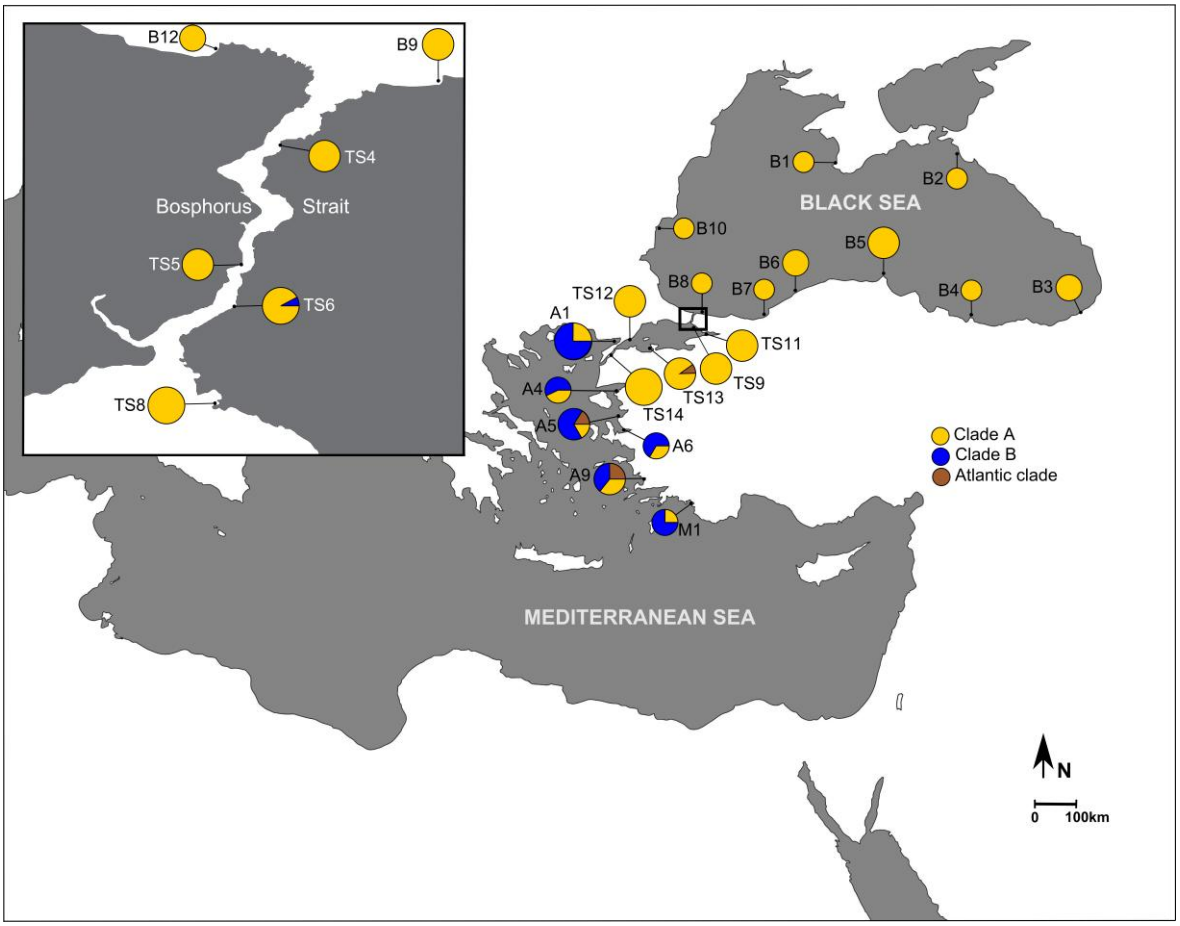


Figure 3.2. The COIII clade frequencies.

The neighbor-joining and the maximum likelihood trees showed two major groups (Group I and II) with very low bootstrap values (less than 20%) (Figures 3.3, 3.4). Group I in the trees corresponded to the haplotypes of clade A in the haplotype network and group II in the trees included the haplotypes of clades B, C and the Atlantic in the network.

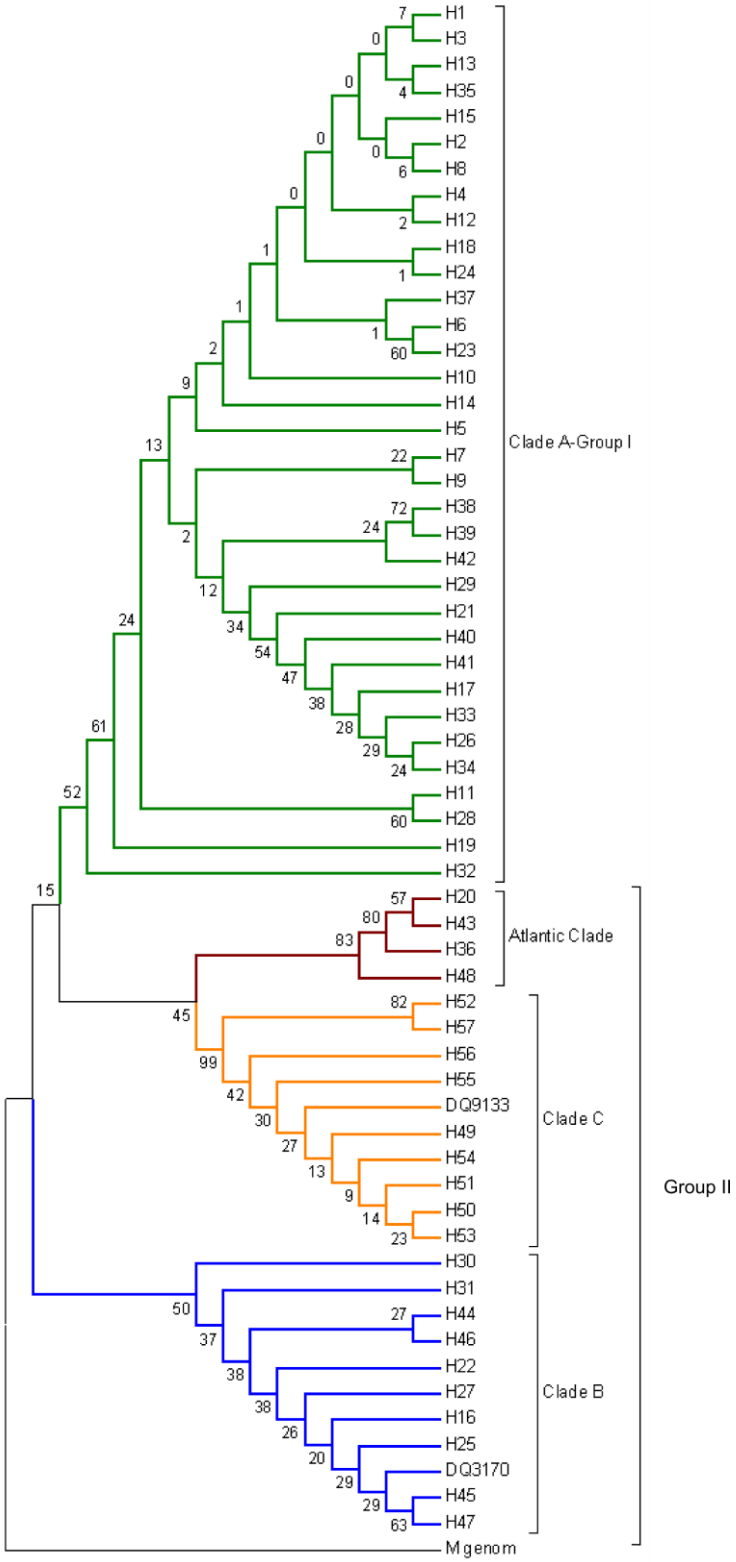


Figure 3.3 Maximum-likelihood tree of 57 haplotypes of *Mytilus galloprovincialis* from the Black Sea, Turkish Straits System and the Aegean. The identified clades on the tree correspond to the groups in the haplotype network. The numbers corresponds to the bootstrap values of maximum-likelihood tree.

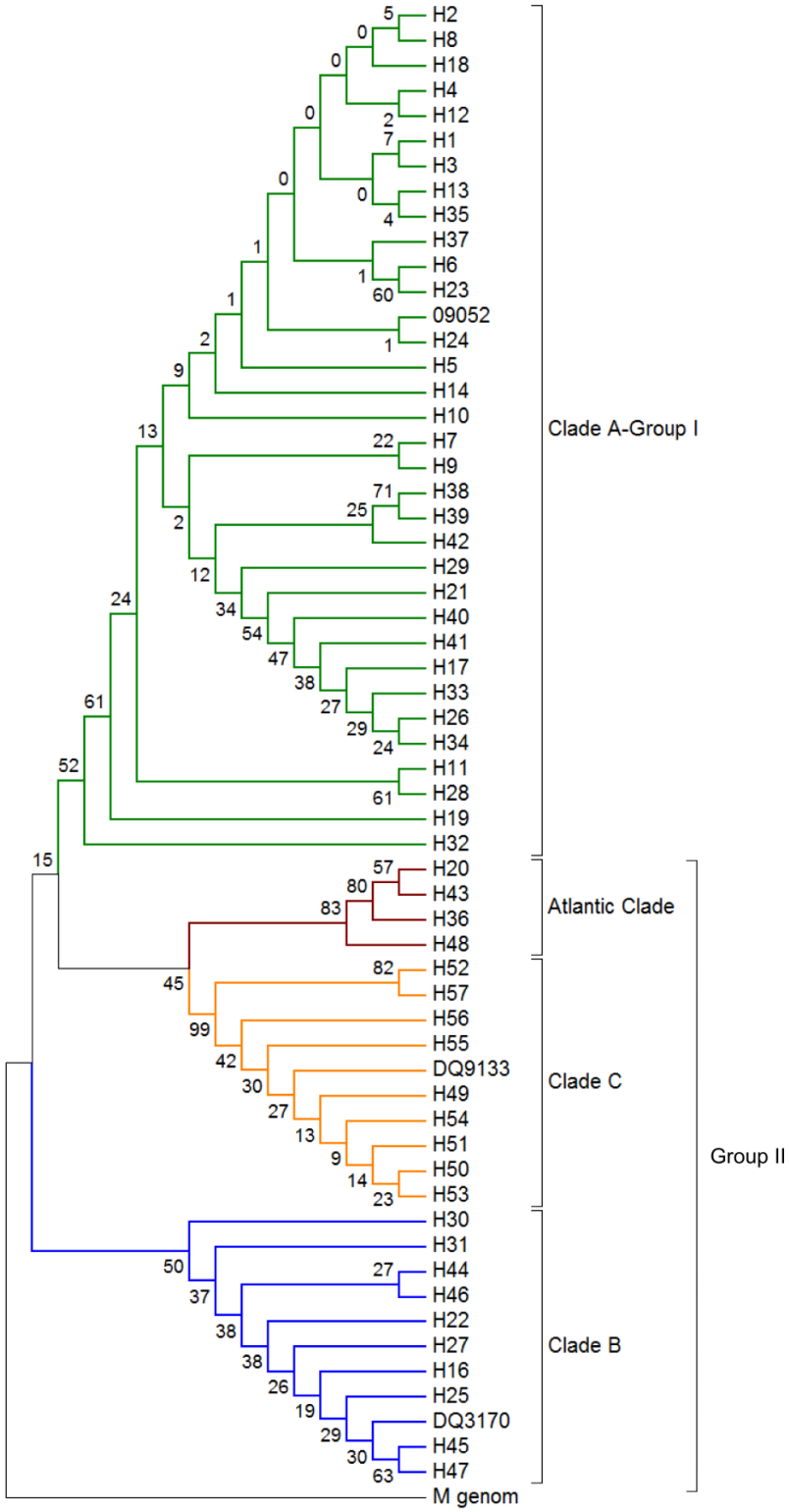


Figure 3.4. Neighbour-joining tree of 57 haplotypes of *Mytilus galloprovincialis* from the Black Sea, Turkish Straits System and the Aegean. The identified clades on the tree correspond to the groups in the haplotype network. The numbers corresponds to the bootstrap values of neighbour-joining tree.

3.1.2. Mitochondrial Population Structure

Considering all populations the mean haplotype diversity h was 0.64 ±0.034, and mean nucleotide diversity π was 0.0123 ±0.00108 (see Table 3.2 for the h and π values of individual populations). Comparing the water bodies, the Aegean Sea had the highest (h= 0.73 ±0.063, π= 0.0181 ±0.0019) and TSS had the lowest haplotype and nucleotide diversities (h= 0.36 ±0.057, π= 0.0031 ±0.001). The overall average haplotype and nucleotide diversity of the Black Sea populations were h= 0.49 ±0.081 and π= 0.0014 ±0.0003, respectively.

The analysis of molecular variance (AMOVA) indicated high levels of genetic structure among the populations (Φ_{st}= 0.39), when populations were grouped as Black Sea, TSS and the Aegean. The analysis also showed that 34.09% of the variation was among groups, 4.57% was among populations within groups, and 61.33% was within populations. Pairwise Φ_{st} values between individual sites are given in Table 3.3. Significant and high values show strong genetic differentiation between the Black Sea and the Aegean, and the TSS and the Aegean. While 46 values out of 66 were found to be significant between the Black Sea and the Aegean, all pairwise Φ_{st} values were significant between the TSS and the Aegean.

As mentioned above, because the haplotype network showed two different clades, A and B, I also analyzed the data set to quantify the differences between these two clades. After pooling individuals based on whether they belonged to clade A or clade B, AMOVA analysis showed that 71.78% of molecular variance was among groups, 28.24% within populations and 0.02% among populations within groups. Hence, the analysis indicated a high level of genetic differentiation among groups based on whether the individuals carried the Aegean haplotypes (clade B) or the ‘common’ haplotypes (clade A). In addition, relatively high and significant pairwise Φ_{ST} values between Black Sea-Aegean and TSS-Aegean strengthens the idea of isolation of these populations.

Table 3.2. Molecular diversity indices and neutrality tests for *Mytilus galloprovincialis* populations based on COIII region sequences. Population code; n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; P, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses.

Sampling site	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B1	5	4	3	0.900 (0.161)	0.00209 (0.00056)	-1.04849	-1.938	0.1633
B2	4	3	5	0.833 (0.222)	0.00465 (0.00195)	-0.21249	0.556	0.3528
B3	7	4	5	0.810 (0.130)	0.00316 (0.00079)	-0.56143	-0.324	0.1741
B4	3	3	2	1.000 (0.272)	0.00233 (0.00078)	-	-1.216	0.2357
B5	12	4	4	0.491 (0.175)	0.00127 (0.00056)	-1.71166	-1.415	0.1928
B6	6	1	0	0	0	-	-	-
B7	2	1	0	0	0	-	-	-
B8	2	1	0	0	0	-	-	-
B9	11	1	0	0	0	-	-	-
B10	2	1	0	0	0	-	-	-
B12	5	3	2	0.700 (0.218)	0.00140 (0.00053)	-0.97256	-0.829	0.2449
TS4	13	3	2	0.410 (0.154)	0.00076 (0.00031)	-0.90920	-0.790	0.1507
TS5	11	2	1	0.182 (0.144)	0.00032 (0.00025)	-1.12850	-0.410	0.2875
TS6	16	4	19	0.350 (0.148)	0.00714 (0.00362)	-1.1432	3.837	0.0990
TS6 with clade A haplotypes	(14)	2	2	0.143 (0.119)	0.00050 (0.00041)	-1.48074	0.296	0.2575
TS8	17	3	2	0.228 (0.129)	0.00041 (0.00024)	-1.50358	-1.680	0.1611
TS9	14	2	1	0.143 (0.129)	0.00025 (0.00021)	-1.15524	-0.595	0.2575

Table 3.2. Molecular diversity indices and neutrality tests for *Mytilus galloprovincialis* populations based on COIII region sequences. Population code; n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; P, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses (cont.)

Sampling site	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
TS11	12	5	6	0.576 (0.163)	0.00175 (0.00065)	**-1.89423**	-1.899	0.1273
TS12	13	1	0	0	0	-	-	-
TS13	10	5	31	0.727 (0.144)	0.01336 (0.00588)	**-1.39143**	1.857	0.1283
TS14	16	7	10	0.625 (0.139)	0.00371 (0.00124)	**-1.09976**	-1.531	0.0978
A1	21	7	35	0.5411 (0.125)	0.01523 (0.00373)	-0.45144	4.558	0.1257
A1 with clade B haplotypes	(18)	(5)	32	0.3860 (0.139)	0.01029 (0.00443)	-1.49840	4.590	0.1084
A4	8	3	19	0.571 (0.119)	0.01895 (0.00396)	**2.24021**	9.363	0.2857
A4 with clade B haplotypes	(6)	(2)	(19)	0.400 (0.237)	0.01326 (0.00787)	-1.22992	6.045	0.4000
A5	6	3	22	0.600 (0.215)	0.01315 (0.00685)	-1.36789	4.020	0.2992
A6	8	6	24	0.893 (0.111)	0.01957 (0.00431)	1.11121	1.160	0.2096
A6 with clade B haplotypes	(5)	(3)	(2)	0.700(0.218)	0.00140 (0.00053)	-0.97256	-0.829	0.2449
A9	13	11	43	0.978 (0.035)	0.02528 (0.00229)	0.12468	-1.500	0.1494
A9 with clade B haplotypes	(9)	(7)	(33)	0.944 (0.070)	0.01949 (0.00514)	-0.54109	0.512	0.1434
M1	4	2	20	0.500 (0.265)	0.01745 (0.00926)	**-0.85430**	5.652	0.4330
M1 with clade B haplotypes	(2)	(1)	-	-	-	-	-	-
Total	242	57	65	0.638 (0.034)	0.01227 (0.00108)	-1.08676	-13.421	0.0528
Total_with clade A haplotypes	(195)	(34)	(52)	0.468 (0.468)	0.468 (0.00063)	**-2.46765**	**-32.990**	**0.0175**

Table 3.2. Molecular diversity indices and neutrality tests for *Mytilus galloprovincialis* populations based on COIII region sequences. Population code; n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; P, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses (cont.)

Sampling site	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
Black Sea	60	15	5	0.495 (0.081)	0.00141 (0.00033)	**-2.42937**	**-15.227**	**0.0324**
Turkish Strait System	122	20	39	0.365 (0.057)	0.00310 (0.00100)	**-2.34649**	**-10.697**	**0.0292**
TSS_ with clade A haplotypes	(120)	(18)	(39)	0.344(0.057)	0.00224 (0.00084)	**-2.55255**	**-11.871**	**0.0272**
Aegean Sea	60	24	53	0.731 (0.063)	0.01813 (0.00193)	-0.34782	-1.607	0.0932
Aegean Sea with clade B haplotypes	(46)	(9)	(46)	0,394 (0,097)	0.00126 (0,00042)	**-2.27650**	-6.327	0.0637

Table3.3. Gene flow among *M. galloprovincialis* populations represented by Φ_{ST} values. Significant P values (<0.05) are indicated in bold.

		Black Sea											**Turkish Straits System**									**Aegean Sea**					
		B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	A1	A2	A3	A4	A5	A6
Black Sea	B1	0.00																									
	B2	0.03	0.00																								
	B3	-0.03	0.08	0.00																							
	B4	-0.18	0.01	**-0.03**	0.00																						
	B5	0.14	0.35	**0.14**	**0.24**	0.00																					
	B6	-0.04	0.11	-0.01	-0.02	0.03	0.00																				
	B7	-0.02	0.25	0.00	0.08	-0.28	-0.14	0.00																			
	B8	-0.02	0.25	**0.00**	0.08	-0.28	-0.14	0.00	0.00																		
	B9	**0.44**	**0.68**	0.38	0.64	0.05	0.25	0.00	0.00	0.00																	
	B10	-0.02	0.25	0.00	**0.08**	-0.28	-0.14	0.00	0.00	0.00	0.00																
	B11	-0.05	0.10	**-0.02**	-0.03	0.02	-0.08	-0.16	-0.16	0.31	**-0.16**	0.00															
Turkish Straits System	TS1	0.09	0.31	**0.12**	0.16	-0.02	0.02	-0.22	-0.22	0.09	-0.22	0.00	0.00														
	TS2	**0.26**	0.49	**0.24**	0.40	-0.03	0.11	-0.33	**-0.33**	0.00	**-0.33**	0.12	0.01	0.00													
	TS3	**0.16**	0.37	**0.16**	**0.26**	-0.04	0.04	-0.27	-0.27	0.03	-0.27	0.03	-0.01	-0.03	0.00												
	TS4	**0.27**	**0.46**	**0.25**	0.39	-0.03	0.11	-0.30	-0.30	0.00	-0.30	0.11	0.01	-0.04	-0.02	0.00											
	TS5	**0.33**	**0.53**	0.30	0.48	-0.01	0.16	-0.33	-0.33	-0.02	-0.33	0.18	0.04	-0.04	-0.01	-0.05	0.00										
	TS6	0.00	0.15	0.01	**0.07**	-0.02	-0.04	-0.19	-0.19	0.13	-0.19	-0.05	-0.01	0.03	0.00	0.02	0.05	0.00									
	TS7	**0.48**	**0.71**	**0.42**	0.68	0.07	0.29	0.00	0.00	0.00	0.00	0.35	0.11	0.02	0.05	0.01	**-0.01**	**0.15**	0.00								
	TS8	-0.03	0.11	-0.04	-0.01	0.03	-0.05	-0.12	**-0.12**	0.20	-0.12	-0.06	**0.02**	0.10	0.05	**0.11**	0.14	-0.04	**0.23**	0.00							
	TS9	0.01	0.15	0.03	0.05	0.00	-0.04	-0.16	-0.16	0.12	-0.16	-0.05	-0.02	0.05	0.01	0.05	0.08	-0.03	**0.14**	-0.03	0.00						
Aegean Sea	A1	**0.36**	**0.40**	0.37	0.36	**0.54**	**0.41**	**0.55**	**0.55**	**0.67**	**0.55**	**0.42**	**0.52**	**0.61**	**0.55**	**0.61**	**0.64**	**0.45**	**0.69**	**0.34**	**0.42**	0.00					
	A2	**0.28**	**0.24**	0.31	0.28	**0.56**	**0.36**	**0.55**	**0.55**	**0.77**	**0.55**	**0.37**	**0.53**	**0.66**	**0.57**	**0.66**	**0.70**	**0.43**	**0.79**	**0.31**	**0.38**	0.10	0.00				
	A3	**0.26**	**0.30**	0.29	0.25	**0.56**	**0.34**	**0.54**	**0.54**	**0.78**	**0.54**	**0.35**	**0.52**	**0.66**	**0.57**	**0.66**	**0.71**	**0.41**	**0.81**	**0.28**	**0.38**	-0.04	0.05	0.00			
	A4	**0.06**	0.07	0.10	0.03	**0.34**	0.13	0.24	**0.24**	**0.55**	**0.24**	**0.13**	**0.31**	**0.43**	**0.36**	**0.45**	**0.49**	**0.21**	**0.58**	**0.10**	**0.19**	0.07	0.00	0.00	0.00		
	A5	**0.06**	0.06	0.10	0.02	**0.33**	**0.14**	**0.24**	**0.24**	**0.49**	**0.24**	**0.14**	**0.31**	**0.40**	**0.35**	**0.43**	**0.45**	**0.22**	**0.51**	**0.13**	**0.20**	**0.19**	0.10	**0.10**	0.00	0.00	
	A6	**0.28**	**0.33**	0.32	0.28	**0.61**	0.37	0.64	**0.64**	**0.87**	**0.64**	**0.39**	**0.56**	**0.73**	**0.61**	**0.71**	**0.77**	**0.45**	**0.89**	**0.31**	**0.41**	-0.08	0.05	-0.11	-0.01	**0.11**	0.00

Considering the neutrality tests of Tajima's D, Fu's Fs, and Ramos-Onsins and Rozas's R_2, most of values were not significant (Table 3.2). However, after pooling samples together for clade A, the neutrality tests were all significant and negative. For the Aegean samples, pooled analyses showed significance for clade B in Tajima's D only (Table 3.2). Tajima's D and Fu's are sensitive to factors such as bottlenecks or population expansion (Martel et al., 2004; Tajima, 1996) and Fu's Fs is more sensitive to recent population growth than Tajima's D (Fu, 1997). Indeed, significant negative values of these two statistics indicated that clade A had experienced population expansion. Examining the expansion using mismatch distributions, The Black Sea, TSS and all of the clade A sequences (Figure 3.5a), as well as the Aegean clade B haplotypes (Figure 3.5b) showed unimodal distributions of pairwise differences, indicative of population expansion.

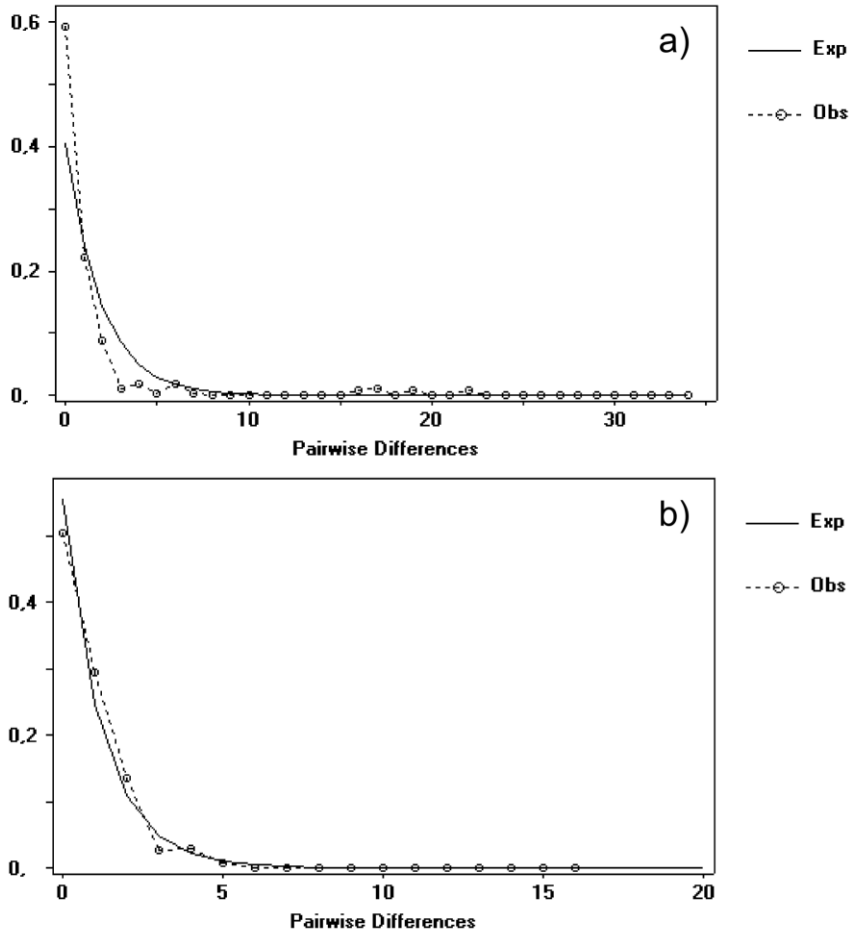


Figure 3.5. Mismatch distribution obtained from COIII gene sequence data for (a) clade A and (b) clade B. The lines with empty circles represent the observed distribution and the black lines represent the expected distribution under a sudden expansion model.

3.1.3. Nuclear Diversity and Structure

Five microsatellite primer pairs were amplified for 227 *M. galloprovincialis* individuals (Table 3.4). Observed heterozygosity varied between 0.15 to 0.50, and the expected heterozygosity was between 0.63 to 0.92, indicating a heterozygote deficiency (Table 3.4). The results showed that Hardy-Weinberg equilibrium was rejected due to heterozygote deficiency for all loci (Mgμ2, Mgμ3, Mgμ4, Mgμ6 and Mgμ181). Fisher exact test for linkage disequilibrium within samples between the five loci indicated no significant association of loci (all $P>0.13$) after Bonferroni correction, except between Mgμ2-Mgμ3 and Mgμ181-Mgμ6. Analyses of genotypes with MICRO-CHECKER confirmed the validity of excessive homozygote size classes and high levels of heterozygote deficiency were found at each locus, with alleles of one repeat unit differences that might be due to stuttering at two loci (Mgμ4 and Mgμ181). No evidence for large allele dropout was found at any locus, however we detected null alleles at all loci.

Considering allelic diversity, the five microsatellite loci did not vary greatly in terms of number of alleles per locus, across all sampling sites (mean= 6-10). The effective number of alleles (N_e) was between 3.3 and 5.7. For subsequent analyses, the microsatellite data set was separated into two population groups based on the haplotype network (clade A and clade B). When those groups were compared in terms of N_a, N_e and number of private alleles, clade A had higher values than clade B (Table 3.4).

Table 3.4. Levels of genetic variability at the five microsatellite loci for *Mytilus galloprovincialis* populations. n, sample size; Na, number of alleles; Ne, number of effective alleles; He, expected heterozygosity; Ho, observed heterozygosity.

Locus	**2**	**3**	**4**	**6**	**181**	**Mean**
Black Sea						
n	196	200	185	178	188	189.4
Na	19	13	39	26	19	23.20
Ne	4.53	2.76	9.54	11.65	5.93	6.88
Ho	0.40	0.24	0.31	0.45	0.31	0.34
He	0.80	0.64	0.89	0.91	0.83	0.81
Number of private alleles	3	1	6	1	4	3
Turkish Straits System						
n	168	170	167	162	173	168
Na	17	11	41	30	12	22.2
Ne	4.41	2.87	12.43	13.00	3.51	7.24
Ho	0.45	0.25	0.28	0.44	0.31	0.35
He	0.77	0.65	0.92	0.92	0.65	0.80
Number of private alleles	3	1	13	4	2	4.6
Aegean Sea						
n	117	122	114	115	118	117.2
Na	19	10	27	26	10	18.40
Ne	5.66	3.31	6.31	9.27	2.68	5.45
Ho	0.50	0.30	0.15	0.23	0.20	0.28
He	0.82	0.70	0.84	0.89	0.63	0.78
Number of private alleles	3	1	4	3	2	2.6
Clade A-Common group						
n	171	177	164	162	172	169.2
Na	21	10	40	28	16	23
Ne	21	10	40	28	16	7.147
Ho	0.44	0.30	0.32	0.46	0.33	0.37
He	0.81	0.65	0.92	0.91	0.75	0.81
Number of private alleles	8	3	23	10	12	11.20

Table 3.4. Levels of genetic variability at five microsatellite loci for *Mytilus galloprovincialis* populations. n, sample size; Na, number of alleles; Ne, number of effective alleles; He, expected heterozygosity; Ho, observed heterozygosity (cont.)

Locus	**2**	**3**	**4**	**6**	**181**	**Mean**
Clade B-Aegean group						
n	37	40	37	38	40	38.4
Na	14	8	21	20	4	13.4
Ne	7.20	2.95	12.98	10.46	2.26	7.17
Ho	0.38	0.30	0.16	0.32	0.22	0.28
He	0.86	0.66	0.92	0.90	0.56	0.78
Number of private alleles	1	1	4	2	0	1.60

Based on AMOVA using all loci, the highest percentage of variation was found among individuals (86%). The percentage of variation among populations within groups was 14%. Multilocus estimates of overall R_{ST} and F_{ST} values were 0.14 and 0.04, respectively. Pairwise R_{ST} and F_{ST} values were estimated among all populations and are reported in Table 3.5. Within the different water bodies (the Black Sea, the TSS and the Aegean Sea) majority of R_{ST} estimates were significant (52 out of 60 between the Black Sea and the Aegean, and 43 out of 54 between the TSS and the Aegean) and all significant values were less than 0.06 (Table 3.5). In addition, about half of the F_{ST} values between the Black Sea and the Aegean (29 out of 60) and majority of the R_{ST} values between the TSS and the Aegean (40 out of 54) were found to be significant, but all values were found to be less than 0.06. In summary, though most of the pairwise microsatellite comparisons between the populations were significant, the actual values were low.

Finally, 20 individuals from each clade (clade A and clade B) were selected to amplify a part of a non-repetitive nuclear region using a diagnostic marker for identification of three *Mytilus* species (*M. galloprovincialis*, *M. edulis* and *M. trossulus*). PCR analysis indicated that all samples exhibited a single (122 bp) band, confirming that all of the individuals analyzed were *M. galloprovincialis* and there was no differentiation at this locus between the individuals belonging to clade A and B.

Table 3.5. Above diagonal: Pairwise F_{ST} values between populations of *Mytilus galloprovincialis*. Below diagonal: Pairwise R_{ST} values between populations of *Mytilus galloprovincialis*. Significant P values (P<0.05) are indicated in bold.

		Black Sea										**Turkish Straits System**									**Aegean Sea**					
		B1	B2	B3	B4	B5	B6	B7	B9	B10	B11	TS1	TS2	TS3	TS4	TS5	TS6	TS7	TS8	TS9	A1	A2	A3	A4	A5	A6
Black Sea	B1		0.13	0.36	0.24	0.14	0.36	0.37	0.40	0.62	0.48	0.18	0.35	0.10	0.40	0.13	0.25	0.27	0.28	0.13	0.18	0.42	0.36	0.25	0.14	0.39
	B2	0.05		0.20	**0.001**	**0.001**	**0.001**	**0.002**	**0.05**	0.14	0.00	**0.01**	**0.07**	0.00	0.14	0.05	0.00	0.02	0	0	0	0.02	0.05	0	0.05	0
	B3	0.09	**0.04**		0.29	0.35	0.34	0.24	0.39	0.57	0.34	0.19	0.41	0.36	0.47	0.05	0.31	0.26	0.30	0.28	0.27	0.34	0.42	0.29	0.26	0.12
	B4	0.08	**0.001**	**0.04**		0.07	0.17	**0.04**	0.25	**0.002**	0.17	0.20	0.27	0.16	0.34	0.21	**0.001**	0.22	**0.001**	**0.001**	0.17	0.22	0.24	**0.002**	0.24	**0.001**
	B5	0.06	**0.02**	**0.04**	**0.001**		**0.001**	**0.002**	**0.04**	0.49	0.17	0.06	0.12	**0.001**	**0.02**	0.09	0.11	**0.06**	**0.001**	**0.001**	**0.01**	0.06	**0.001**	**0.001**	**0.001**	**0.001**
	B6	0.07	**0.01**	**0.03**	**0.02**	**0.03**		**0.01**	**0.001**	0.58	**0.02**	**0.001**	**0.01**	**0.001**	**0.001**	**0.02**	0.18	**0.002**	**0.001**	0.06	**0.002**	**0.002**	**0.001**	**0.001**	**0.001**	**0.001**
	B7	0.07	**0.003**	**0.03**	**0.01**	**0.001**	**0.01**		**0.001**	0.53	0.15	**0.001**	**0.05**	**0.006**	**0.001**	**0.001**	0.10	**0.001**	**0.001**	**0.001**	**0.002**	**0.001**	**0.001**	**0.001**	**0.001**	0.10
	B9	0.06	**0.001**	**0.02**	**0.01**	**0.02**	**0.02**	**0.01**		0.62	**0.003**	**0.001**	**0.001**	0.05	**0.001**	0.05	0.23	**0.002**	**0.002**	0.12	**0.001**	**0.001**	**0.03**	**0.01**	**0.03**	**0.001**
	B10	0.11	**0.03**	0.08	**0.001**	0.05	0.06	0.06	**0.06**		0.55	0.55	0.63	0.56	0.70	0.51	0.03	0.57	0.39	0.26	0.52	0.60	0.64	0.42	0.61	0.47
	B11	0.08	**0.01**	0.05	**0.02**	**0.02**	**0.03**	**0.01**	**0.01**	0.09		**0.001**	**0.001**	0.18	0.21	**0.05**	0.19	**0.001**	0	0.14	**0.009**	**0.007**	0.25	0	0.17	**0.001**
Turkish Straits System	TS1	0.24	0.21	0.20	0.21	0.18	0.19	0.21	0.17	0.24	0.20		**0.001**	**0.05**	**0.03**	**0.001**	0.22	**0.007**	**0.01**	0.11	**0.001**	**0.001**	**0.001**	**0.002**	**0.001**	**0.001**
	TS2	0.05	**0.02**	**0.04**	**0.02**	**0.03**	**0.03**	**0.03**	**0.01**	0.07	**0.01**	0.16		0.09	0.12	**0.06**	0.25	**0.005**	**0.04**	0.14	**0.001**	**0.001**	0.06	**0.02**	0.06	**0.001**
	TS3	0.05	**0.02**	**0.03**	**0.02**	**0.02**	**0.03**	**0.02**	**0.01**	0.06	**0.02**	0.16	**0.01**		**0.001**	**0.07**	0.18	0.06	**0.03**	**0.02**	**0.02**	0.06	**0.002**	**0.001**	**0.001**	**0.001**
	TS4	0.08	**0.04**	0.06	**0.05**	**0.05**	**0.05**	**0.04**	**0.04**	0.09	0.06	0.14	**0.03**	**0.03**		**0.07**	0.29	**0.01**	**0.07**	0.12	**0.001**	**0.001**	**0.001**	**0.03**	**0.002**	**0.001**
	TS5	0.10	0.06	**0.04**	0.06	**0.04**	0.05	**0.04**	0.05	0.09	0.07	0.12	**0.04**	**0.04**	0.05		0.23	**0.001**	**0.07**	0.12	**0.02**	**0.03**	**0.05**	**0.05**	**0.002**	**0.001**
	TS6	0.07	**0.001**	**0.04**	**0.001**	**0.03**	**0.04**	**0.03**	**0.02**	**0.02**	**0.04**	0.17	**0.03**	**0.02**	0.05	0.06		0.22	**0.04**	**0.001**	0.19	0.21	0.22	**0.05**	0.23	**0.001**
	TS7	**0.05**	**0.001**	**0.01**	**0.001**	**0.01**	**0.01**	**0.002**	**0.001**	**0.05**	**0.001**	0.16	**0.001**	**0.001**	**0.03**	**0.04**	**0.02**		**0.001**	0.11	**0.002**	**0.001**	**0.02**	**0.001**	**0.001**	**0.001**
	TS8	0.06	**0.001**	**0.03**	**0.001**	**0.01**	**0.02**	**0.01**	**0.002**	**0.04**	**0.001**	0.17	**0.01**	**0.02**	**0.04**	**0.05**	**0.01**	**0.001**		**0.001**	**0.001**	**0.001**	0.06	**0.001**	0.07	**0.001**
	TS9	**0.03**	**0.001**	**0.04**	**0.004**	**0.02**	**0.02**	**0.02**	**0.02**	**0.02**	**0.03**	0.17	**0.02**	**0.01**	**0.04**	**0.05**	**0.01**	**0.01**	**0.02**		**0.08**	**0.11**	**0.07**	**0.001**	0.09	**0.001**
Aegean Sea	A1	**0.02**	**0.001**	**0.02**	**0.001**	**0.01**	**0.01**	**0.001**	**0.001**	**0.04**	**0.01**	0.15	**0.001**	**0.01**	**0.03**	**0.04**	**0.01**	**0.001**	**0.001**	**0.001**		**0.003**	**0.001**	**0.001**	**0.001**	**0.001**
	A2	**0.04**	**0.001**	**0.03**	**0.02**	**0.03**	**0.01**	**0.03**	**0.01**	0.06	**0.02**	0.18	**0.02**	**0.02**	**0.05**	0.06	**0.01**	**0.01**	**0.01**	**0.02**	**0.001**		0.08	**0.00**	**0.04**	0
	A3	0.05	**0.01**	**0.01**	**0.01**	**0.02**	**0.01**	**0.01**	**0.01**	0.06	**0.02**	0.18	**0.001**	**0.01**	**0.04**	**0.04**	**0.03**	**0.01**	**0.01**	**0.02**	**0.01**	**0.02**		**0.02**	**0.00**	**0.001**
	A4	**0.04**	**0.003**	**0.03**	**0.01**	**0.02**	**0.02**	**0.02**	**0.01**	**0.04**	**0.03**	0.18	**0.02**	**0.01**	**0.04**	**0.05**	**0.01**	**0.01**	**0.01**	**0.00**	**0.001**	**0.006**	**0.01**		**0.03**	**0.001**
	A5	0.06	**0.02**	**0.02**	**0.02**	**0.01**	**0.02**	**0.01**	**0.01**	0.06	**0.01**	0.14	**0.01**	**0.005**	**0.04**	**0.04**	**0.03**	**0.001**	**0.01**	**0.02**	**0.01**	**0.02**	**0.01**	**0.02**		0
	A6	0.08	**0.001**	**0.04**	**0.001**	**0.01**	**0.04**	**0.001**	**0.02**	0.08	**0.04**	0.27	0.05	0.02	0.06	0.09	**0.05**	**0.001**	**0.001**	**0.03**	**0.01**	**0.04**	**0.01**	**0.03**	0	

3.1.4. Mitochondrial Phylogeography and Dispersal Patterns of *Mytilus galloprovincialis*

Understanding the origin of the genetic diversity can be particularly difficult in the marine realm, as the species with high dispersal potential (e.g. with planktonic larvae) are expected to show little genetic structure and high levels of gene flow (Palumbi, 1994). Gene flow is related to the potential of larval dispersal, which influences the geographic range and the genetic structuring of populations (Levin, 2006; Hedgecock, 1994,). Especially marine bivalve molluscs, such as scallops, oysters and mussels are all sessile or sedentary as adults, but they have high fecundity and pelagic larvae. Therefore, in these species, large population sizes and relatively high rates of gene flow between distant localities can be expected. However, many species have pelagic larval durations of only a few hours or days (Tioho et al., 2001; Knowlton and Keller, 1986; Olson, 1985) that would not be conducive to dispersal over large geographical distances, and may result in local differentiation. For instance, the maximum effective dispersal distance of mussel larvae is considered to be not more than 100 km (Becker et al., 2007; McQuaid and Phillips, 2000) Many studies show genetic differentiation of populations over large geographic scales, along open coasts [e.g. oysters *Pecten maximus* (Heipel et al., 1999); *Pinctada mazatlanica* (Arnaud et al., 2000); *Ostrea edulis* (Launey et al., 2002), as well as at small geographic scales [e.g. the mussel *Mytilus edulis* (Ridgway, 2001), the clam *Macoma balthica* (Luttikhuizen et al., 2003)].

Ladoukakis et al. (2002) showed genetic differentiation within *Mytilus galloprovincialis* geographically, despite the species' inherent ability to disperse through its planktonic larval stage. They had observed the differentiation of the populations of the species between the Mediterranean (Adriatic, Ionian, and southern, middle and northern Aegean) and northern Black Sea (Sevastopol, Ukraine). The differentiation found between the Black Sea and the Mediterranean was significant, however the sampling gap between northern Aegean and Ukraine did not make it possible to determine where the actual genetic break took place. The role of the Bosphorus Strait as a potential barrier differentiating these populations was investigated by Kalkan et al. (2011). This study had shown that the strait did not comprise a hydrographic barrier preventing gene flow between the Black Sea and the Sea of Marmara.

Here, we tried to trace the origin of the differentiation detected by Ladoukakis et al. (2002) with an intense sampling strategy along the Turkish coast, Bulgaria, Ukraine and Russia, and using both the mitochondrial COIII gene and five microsatellite loci. Our COIII results confirm the genetic differentiation detected by Ladoukakis et al. (2002) in the populations of *Mytilus galloprovincialis*. Three main haplogroups were detected in the mtDNA haplotype network. One of the clades (C) belongs to the mussel-specific C-genome, and was excluded from the phylogeographic analyses. Kalkan et al. (2011) had previously detected four C-genome haplotypes in seven individuals. In this study, five additional haplotypes were observed. Although we did our extractions from gill-tissue that is supposed to only contain F-genome mitochondrial DNA, sperm contamination of gill tissue during extraction could be the explanation of the C-genomes we encountered. This finding supports Kalkan et al. (2011), showing that mutational differences can accumulate within the C-genome and the genome is not monomorphic. C-genome specific sperm sampling and subsequent analyses are needed to get a clearer picture of the evolution of this genome, and evaluate its potential use for phylogeographic purposes.

The results of the expansion tests combined with phylogeographic evaluations give an idea about the direction of the connectivity of populations in the Black Sea and the Aegean. The difference between two clades may be explained with a scenario where the populations of *M. galloprovincialis* were differentiated during the Pleistocene. *M. galloprovincialis* is believed to have evolved during the isolation of *M. edulis* populations in the Mediterranean Sea around two million years ago (Seed, 1992; Gosling, 1984; Barsotti and Meluzzi, 1968). According to Quesada et al. (1995), the evolution of *M. galloprovincialis* in the Mediterranean could be more recent if a molecular clock rate of 2 % per million years is assumed. Over the past few million years, numerous ocean-climate events caused population subdivisions, population declines and local extinctions (Magoulas et al., 2006). In the early Pleistocene (1.5-3 Mya), the ancient basin including the broader geographical area of the modern Black Sea and the Caspian Sea lost its connection to the world ocean and regained it in the Riss-Würm interglacial period (100,000-150,000 ya) (Zaitsev and Mamaev, 1997). According to our scenario, the clades A and B were formed in the Black Sea and the Aegean, respectively during the isolation of *M. galloprovincialis* populations in the Pleistocene. At the end of the Riss-Würm interglacial period (100,000-150,000 ya), following the opening the Dardanelles for the

first time since the formation of the Tethys Sea, the Black Sea got connected to the Mediterranean (Zaitsev and Mamaev, 1997). After the connection was established, *M. galloprovincialis* populations in the Black Sea dispersed into the Mediterranean with massive water outflow from the Black Sea, and this resulted in the movement of individuals with the main clade A mtDNA haplotypes in a first round of colonization. At the end of the last Würm Glaciation (18,000-20,000 ya), the Black Sea again lost its connection with the Mediterranean (Zaitsev and Mamaev, 1997). During this second isolation, we hypothesize that the sub-clade A2, that is slightly differentiated from the main A1 clade (by three bp), was formed in the Aegean Sea, and this process of differentiation likely continued until approximately 7000 ya, when the Black Sea turned to its current phase and its connection with the Mediterranean was re-established through the Turkish Strait System (TSS) (Zaitsev and Mamaev, 1997; Herman, 1988), and gene flow ensued. The observation that only one of the 13 sub-clade A2 haplotypes is found in the Black Sea supports this scenario. After the connection was established, the planktonic larvae were able to move in one direction only, following the surface current from the Black Sea to the Aegean through the TSS. The movement in the other direction (Aegean to the Black Sea, through the Dardanelles) was not possible due to the current regime of the TSS. Hence, although sub-clade A1 was able to colonize the Aegean from the Black Sea, clade B and sub-clade A2 were not able to colonize the TSS and the Black Sea in the reverse direction, with the barrier effect coinciding with the Dardanelles.

It should also be noted that the mtDNA of six individuals from the study area clustered with Atlantic haplotypes. Five of these individuals were collected from three different Aegean sampling sites (Saros, Foca and Gulluk) and the last one was from the TSS (Erdek). Because the Mediterranean Sea has sustained extensive maritime activity, many organisms can be easily transported via ballast water and hull fouling (Wachsmann, 2008; Minchin, 2002; Knapp, 1993). Therefore, these six individuals that grouped with the Atlantic haplotypes were probably transported by the ballasts of ships from the Atlantic.

3.1.5. Mitochondrial and Microsatellite Discordance and Heterozygote Deficiency

Microsatellites comprise a valuable tool in the detection of differentiated populations, and many studies show the effectiveness of these markers when investigating potential presence of a genetic barrier between populations (Lemaire et al., 2005; Reilly et al., 1999; Shaw et al., 1999). In this study, we did not detect differentiation between the populations in the Black Sea, TSS and the Aegean, using five microsatellite loci, although monophyletic mtDNA clades (A and B) were found. There are various possible reasons for this observation. Although female philopatry and male dispersal can be a behavioural factor in mammals (Greenwood, 1980), such behavioral imprint on genetic architecture is not the case for marine invertebrates with pelagic-larval stages, such as *M. galloprovincialis*. Another reason for not detecting differentiation in the nuclear genome could be the gene flow having decreased recently among sampling sites, which would prohibit the divergence and differential sorting of nuclear alleles (Friesen et al., 1996). However, our analyses support a scenario of long-term isolation rather than a recent separation. It should be noted that nuclear DNA is usually diploid and bipaternally inherited, whereas mtDNA is mostly haploid and maternally inherited (Hoarau et al., 2004). Because of these differences, mitochondrial effective population size [Ne(mt)] is four times smaller than that of nuclear loci (Hefti-Gautschi et al., 2009). Therefore, mitochondrial markers are expected to reach equilibrium more rapidly than nuclear markers (Friesen et al., 1996), and contrasting patterns of genetic structure may be seen in mtDNA, especially if the nuclear markers have not yet reached the equilibrium state (Harris and Hey, 1999). Also, drift might be too small to detect in nuclear DNA, but mtDNA will reach migration-drift equilibrium sooner of nuclear DNA (Birky-Jr et al., 1989). Hence mtDNA is likely to be more effective in detecting population differentiation than nuclear DNA (Birky-Jr et al., 1989), and the discrepancy we observed between mtDNA and nuclear markers could be reflecting this phenomenon.

The lack of genetic differentiation found in the five microsattalite loci could also be due to the mussel populations (representing clades A and B) not having established complete reproductive isolation. Our analyses of a diagnostic non-repetitive nuclear region (Inoue et al., 1995), which showed no base differences between individuals belonging to clades A and B confirmed that all of the individuals analyzed were *M. galloprovincialis*.

Hence although the isolation process between the Black Sea and the Aegean resulted in mtDNA differentiation of the respective populations, the subsequent connection that was made through the TSS helped to establish gene flow between these mitochondrially differentiated populations and homogenize their nuclear gene pools, as reproductive isolation was not formed during the course of this isolation.

There are a number of investigations about the heterozygote deficiency in marine organisms (Raymond et al., 1997; Waldman and McKinnon, 1993, Zouros and Foltz, 1984) but there is no consensus on this issue. Heterozygote deficiencies relative to Hardy-Weinberg expectations in bivalve populations have been commonly reported (Borsa et al.,1991; Fairbrother and Beaumont, 1993; Gaffney, 1990; Gallardo et al., 1998; Gosling, 1992; Huvet et al., 2000; Laudien et al., 2003; Passamonti et al., 1999; Raymond et al., 1997; Zouros and Foltz 1986). Heterozygote deficiency could be directly generated by genotyping or sampling artefacts (e.g. null alleles, Wahlund effect) and biological processes such as selection or inbreeding (Bierne et al. 1998; Hoarau, 2004; Van Oosterhout, 2004; McGoldrick et al. 2000).

Here, no genetic differentiation was detected in the five microsatellite loci genotyped, suggesting extensive gene flow, and the excess homozygosity at each locus was most likely due to the presence of null alleles. Non-amplified alleles due to the mutations in the primer sites are the main reasons of null alleles and the presence of null alleles could confuse the analysis of population structure in population genetic studies as observed here (McGoldrick et al. 2000; Reece et al. 2004). According to Chapuis and Estoup (2007), the presence of null alleles can lead to the overestimation of both F_{ST} and genetic distances in cases of significant population differentiation, and F_{ST} estimates are unbiased in the absence of population structure. In this study, as genetic differentiation was not detected, the presence of null alleles in our data set probably did not bias our F_{ST} estimates to affect our inferences.

3.2. *Palaemon elegans* Rathke, 1837

3.2.1. Haplotype Diversity

The genetic diversity of *Palaemon elegans* along the Turkish coasts were assessed by sequencing a 621bp fragment of the mitochondrial CO1 gene and 520bp fragment of the 16S rRNA in 210 specimens from 21 sampling sites. The CO1 and the 16S fragments were successfully amplified in 186 and 203 individuals, respectively (Table 3.1).

For the CO1 gene a total of 78 unique haplotypes were found in 186 specimens. 59 sites were polymorphic and 34 were parsimony informative in this fragment (Appendix C). Overall, 78 unique haplotypes resulted in high haplotype diversity (h= 0.907 ±0.015). Nucleotide diversity (π) was found to be 0.03946 ±0.00068. The mean number of pairwise differences (k) was 13.53. The analyses of 16S rRNA showed 29 haplotypes and 26 variable sites, 11 of which were parsimony informative (Appendix C). High haplotype diversity (h= 0.763 ±0.018) were detected again and π was found to be 0.01001 ±0.00038. For the 16S gene, the mean number of pairwise differences was 2.45.

3.2.2. Phylogenetic Trees

The neighbor-joining and the maximum likelihood trees of CO1 gene showed two main groups (Type A and Type B) with very low bootstrap values (4%) (Figure 3.6, 3.7). Same groups were also observed at the phylogenetic trees of 16S rRNA gene with high bootstrap values (more than 65%) (Figure 3.8, 3.9). Low bootstrap values are probably due to high diversity levels and reversals in the CO1 data set.

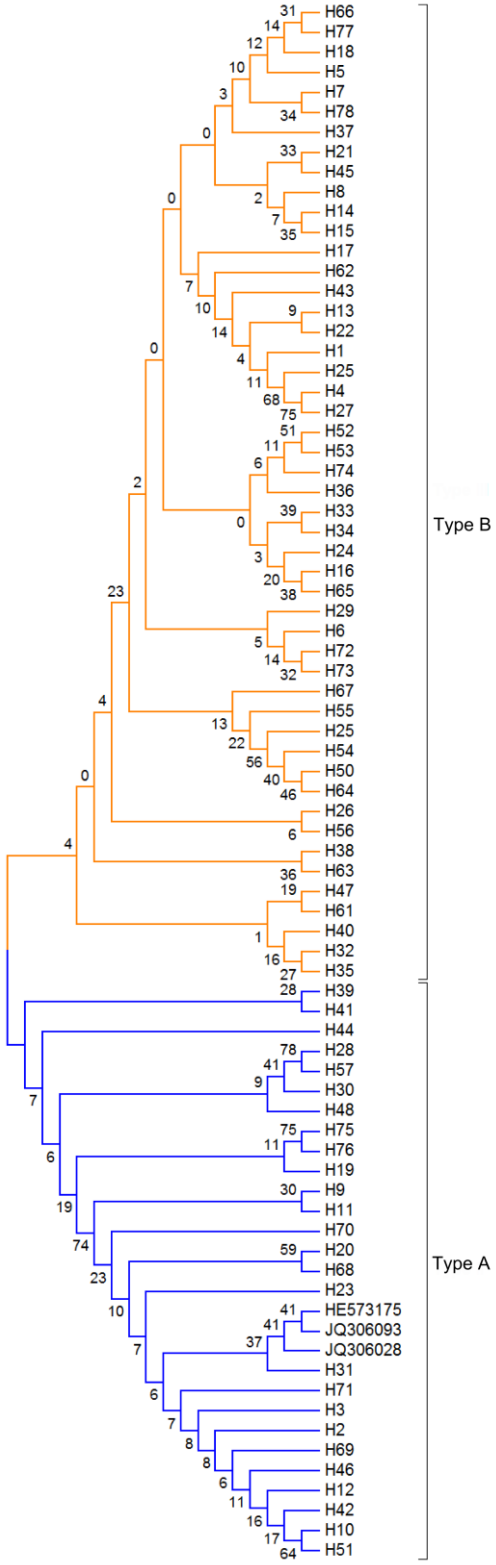


Figure 3.6. Maximum-likelihood tree of 78 haplotypes belonging to CO1 dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. HE573175, JQ306093 and JQ306028 are sequences retrieved from GenBank. The numbers corresponds to the bootstrap values of maximum-likelihood tree.

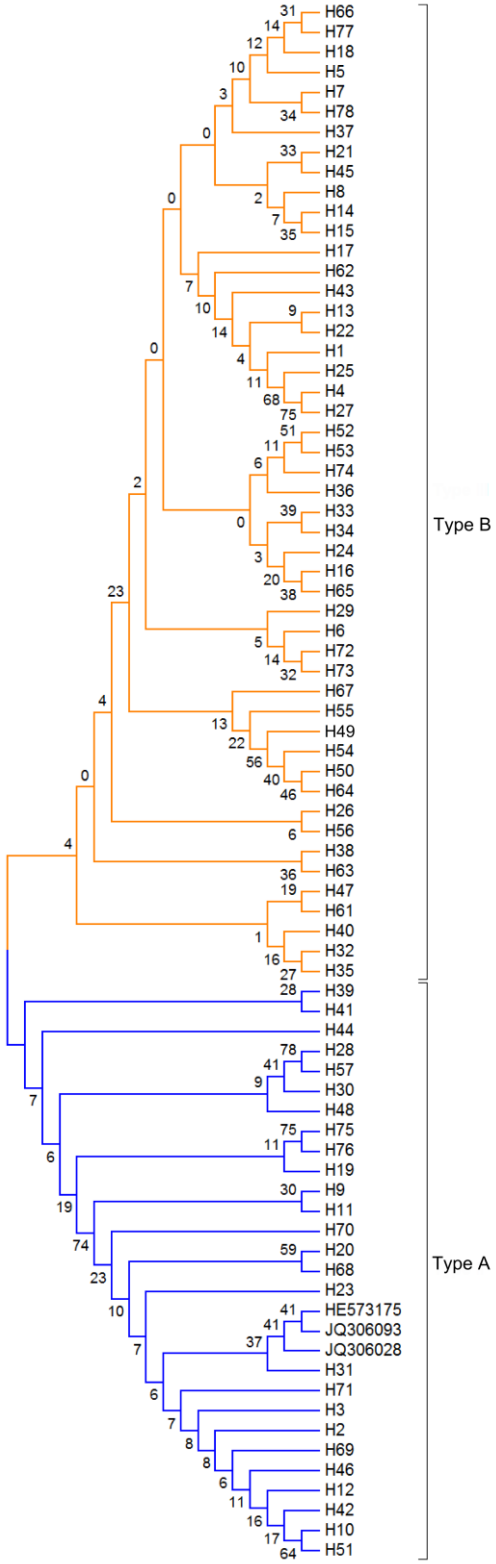


Figure 3.7. Neighbour-joining tree of 78 haplotypes belonging to CO1 dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. HE573175, JQ306093 and JQ306028 are sequences retrieved from GenBank. The numbers corresponds to the bootstrap values of neighbour-joining tree.

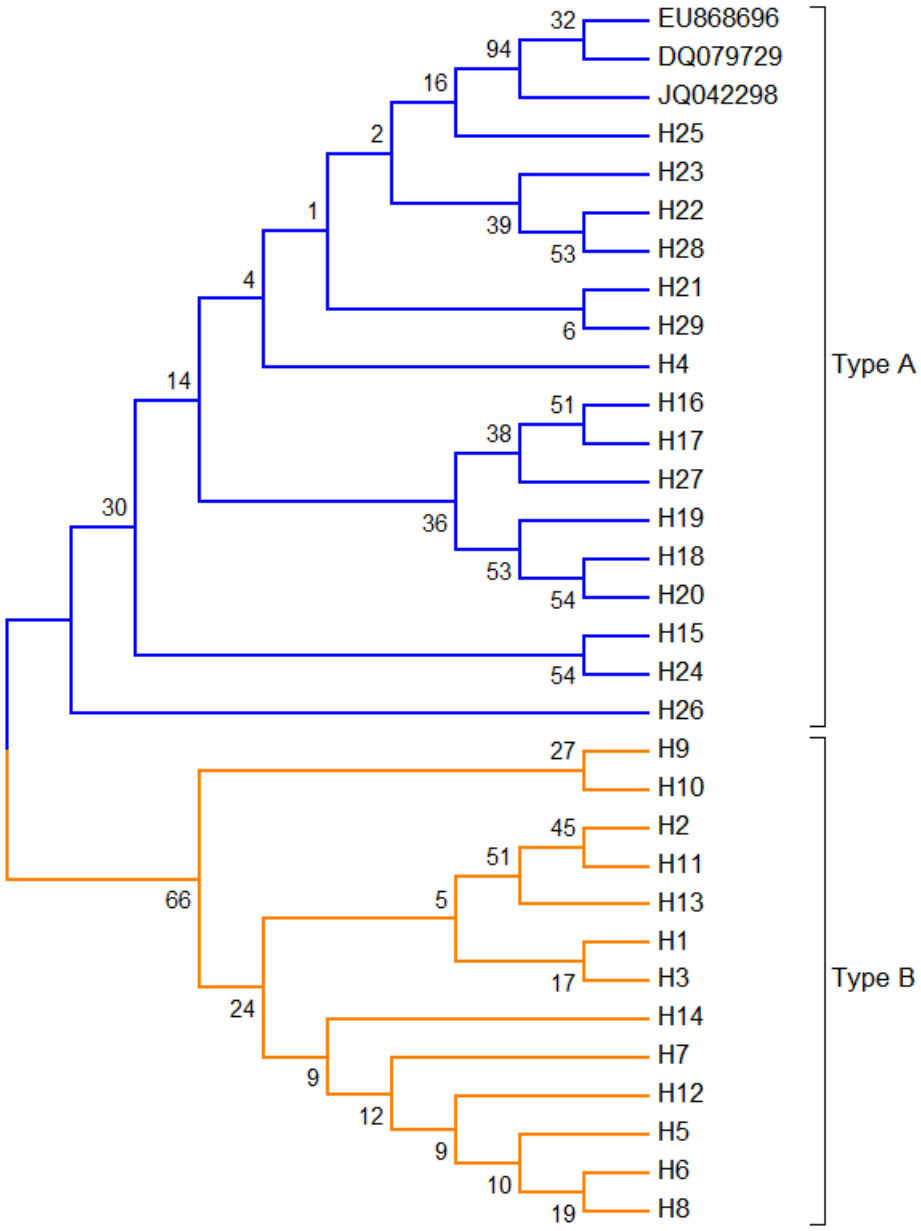


Figure 3.8. Maximum-likelihood tree of 29 haplotypes belonging to 16S data set from the Black Sea, Turkish Straits System and the Mediterranean Sea. EU868696, DQ079729 and JQ042298 are sequences retrieved from GenBank. The numbers corresponds to the bootstrap values of maximum-likelihood tree.

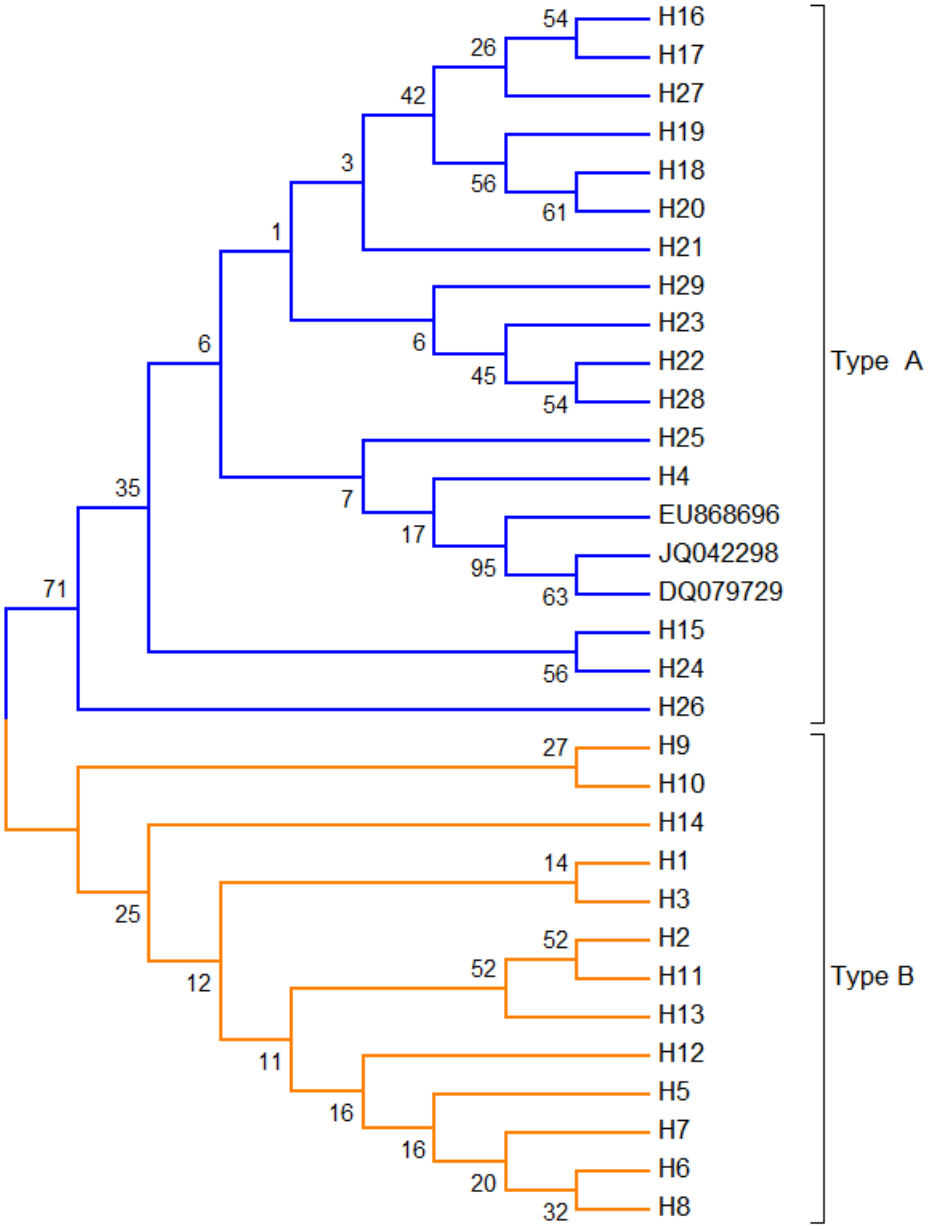


Figure 3.9. Neighbour-joining tree of 29 haplotypes belonging to 16S dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. EU868696, DQ079729 and JQ042298 are sequences retrieved from GenBank. The numbers corresponds to the bootstrap values of neighbour-joining tree.

3.2.3. Haplotype Network

Within *Palaemon elegans*, two main haplogroups (referred as Type A and Type B) were also recovered for both of the genes in the haplotype networks (Figure 3.10, 3.12). However, the separation of two groups in the haplotype networks was not as extensive, and therefore the determination of the separation points between the groups (Type A and Type B) was done based on the phylogenetic trees. Each group had one common haplotype (H1 and H2 for CO1, H4 and H5 for 16S) at a central position in the networks. In the CO1 gene, two groups were differentiated from each other by six mutations. Out of the 186 specimens, 79 were grouped in Type A. Out of these, 46 shared a common haplotype, which represented the most ancestral haplotype (H2). Type B, on the other hand, included 107 specimens, 46 out of which represented the other most commom ancestral haplotype, H1. While Type A was dominated by Mediterranean Sea specimens (65 of 79), Type B was observed in the Black Sea, the TSS and the Mediterranean Sea. The frequency of Type B in the Black Sea, the TSS and the Mediterranean were ~40% (42 out of 107), 30% (33 out of 107) and ~30% (32 out of 107), respectively (Figure 3.11). Considered separately, Type A and Type B had 22 and 54 haplotypes, respectively. The haplotype and nucleotide diversities were h= 0.695 ±0.062, π= 0.00419 ±0.00119 (Type A) (Table 3.6) and h= 0.746 ±0.052, π= 0.00914 ±0.00184 (Type B) (Table 3.7). The highest haplotype and nucleotide diversities in Type A were found in the TSS (h= 0.9 ±0.161, π= 0.0046 ±0.00138). On the other hand, within Type B the Black Sea populations showed the highest haplotype and nucleotide diversities (h= 0.959 ±0.02, π= 0.0234 ±0.00132).

Table 3.6. Molecular diversity indices and neutrality tests for *Palaemon elegans*-Type A populations based on CO1 region sequences. n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; π, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses.

Sampling site of *P.elegans*- Type A	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B3	2	-	-	-	-	-	-	-
B4	2	-	-	-	-	-	-	-
B6	3	-	-	-	-	-	-	-
B7	2	-	-	-	-	-	-	-
TS14	5	4	4	0.900 (0.161)	0.00462 (0.00138)	-1.09380	-1.405	0.1871
A3	19	9	10	0.914 (0.047)	0.00528 (0.00095)	-1.54313	-4.898	0.0891
A4	3	-	-	-	-	-	-	-
A8	3	-	-	-	-	-	-	-
A10	2	-	-	-	-	-	-	-
A11	4	3	2	0.833 (0.222)	0.00289 (0.00098)	-0.70990	-0.887	0.2500
M1	9	3	2	0.464 (0.200)	0.00145 (0.000699	-1.31009	-0.999	0.2165
M2	11	5	5	0.618 (0.164)	0.00263 (0.00095)	**-1.79107**	**-2.310**	0.1311
M3	2	-	-	-	-	-	-	-
M4	12	2	1	0.182 (0.144)	0.00053 (0.00041)	-1.12850	-0.410	0.2875
Total	79	22	30	0.695 (0.062)	0.00419 (0.00119)	**-2.48896**	**-13.755**	**0.0536**
Black Sea	9	6	27	0.852 (0.096)	0.02733 (0.00943)	-0.82114	0.048	0.1927
Turkish Straits System	5	4	4	0.900 (0.161)	0.00462 (0.00138)	-1.09380	-1.405	0.1871
Mediterranean Sea	65	16	15	0.677 (0.066)	0.00285 (0.00046)	**-2.17934**	**-13.293**	**0.0390**

Table 3.7. Molecular diversity indices and neutrality tests for *Palaemon elegans*-Type B populations based on CO1 region sequences. n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; π, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D. Fs and R_2 values are given in bold. Standard deviations are given in parentheses.

Sampling site of *P.elegans*- Type B	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B3	6	5	23	1.000 (0.177)	0.03527 (0.01439)	-0.31054	0.561	0.3381
B4	2	-	-	-	-	-	-	-
B5	6	5	15	1.000 (0.126)	0.02151 (0.00510)	0.20309	-0.752	0.2049
B6	20	3	3	0.600 (0.215)	0.00347 (0.00157)	-0.44736	0.117	0.2639
B7	8	4	9	1.000 (0.177)	0.01301 (0.00428)	-0.82943	-0.664	0.2406
TS2	6	3	5	0.600 (0.215)	0.00482 (0.00205)	-1.33698	0.688	0.2427
TS3	11	4	4	0.643 (0.184)	0.00289 (0.00112)	-1.53470	-1.236	0.1768
TS9	16	6	6	0.604 (0.150)	0.00433 (0.00143)	-1.18462	-1.765	0.1881
A2	25	9	14	0.696 (0.096)	0.00651 (0.00143)	-1.39737	-2.277	0.0795
A3	4	3	4	0.833 (0.222)	0.00578 (0.00196)	-0.78012	0.134	0.2500
A4	3	-	-	-	-	-	-	-
Total	107	54	27	0.746±0.052	0.00914±0.00184	-0.25031	-35.046	0.0961
Black Sea	42	24	25	0.959 (0.022)	0.02342 (0.00132)	0.93185	-9.103	0.1562
Turkish Straits System	33	12	17	0.623 (0.107)	0.00478 (0.00121)	**-2.22093**	**-6.941**	**0.0696**
Mediterranean Sea	32	10	16	0.664 (0.090)	0.00595 (0.00125)	-1.66410	-3.024	0.0648

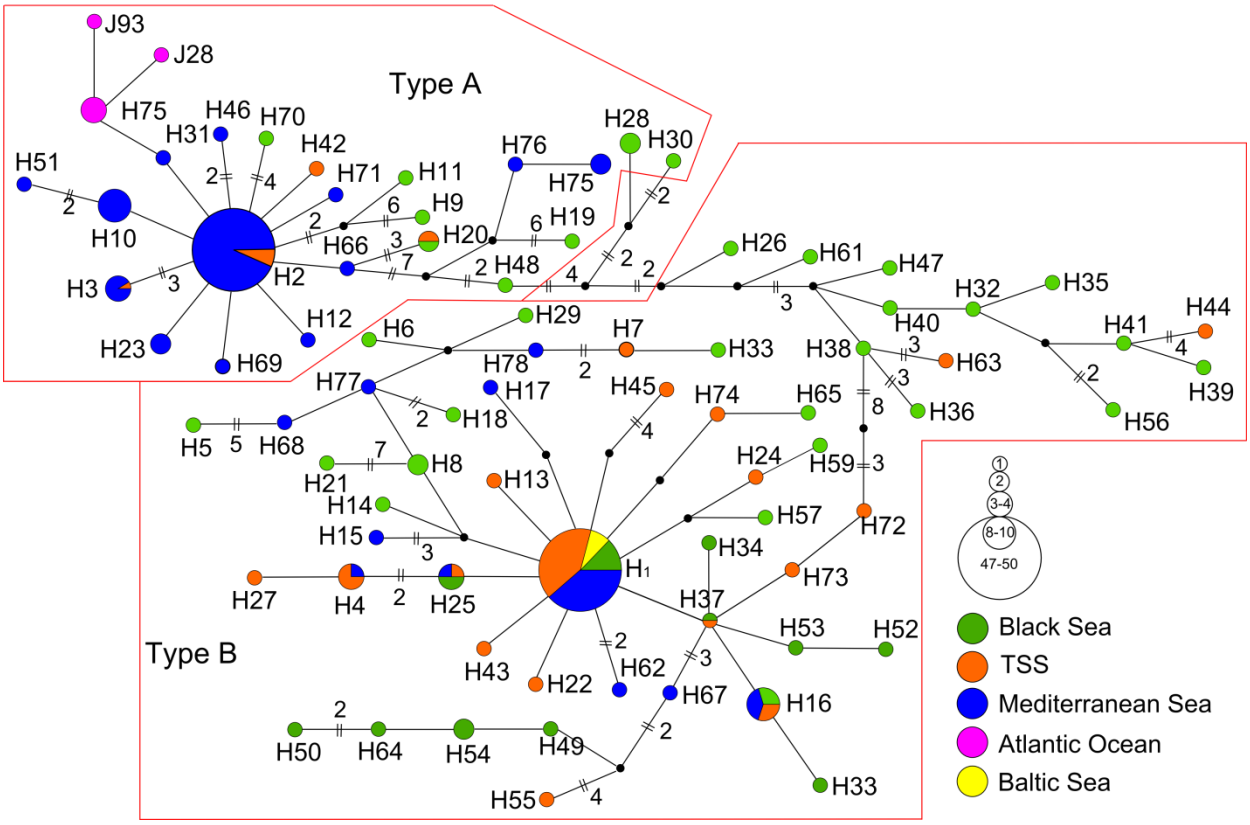


Figure 3.10. The haplotype network based on CO1 sequences of *Palaemon elegans*. The partitions inside a circle represent the frequency of the haplotype for each population and its diameter is proportional to the frequency of the haplotype. Small black circles represent missing haplotypes and each line represents a single mutational change.

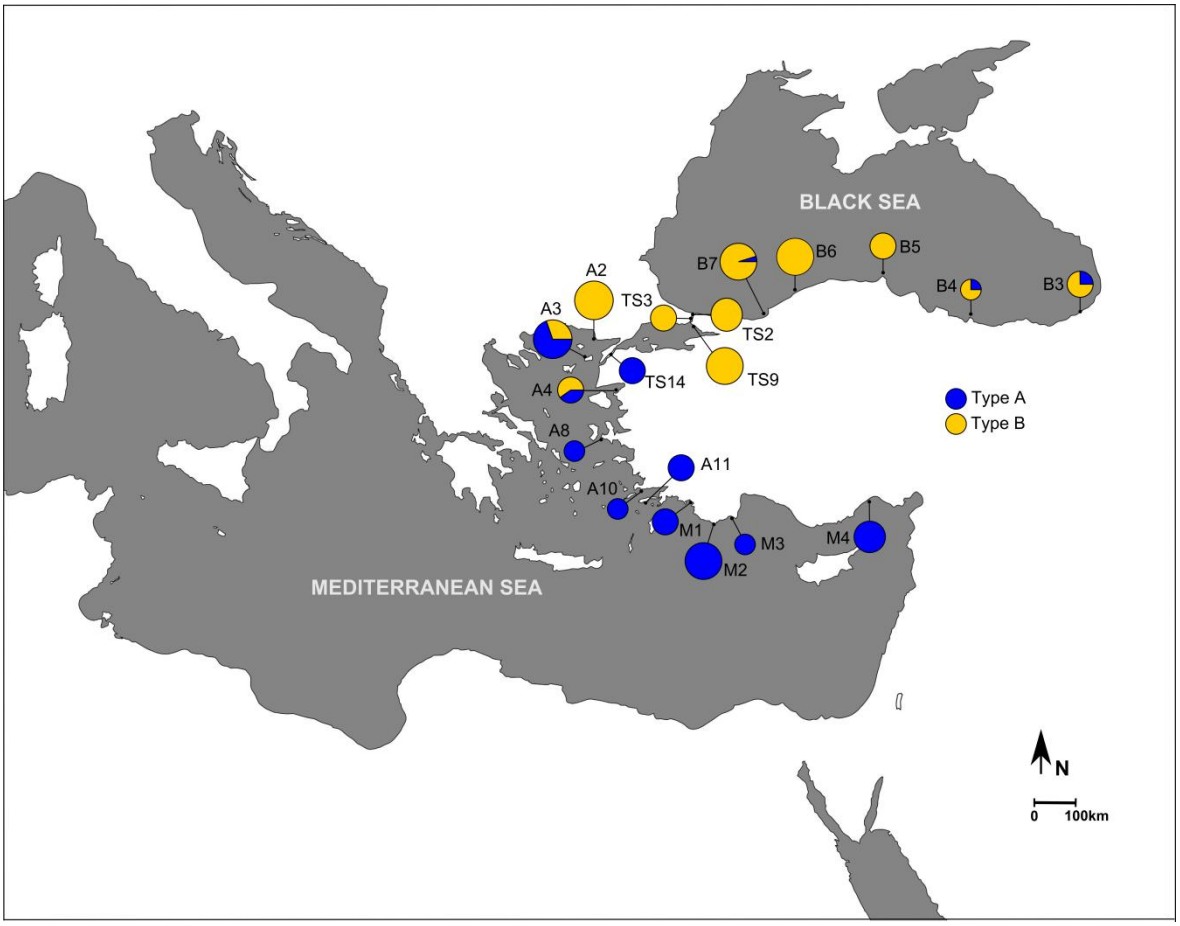


Figure 3.11. The CO1 clade frequencies of Type A and Type B.

For the 16S gene, Type A differed by at least three mutation steps from Type B. H9 was grouped into Type B based on phylogenetic trees (Figure 3.8, 3.9). While Type A consisted of 17 haplotypes (86 specimens), Type B included 13 haplotypes (113 specimens) (Table 3.8, 3.9). Within Type A the common haplotype H4 was recovered from 66 specimens mainly from the Mediterranean Sea. In addition, the common haplotype (H5) of Type B included 65 specimens representing the Black Sea, the TSS and the Mediterranean Sea. The Type B frequency in the Black Sea, the TSS and the Mediterranean were ~32% (21 out of 65), ~36% (23 out of 65) and ~32% (21 out of 65), respectively (Figure 3.13). The haplotype and nucleotide diversities were h= 0.412 ±0.068, π= 0.00286 ±0.00065 for Type A, and h= 0.606 ±0.041, π= 0.00329 ±0.00037 for Type B (Table 3.8. 3.9). The highest haplotype and nucleotide diversities were found in the TSS for Type A (h= 0.727 ±0.144. π= 0.00572 ±0.00169), and the Black Sea had the highest haplotype and nucleotide diversities for Type B (h= 0.969 ±0.017, π= 0.02460 ±0.00197) in a similar manner to the CO1 data set (Table 3.8, 3.9).

Table 3.8. Molecular diversity indices and neutrality tests for *Palaemon elegans*-Type A populations based on 16S region sequences. n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; π, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses.

Sampling site of *P.elegans* Type A	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B3	2	2	2	-	-	-	-	-
TS3	1	1	5	-	-	-	-	-
TS9	2	-	-	-	-	-	-	-
TS14	7	4	4	0.714 (0.181)	0.00461 (0.00168)	-1.43414	-1.217	0.1821
A1	1	-	-	-	-	-	-	-
A2	1	-	-	-	-	-	-	-
A3	8	3	6	0.607 (0.164)	0.00674 (0.00355)	-1.28011	1.200	0.2779
A10	3	-	-	-	-	-	-	-
A11	11	3	2	0.345 (0.172)	0.00146 (0.00077)	-1.42961	-1.246	0.1928
M1	7	1	0	0	0	0	0	0
M2	11	4	4	0.533 (0.180)	0.00321 (0.00137)	-1.66706	-1.345	0.1658
M3	17	4	9	0.331 (0.143)	0.00427 (0.00224)	**-2.17089**	**-0.061**	0.1432
M4	15	3	2	0.275 (0.148)	0.00114 (0.00065)	-1.48074	-1.475	0.1750
Total	86	17	18	0.412 (0.068)	0.00286 (0.00065)	**-2.46737**	**-19.115**	**0.0290**
Black Sea	2	-	-	-	-	-	-	-
Turkish Straits System	11	6	7	0.727 (0.144)	0.00572 (0.00169)	-1.64995	-2.508	0.1141
Mediterranean Sea	73	12	11	0.348 (0.073)	0.00227 (0.00066)	**-2.22588**	**-11.827**	**0.0358**

Table 3.9. Molecular diversity indices and neutrality tests for *Palaemon elegans*-Type B populations based on 16S region sequences. n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; π, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses.

Sampling site of *P.elegans* Type B	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B3	14	2	1	0.363 (0.130)	0.00147 (0.00053)	0.32440	0.643	0.1813
B4	11	2	4	0.691 (0.128)	0.00350 (0.00078)	0.85048	-1.026	0.2182
B5	3	-	-	-	-	-	-	-
B6	12	3	3	0.439 (0.158)	0.00188 (0.00074)	-0.84971	-0.725	0.1576
B7	13	6	6	0.795 (0.085)	0.00515 (0.00118)	-1.24415	-2.375	0.1126
B11	2	-	-	-	-	-	-	-
TS2	2	-	-	-	-	-	-	-
TS3	10	3	3	0.378 (0.181)	0.00240 (0.00132)	-1.56222	-0.459	0.2134
TS9	14	2	1	0.264 (0.136)	0.00105 (0.00054)	-0.34144	0.186	0.1319
A1	3	-	-	-	-	-	-	-
A2	20	6	7	0.574 (0.122)	0.00375 (0.00122)	-1.72678	-2.464	0.1048
A3	3	-	-	-	-	-	-	-
A4	2	-	-	-	-	-	-	-
A11	1	-	-	-	-	-	-	-
M2	1	-	-	-	-	-	-	-
Total	113	47	36	0.823 (0.040)	0.01750 (0.00187)	-0.82675	-30.654	0.0805
Black Sea	55	29	5	0.969 (0.017)	0.02460 (0.00197)	0.53367	-13.892	0.1377
Turkish Straits System	26	4	4	0.286 (0.112)	0.00151 (0.00068)	-1.70705	-1.966	0.1074
Mediterranean Sea	29	8	9	0.525 (0.110)	0.00319 (0.00095)	**-2.04123**	**-5.086**	**0.0722**

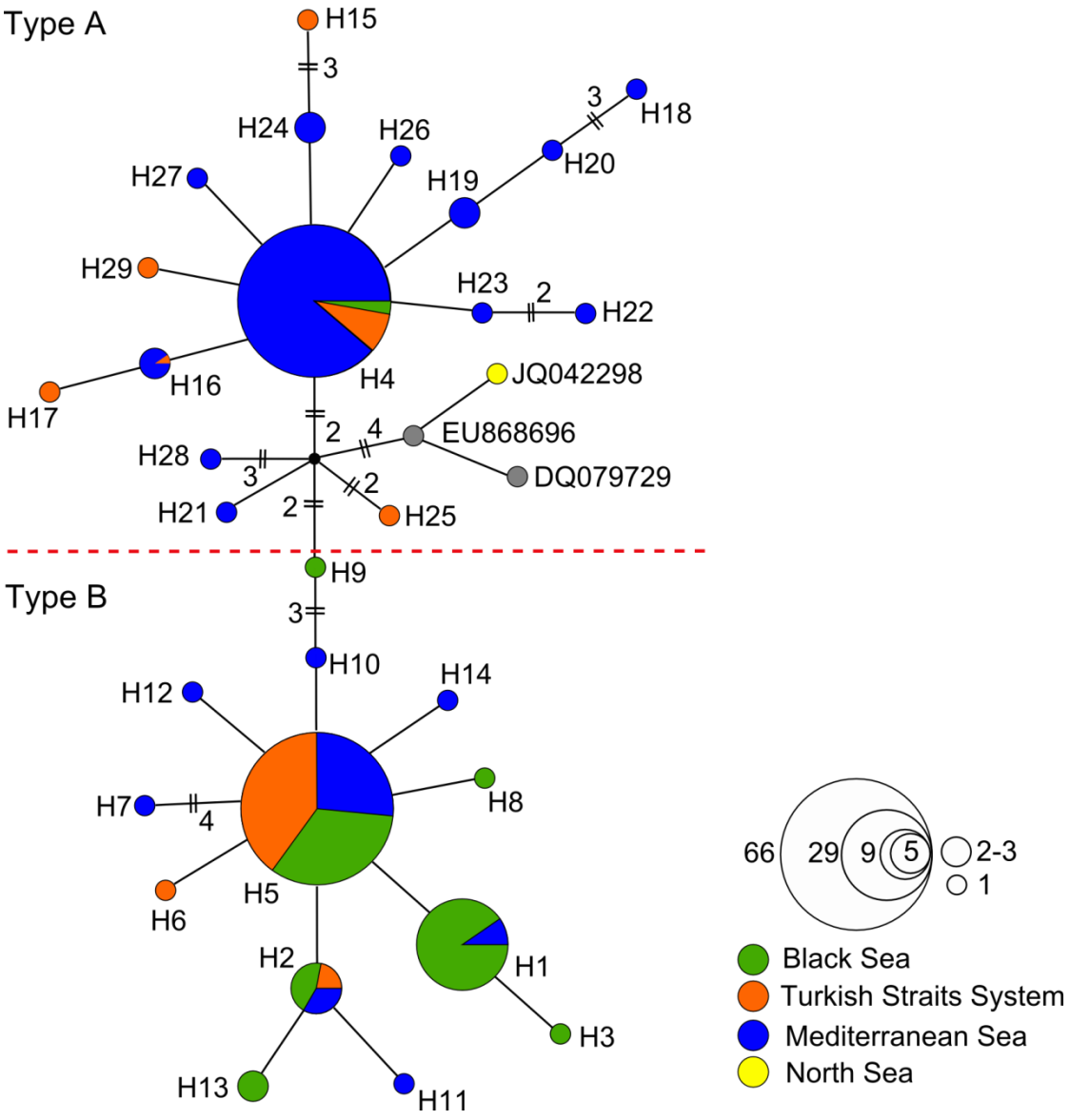


Figure 3.12. The haplotype network based on the 16S rRNA sequences of *Palaemon elegans*. The partitions inside a circle represent the frequency of the haplotype for each population and its diameter is proportional to the frequency of the haplotype. Small black circles represent missing haplotypes and each line represents a single mutational change. The dashed-line shows the separation of the two groups.

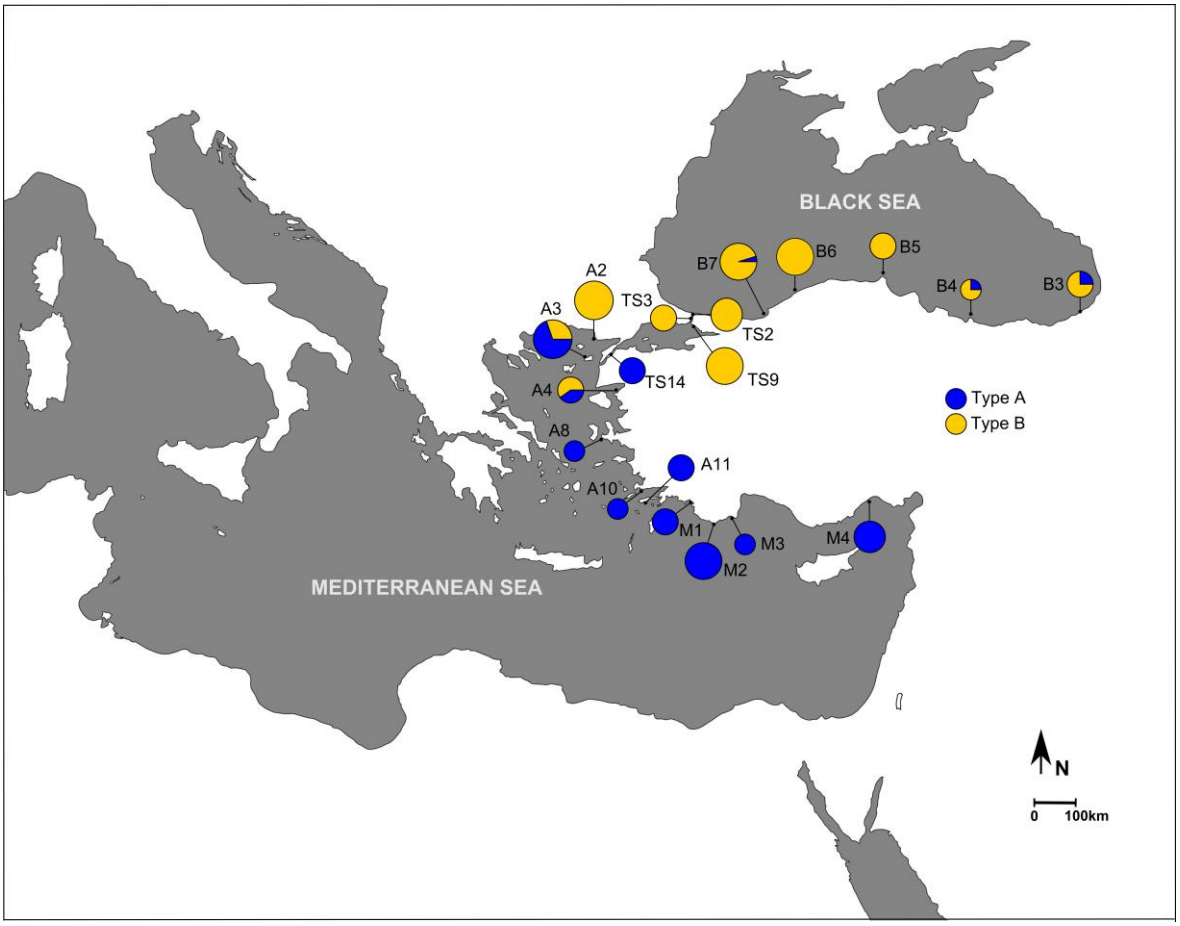


Figure 3.13. The 16S rRNA clade frequencies of Type A and Type B.

3.2.4. Population Structure

In general, the analysis of molecular variance (AMOVA) of the CO1 and 16S datasets among different water bodies (Black Sea, TSS and Mediterranean) showed high genetic variability; $\Phi_{st} = 0.63$ and $\Phi_{st} = 0.70$, respectively. While the molecular variance between seas was 13.93%, it was 49.54% among populations within groups and 36.52% within populations for the CO1 dataset. In addition, AMOVA analysis for the 16S dataset showed that 31.22% of molecular variance was among seas, 29.85% within populations and 38.93% among populations within seas. Pairwise Φ_{st} values between individual sites are given for the CO1 and 16S datasets in Tables 3.10, 3.11, respectively.

Buraya pairwise CO1 tablosu

Buraya pairwise 16S tablosu

The analysis of molecular variance (AMOVA) of the CO1 dataset between two subpopulations (Type A and Type B) revealed high levels of genetic structure (Φ_{st} = 0.907), the molecular variance between two types being 87.30% among populations, 3.40% within types, and 9.30% within populations. Pairwise Φ_{st} values between individual sites are given in Table 3.12. Significant and high values supported the strong genetic differentiation, which was detected in the haplotype network between the two types. Out of the 195 pairwise Φ_{st} values, 107 were found to be significant between populations of Type A and Type B.

The AMOVA analysis was also performed for Type A and Type B, separately. When populations were grouped as Black Sea, TSS and the Mediterranean, the AMOVA indicated high levels of genetic structure among Type A populations (Φ_{st}= 0.71), but low genetic structure in Type B populations (Φ_{st}= 0.20). The AMOVA also showed that 65.48% of molecular variance was among groups, 28.95% within populations and 5.56% among populations within groups for Type A, and 0.08% of molecular variance was among groups, 79.70% within populations and 20.21% among populations within groups for Type B. Pairwise Φ_{st} values for Type A and Type B among different water bodies (Black Sea, TSS and Mediterranean) are given in Table 3.13 and Table 3.14, respectively. Pairwise Φ_{st} values for the Type A were found to be higher than 0.25 between the Black Sea and the Mediterranean Sea, but only one value was found to be significant. 13 pairwise Φ_{st} values out of 30 were found to be significant between the Black Sea and the TSS-Mediterranean Sea for the Type B.

Similar results were obtained from the AMOVA of the 16S data set, which showed significant levels of differentiation among Type A and Type B (Φ_{st}= 0.71); the molecular variance between two types was 65.49%, among populations within types 5.56% and within populations 28.95%. Pairwise Φ_{st} values between individual sites are given in Table 3.15. 111 pairwise Φ_{st} values out of 195 were found to be significant between Type A and Type B.

The AMOVA, after pooling the sampling sites in the Black Sea, the TSS and the Mediterranean Sea for 16S dataset of Type A samples revealed a mean overall Φ_{st} value of 0.29 with 70.29% of the genetic variance within populations, 14.72% among populations

within groups and 14.98% among groups. When the AMOVA analysis was performed for 16S dataset of the Type B populations, low genetic structure was detected (Φ_{st}= 0.07) with 93.29% of the genetic variance within populations, 4.30% among populations within groups and 2.41% among groups. Pairwise Φ_{st} values between individual sites for Type A and Type B are given in Table 3.16 and Table 3.17, respectively.

Table 3.12. Pairwise Φ_{st} values of the CO1 dataset between two subpopulations (Type A and Type B). Significant P values ($P < 0.05$) are indicated in bold.

		TYPE A													**TYPE B**														
		B3	TS3	TS9	TS14	A2	A1	A3	A10	A11	M1	M2	M3	M4	B3	B4	B5	B6	B7	B11	TS3	TS2	TS9	A2	A1	A3	A4	A11	M2
TYPE A	B3	0																											
	TS3	0	0																										
	TS9	0.2	-1.0	0																									
	TS14	**0.6**	-1.0	**0.3**	0																								
	A2	0	0	-1	-1	0																							
	A1	0.2	1	-0.5	0.2	1	0																						
	A3	**0.6**	-0.9	0.3	0	-1	0.2	0																					
	A10	0.5	0	0.3	-0.2	0	1	-0.1	0																				
	A11	**0.7**	-1	**0.6**	0	-1	0.7	0	-0.2	0																			
	M1	0.7	0	**0.5**	0	0	1	0	0	0	0																		
	M2	0.5	-0.9	0.2	0	-1	0.1	0	-0.1	0	0	0																	
	M3	**0.8**	-1	**0.6**	0	-1	0.6	0	-0.2	0	0	0	0																
	M4	**0.8**	-1	**0.6**	0	-1	0.7	0.1	-0.2	0	0	0.1	0	0																
TYPEB	B3	**0.5**	1	**0.9**	**0.9**	1	**1**	**0.9**	**1**	**1**	**1**	**0.9**	**1**	**1**	0														
	B4	0.3	0.9	**0.8**	**0.9**	0.9	0.9	**0.9**	**0.9**	**0.9**	**0.9**	**0.8**	**0.9**	**0.9**	**0.2**	0													
	B5	0.1	1	0.7	**0.9**	1	1	**0.8**	1	**1**	**1**	**0.8**	**0.9**	**1**	0	0	0												
	B6	0.3	0.9	**0.9**	**0.9**	0.9	0.9	**0.9**	**0.9**	**0.9**	**1**	**0.9**	**0.9**	**0.9**	**0.8**	**0.4**	**0.7**	0											
	B7	0.2	0.9	**0.8**	**0.9**	0.9	0.9	**0.8**	**0.9**	**0.9**	**0.9**	**0.8**	**0.9**	**0.9**	**0.3**	0.1	0.1	0.1	0										
	B11	-0.2	1	0.7	**0.9**	1	1	**0.8**	1	**1**	1	0.8	**0.9**	**1**	**1**	0.3	**1**	-0.3	-0.1	0									
	TS3	0.3	0.9	**0.9**	**0.9**	0.9	1	**0.9**	**1**	**1**	**1**	**0.9**	**0.9**	**1**	**0.8**	**0.4**	**0.7**	0	0.1	-0.3	0								
	TS2	-0.2	1	0.7	**0.9**	1	1	0.8	1	**1**	**1**	**0.8**	**0.9**	**1**	**1**	0.3	**1**	-0.3	-0.1	0	-0.3	0.0							
	TS9	0.4	1	**0.9**	**0.9**	1	1	**0.9**	**1**	**1**	**1**	**0.9**	**1**	**1**	**1**	**0.5**	**0.9**	0	**0.2**	0	0	-0.3	0						
	A2	0.3	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.6**	**0.3**	**0.5**	0	**0.1**	-0.3	0	-0.3	0	0					
	A1	-0.1	0.9	0.7	**0.9**	0.9	0.9	**0.8**	1	**0.9**	**1**	**0.8**	**0.9**	**1**	**0.8**	0.1	0.5	0	0	0	0	0	0.2	0	0				
	A3	0	0.9	0.7	**0.9**	0.9	0.9	**0.8**	1	**0.9**	**1**	**0.8**	**0.9**	**1**	**0.9**	**0.4**	0.7	0.1	0.1	-0.2	0.1	-0.2	0.2	0	0	0			
	A4	-0.2	1	0.7	**0.9**	1	1	**0.8**	**1**	**1**	**1**	**0.8**	**0.9**	**1**	**1**	0.3	1	-0.3	-0.1	0	-0.3	0	-0.3	-0.3	-0.2	-0.2			
	A11	-1	1	0.3	0.9	1	1	0.8	1	1	1	0.8	0.9	1	1	0.1	1	-1	-0.5	0	-1	0	-1	-0.9	-1	-1	0	0	
	M2	-0.8	1	0.2	0.8	1	1	0.8	1	0.9	1	0.8	0.9	1	0	-0.6	0	0.6	-0.3	1	0.6	1	0.9	0.4	0	0.5	1	1	0

Table 3.13. Pairwise Φ_{st} values among the Black Sea, the TSS and the Mediterranean Sea populations of the Type A for CO1 dataset.

		Black Sea			**TSS**	**Mediterranean Sea**							
		B3	B4	B7	TS14	A3	A4	A8	A10	A11	M1	M2	M4
Black Sea	B3	0											
	B4	1	0										
	B7	1	1	0									
TSS	TS14	0.79	0.86	0.89	0								
Mediterranean Sea	A3	0.65	0.76	0.82	-0.04	0							
	A4	1	1	1	-0.29	-0.29	0						
	A8	1	1	1	-1	-0.93	0	0					
	A10	1	1	1	-1	-0.93	0	0	0				
	A11	0.87	0.91	0.93	-0.10	-0.13	-0.26	-1	-1	0			
	M1	0.93	0.95	0.97	0.05	0.01	-0.32	-1	-1	0.05	0		
	M2	0.81	0.87	**0.91**	0.05	-0.01	-0.25	-0.83	-0.83	-0.09	0.03	0	
	M4	0.97	0.98	0.99	**0.12**	0.03	-0.32	-1	-1	0.16	0.02	0.06	0

Table 3.14. Pairwise Φ_{st} values among the Black Sea, the TSS and the Mediterranean Sea populations of the Type B for CO1 dataset.

		Black Sea					**TSS**			**Mediterranean**		
Black Sea		B3	B4	B5	B6	B7	TS2	TS3	TS9	A2	A3	A4
	B3	0										
	B4	-0.22	0									
	B5	0.01	0.21	0								
	B6	**0.40**	0.91	0.20	0							
	B7	0.14	0.61	-0.02	0.05	0						
TSS	TS2	**0.45**	0.93	**0.24**	**-0.02**	0.13	0					
	TS3	**0.38**	0.88	**0.18**	**0.03**	0.13	-0.02	0				
	TS9	**0.45**	**0.85**	**0.24**	-0.06	0.06	-0.01	0.03	0			
Med.	A2	**0.49**	**0.83**	**0.25**	-0.03	0.08	-0.03	-0.02	0	0		
	A3	**0.25**	0.86	0.07	-0.02	-0.05	-0.04	-0.03	-0.03	-0.06	0	
	A4	0.24	1	0.06	-0.12	-0.01	-0.17	-0.17	-0.14	-0.12	-0.09	0

Table 3.15. Pairwise Φ_{st} values of the 16S dataset between two subpopulations (Type A and Type B). Significant P values ($P < 0.05$) are indicated in bold.

		Type A													**Type B**														
		B3	TS3	TS9	TS14	A2	A1	A3	A10	A11	M1	M2	M3	M4	B3	B4	B5	B6	B7	B11	TS3	TS2	TS9	A2	A1	A3	A4	A11	M2
Type A	B3	0																											
	TS3	0	0																										
	TS9	0.2	-1	0																									
	TS14	0.6	-1	**0.3**	0																								
	A2	0	0	-1	-1	0																							
	A1	0.2	1	-0.5	0.2	1	0																						
	A3	**0.6**	-0.9	**0.3**	0	-0.9	0.2	0																					
	A10	0.5	0	0.2	-0.2	0	1	-0.1	0																				
	A11	**0.7**	-1	**0.6**	0	-1	0.7	0	-0.2	0																			
	M1	0.7	0	**0.5**	0	0	1	0	0	-0.1	0																		
	M2	**0.5**	-1	0.2	0	-0.9	0.1	0	-0.1	0	0	0																	
	M3	**0.8**	-1	**0.6**	0	-1	0.6	0	-0.2	0	-0.1	0	0																
	M4	**0.8**	-1	**0.6**	0	-1	0.7	0.1	-0.2	0	-0.1	0.1	0	0															
Type B	B3	**0.5**	**1**	**0.9**	**0.9**	1	1	**0.9**	**1**	**1**	**1**	**0.9**	**0.9**	**1**	0														
	B4	0.3	**0.9**	**0.8**	**0.9**	0.9	0.9	**0.9**	**0.9**	**0.9**	**0.9**	**0.8**	**0.9**	**0.9**	**0.2**	0													
	B5	0.1	1	0.7	**0.9**	1	1	**0.8**	1	**1**	**1**	**0.8**	**0.9**	**1**	0	0	0												
	B6	0.3	0.9	**0.9**	**0.9**	0.9	0.9	**0.9**	**0.9**	**0.9**	**q**	**0.9**	**0.9**	**0.9**	**0.8**	**0.3**	**0.7**	0											
	B7	0.2	0.9	**0.8**	**0.9**	0.9	0.9	**0.8**	**0.9**	**0.9**	**0.9**	**0.8**	**0.9**	**0.9**	**0.3**	0.1	0.1	0.1	0										
	B11	-0.2	1	0.7	**0.9**	1	1	**0.8**	1	**1**	**1**	**0.8**	**0.9**	**1**	**1**	0.3	1	0.3	0.1	0									

Table 3.15. Pairwise Φ_{st} values of the 16S dataset between two subpopulations (Type A and Type B). Significant P values ($P < 0.05$) are indicated in bold (cont.)

		Type A													**Type B**														
		B3	TS3	TS9	TS14	A2	A1	A3	A10	A11	M1	M2	M3	M4	B3	B4	B5	B6	B7	B11	TS3	TS2	TS9	A2	A1	A3	A4	A11	M2
Type B	TS3	0.3	0.9	**0.9**	**0.9**	0.9	1	**0.9**	**1**	**0.9**	**1**	**0.9**	**0.9**	**1**	**0.8**	**0.4**	**0.7**	0	**0.1**	-0.3	0								
	TS2	-0.2	1	0.7	**0.9**	1	1	**0.8**	1	**1**	1	**0.8**	**0.9**	**1**	**1**	**0.3**	1	-0.3	-0.1	0	-0.3	0							
	TS9	0.4	**1**	**0.9**	**0.9**	**1**	1	**0.9**	**1**	**1**	**1**	**0.9**	**0.9**	**1**	**0.9**	**0.5**	**0.9**	0	**0.2**	-0.3	0	-0.3	0						
	A2	0.3	0.9	**0.8**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.9**	**0.8**	**0.9**	**0.9**	**0.6**	**0.3**	**0.5**	0	**0.1**	-0.3	0	-0.3	0	0					
	A1	-0.1	0.9	0.7	**0.9**	0.9	0.9	**0.8**	1	**0.9**	**1**	**0.8**	**0.9**	**0.9**	**0.8**	0	0.5	0	-0.2	-0.2	0	-0.2	0.2	0	0				
	A3	0	0.9	0.7	**0.9**	0.9	0.9	**0.8**	1	**0.9**	**1**	**0.8**	**0.9**	**0.9**	**0.9**	**0.4**	0.7	0.1	0.1	-0.2	0.1	-0.2	0.2	0	0	0			
	A4	-0.2	1	0.7	**0.9**	1	1	**0.8**	1	**1**	**1**	**0.8**	**0.9**	**1**	**1**	0.3	1	-0.3	-0.1	0	-0.3	0	-0.3	-0.3	-0.2	-0.2	0		
	A11	-1	1	0.3	0.8	1	1	0.8	1	0.9	1	0.8	0.9	1	1	0.1	1	-0.9	-0.5	0	-1	0	-1	-0.9	-1	-1	0	0	
	M2	0	1	0.2	0.8	1	1	0.8	1	0.	1	0.7	0.9	1	0	-1	0	0.6	0	1	0.6	1	0.8	0.4	0	0.5	1	0	0

Table 3.16. Pairwise Φ_{st} values among the Black Sea, the TSS and the Mediterranean Sea populations of the Type A for 16S dataset.

Black Sea			**TSS**			**Mediterranean**								
Black Sea		B3	TS3	TS9	TS14	A2	A1	A3	A10	A11	M1	M2	M3	M4
	B3	0												
TSS	TS3	0	0											
	TS9	-1	-1	0										
	TS14	-1	-1	**0.32**	0									
Mediterranean	A2	0	0	-1	-1	0								
	A1	1	1	-0.50	0.23	1	0							
	A3	-0.89	-0.89	**0.30**	-0.02	-0.89	0.16	0						
	A10	0	0	0.25	-0.17	0	1	-0.13	0					
	A11	-1	-1	**0.57**	-0.01	-1	0.69	0	-0.19	0				
	M1	0	0	**0.54**	-0.04	0	1	-0.01	0	-0.06	0			
	M2	-0.90	-0.90	0.21	0.02	-0.90	0.10	0.041	-0.14	0.03	-0.01	0		
	M3	-1	-1	**0.60**	0.03	-1	0.58	0.043	-0.19	-0.03	-0.08	0.04	0	
	M4	-1	-1	**0.65**	0.03	-1	0.75	0.06	-0.19	-0.02	-0.07	0.07	0	0

Table 3.17. Pairwise Φ_{st} values among the Black Sea, the TSS and the Mediterranean Sea populations of the Type B for 16S dataset.

		Black Sea						**TSS**			**Mediterranean**					
		B3	B4	B5	B6	B7	B11	TS3	TS2	TS9	A2	A1	A3	A4	A11	M2
Black Sea	B3	0														
	B4	0.07	0													
	B5	-0.12	-0.01	0												
	B6	**0.61**	**0.36**	**0.66**	0											
	B7	**0.17**	0.06	0.14	**0.09**	0										
	B11	0.71	0.32	1.00	-0.27	-0.09	0									
TSS	TS3	**0.63**	**0.41**	**0.72**	-0	0.09	-0.32	0								
	TS2	0.71	0.32	1	-0.27	-0.09	0	-0.32	0							
	TS9	**0.76**	**0.52**	**0.88**	-0.03	**0.19**	-0.33	0.01	-0.33	0						
Mediterranean	A2	**0.51**	**0.35**	**0.51**	-0.04	**0.14**	-0.27	0.03	-0.27	-0.02	0					
	A1	0.36	0.06	0.50	-0.03	-0.21	-0.20	-0.06	-0.20	0.24	-0.01	0				
	A3	**0.69**	**0.39**	**0.75**	0.08	0.09	-0.20	0.09	-0.20	0.24	-0.01	0	0			
	A4	0.71	0.32	**1**	-0.27	-0.09	0	-0.32	0	-0.33	-0.27	-0.20	-0.20	0		
	A11	0.66	0.13	1	-0.88	-0.54	0	-1	0	-1	-0.87	-1	-1	0	0	
	M2	-0.83	-0.60	0	0.57	-0.28	1	0.64	1.00	0.86	0.37	0	0.50	1	1	0

3.2.5. Mismatch Analyses

Three neutrality tests (Tajima's D, Fu's Fs, and Ramos-Onsins and Rozas's R_2) and mismatch distribution analyses were used to reconstruct the population demographic history for the populations of Type A and Type B, separately. The separate populations for CO1 and 16S results showed unimodal distributions of pairwise differences, indicative of population expansion (Figure 3.14a-d).

The results of tests for population expansion for CO1 and 16S datasets of Type A and Type B are given in Tables 3.6-3.9. The expansion test for CO1 and 16S datasets of Type A gave significantly negative values of Tajima's D and Fu's Fs. These significant and negative values of the D and Fs indicated that Type A had experienced a population expansion. In addition, after pooling samples together for Type B, the neutrality tests were found to be all insignificant for CO1 datasets. Considering the CO1 gene, the neutrality tests were all found to be significant in the Mediterranean Sea for Type A, and in the TSS for Type B (Table 3.6, 3.7). For the 16S data, they were all found to be significant in the Mediterranean Sea, for both Type A and Type B (Table 3.8, 3.9).

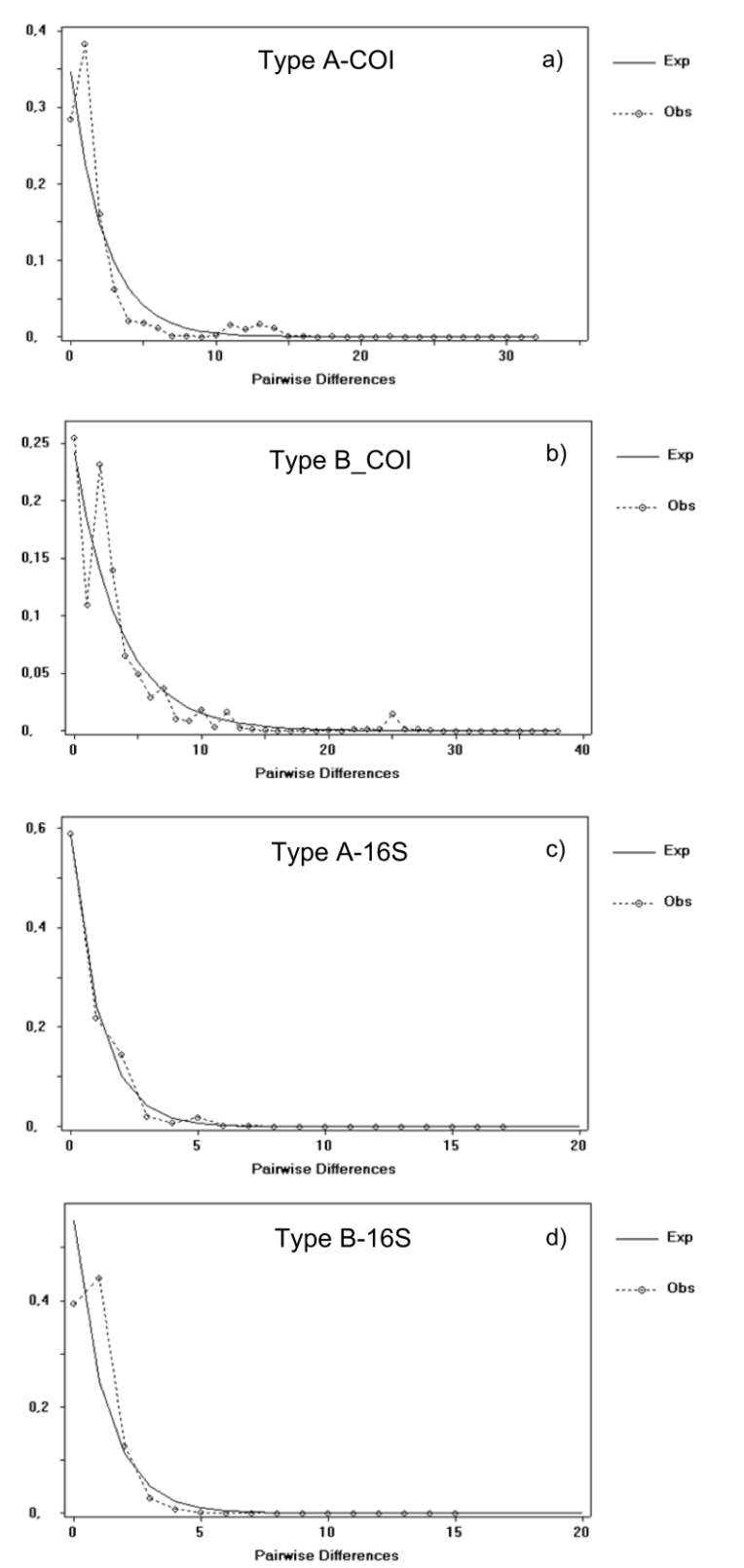


Figure 3.14. (a-d) Mismatch distribution obtained from CO1 and 16S gene sequence data for Type A and Type B. The lines with empty circles represent the observed distribution and the black lines represent the expected distribution under a sudden expansion model.

3.2.6. Discussion on Mitochondrial Phylogeography and Dispersal Patterns

Reuschel et al. (2010) revealed a complex population structure in *Palaemon elegans* and clearly distinct genetic lineages using mitochondrial genes with samples from Northeastern Atlantic, Baltic Sea, entire Mediterranean (especially western basin), Black Sea and Caspian Sea. Their study defined three different haplogroups (referred to as Type I, Type II and Type III) as a result of the comparison of multiple sequences of 16S rRNA and CO1 mitochondrial genes. Geographically speaking, Type I was distributed in Atlantic and Alboran Sea, Type II distributed in the entire Mediterranean, and Type III in Mediterranean, Baltic, Caspian and Black Seas. For the CO1 data set, they found the difference between types I and II, and Type III to be more than 50 mutations. In addition, Types I and II were separated by at least seven mutation steps from Type III according to 16S data. Therefore, they suggested that *P. elegans* is a species complex that includes at least one cryptic species, not differentiable using morphological characters. Here, we undertook a comparative and comprehensive analysis by using new *P. elegans* specimens from the eastern basin of the Mediterranean, the Turkish Straits System and the Black Sea, from where only limited data previously existed. We detected fewer differences between two groups (six bp in CO1 and two bp in 16S; Type A and B in our study corresponding to Type II and Type III, respectively in Reuschel et al. (2010)) for both genes.

While the haplotype networks and AMOVA results sufficiently supported two different groups in Reuschel et al. (2010), our groups could not be distinctly separated from each other in the haplotype networks and phylogenetic trees. However, the differentiation of groups was associated with geography. Type A consists mostly of samples from the Mediterranean, with only a few from the Black Sea and the Turkish Straits System (TSS), and Type B samples are distributed relatively evenly including specimens from all sites. This observed pattern is likely due to the two-layered currents of the TSS flowing in opposite directions (from the Black Sea to the Aegean at the upper-layer, and vice versa at lower-layer): Type B was able to disperse from the Black Sea to the Mediterranean relatively easily after the last ice age, but Type A was not able to colonize the Black Sea in the opposite direction. AMOVA results also supported this scenario; distinct population differentiation among the Black Sea, TSS and the Mediterranean Sea was observed in the overall data (especially for 16S), and in Type A (especially for CO1).

According to Reuschel et al. (2010), *P. elegans* specimens belonging to Type III (Type B in the present work) did not originate from the Black Sea as it had fresh water characteristics before it was flooded with the saline Mediterranean waters around 6800-9630 years ago. However, their calculations showed that the divergence between the Type I-II and Type III happened 6.85±0.85 million year (Myr) ago, so the separation between Type III and the other two types (I and II) occurred at an earlier time.

The Mediterranean Sea was isolated from the world ocean during the Messinian Crisis between 5.96 and 5.33 s Myr ago in the late Miocene (Duggen et al., 2003; Krijgsman et al., 1999). In this period, the sea level of the Mediterranean dramatically dropped because of the evaporation and subsequently a series of saline lakes were formed. According to Reuschel et al.'s scenario, the isolation of Type III ancestors in the Mediterranean from the Atlantic populations must have started before the Messinian Crisis (5.96 Mya). After the connection was established with the Atlantic Ocean, the Atlantic forms of *P. elegans* were re-introduced to the Mediterranean. Afterwards, they were differentiated from the Atlantic population (Type I) due to effects such as isolation by distance and limited gene flow between the populations, and formed Type II in the Mediterranean, which were reproductively isolated from each other.

If we mention two different groups or two different species (Reuschel et al. 2010) combination of our results with those of Reuschel et al. (2010) gives us a different perspective and suggests that Type B/III could have actually originated from the Black Sea. In the Miocene (from 5 to 7 Mya) the Tethys Sea shrunk in size and divided into a number of brackish basins. One of these basins was the Sarmatic Sea, which included modern Black Sea, Azov Sea, Caspian Sea and Aral Sea (Zaitsev and Mamaev, 1997). At this period, shrimps entrapped in the Sarmatic Sea could have been differentiated from the others and formed Type B/III *Palaemon elegans*. In early Pliocene (3-5 Mya), the Sarmatic Sea reduced in size and formed the Maeotic Sea and connected to the oceans again. In this period, many species of marine plants and animals settled in the Maeotic Sea. By the late Pliocene-early Pleistocene (less than one Mya) the connection with the world oceans was lost and the salinity of the sea was reduced again. The connection of the Black Sea with the world ocean had never been established until the Riss-Würm interglacial (100,000-150,000) years ago following the opening the Dardanelles (Zaitsev and Mamaev, 1997).

After the Black Sea-Mediterranean connection was established. *P. elegans* population in the Black Sea, composed of the Type B/III shrimp, likely dispersed into the Mediterranean with massive water outflow from the Black Sea, and this resulted in the movement of individuals belonging to Type B into the Mediterranean Sea.

As mentioned above, during these periods, the salinity of the system changed many times. It is be possible that Type B/III was able to adapt to salinity fluctuations and gained euryhaline characteristics. This situation can explain the occurrence of Type B/III in both marine (Mediterranean Sea) and brackish waters (Black Sea and Baltic Sea). In addition, the colonization of *P. elegans* (Type III) in the Caspian Sea due to the human introduction (Grabowski, 2006; Zenkevich, 1963) is another proof of the adaptation ability of Type III in brackish water. After the introduction of the Atlantic forms into the Mediterranean Sea, Type I was probably isolated in the Mediterranean and diverged to Type II (Type A). According to Reuschel et al. (2010), Type II was not found in the Atlantic and Type I was not detected in the Mediterranean Sea except two sampling sites (Málaga and Granada in Spain), which were in the westernmost part of the Mediterrenean. In addition, Type III was found only in the Mediterranean Sea except the Baltic Sea, where the occurrence of the species might also be a result of human caused introduction (Grabowski, 2006). The non-occurrence of Type II and Type III in the Atlantic and Type I in the Mediterranean are likely to be because of the barrier role of the Almeria-Oran front (Patarnello et al., 2007).

As seen in *Mytilus galloprovincialis* populations, the Turkish Straits System played a semi-permeable role for the populations of *P. elegans* (both for Type A and Type B), as well. While the system let Type B shrimp disperse into the Mediterranean from the Black Sea, it let the limited introduction of Type A in the reverse direction. The results of the expansion tests combined with phylogeographic evaluations support this scenario and give an idea about the direction of the connectivity of populations in the Black Sea and Mediterranean.

3.3. *Pachygrapsus marmoratus* (Fabricius, 1787)

3.3.1. Haplotype Diversity

596 bp and 521 bp fragments of mitochondrial CO1 and 16S rRNA genes were sequenced from 129 specimens of *Pachgrapsus marmoratus* collected from 19 sampling sites along the Black Sea, the Turkish Straits System (TSS) and the eastern Mediterranean coasts of Turkey (Table 3.1). In 129 specimens, a total of 20 haplotypes were found for the CO1 gene, with 21 polymorphic and five parsimony informative sites (Appendix C). A total of six haplotypes were detected for the 16S rRNA gene with five polymorphic sites (none parsimony informative) (Appendix C). Relatively high haplotype diversity (h= 0.71 ±0.028) and low nucleotide diversity (π= 0.0032 ±0.0002) were found in the CO1 dataset. On the other hand, low haplotype and nucleotide diversities were detected in the 16S dataset (h= 0.08 ±0.032, π= 0.0002 ±0.0001). The mean number of pairwise differences (k) was 1.35 for the CO1 dataset and 0.08 for the 16S dataset. The haplotype and nucleotide diversities of each population for the CO1 and the 16S datasets are reported in Table 3.18 and Table 3.19, respectively. In terms of CO1 data, the lowest haplotype diversity was found in the Black Sea and the highest haplotype diversity was found in the Mediterranean Sea (Table 3.18). On the other hand, the haplotype diversity values, in general, were very low in the 16S rRNA dataset in all seas examined (Table 3.19).

Table 3.18. Molecular diversity indices and neutrality tests for *Pachygrapsus marmoratus* based on CO1 region sequences. n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; π, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in parentheses.

Sampling site of *P.marmoratus*	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B3	1	1	-	-	-	-	-	-
B5	1	1	-	-	-	-	-	-
B6	2	1	-	-	-	-	-	-
B7	2	2	-	-	-	-	-	-
B11	2	1	-	-	-	-	-	-
TS2	20	5	5	0.368 (0.135)	0.00117 (0.00051)	**-1.97429**	**-2.991**	**0.1072**
TS3	5	1	0	0	0	-	-	-
TS7	2	2	-	-	-	-	-	-
TS10	3	3	-	-	-	-	-	-
TS14	4	3	3	0.833 (0.222)	0.00389 (0.00104)	0.16766	-0.133	0.2003
A1	1	-	-	-	-	-	-	-
A2	3	2	-	-	-	-	-	-
A3	12	5	5	0.758 (0.093)	0.00354 (0.00043)	-0.31291	-0.879	0.1334
A7	19	5	5	0.405 (0.143)	0.00153 (0.00062)	-1.74211	-2.380	0.0857
A8	2	1	-	-	-	-	-	-
A10	20	6	5	0.726 (0.075)	0.00256 (0.00048)	-0.68305	-1.987	0.1068
A11	8	3	2	0.464 (0.200)	0.00159 (0.00073)	-0.44794	-0.478	0.1970
M2	18	5	4	0.614 (0.117)	0.00186 (0.00048)	-0.94280	-1.844	0.1065
M4	4	2	2	0.667 (0.204)	0.00312 (0.00095)	1.89306	1.530	0.3333
Total	129	20	21	0.706 (0.028)	0.00317 (0.00019)	**-1.83678**	**-14.006**	**0.0329**
Black Sea	8	2	1	0.250 (0.180)	0.00058 (0.00042)	-1.05482	-0.182	0.3307
Turkish Straits System	34	6	6	0.389 (0.106)	0.00152 (0.00047)	-1.58120	-2.922	0.0645
Mediterranean Sea	87	13	11	0.628 (0.054)	0.00239 (0.00026)	-1.41271	-7.897	0.0464

Table 3.19. Molecular diversity indices and neutrality tests for *Pachygrapsus marmoratus* based on 16S rRNA region sequences. n, number of samples; Nh, number of haplotypes; Np, number of polymorphic sites; h, haplotype diversity; π, nucleotide diversity. D, Tajima's D; Fs, Fu's Fs; R_2, Ramos- Onsins and Rozas's R_2. The significant ($P < 0.05$) D, Fs and R_2 values are given in bold. Standard deviations are given in

Sampling site of *P.marmoratus*	**N**	**Nh**	**Np**	**h**	**π**	**D**	**Fs**	**R_2**
B3	2	1	-	-	-	-	-	-
B5	1	1	-	-	-	-	-	-
B6	2	1	-	-	-	-	-	-
B11	2	1	-	-	-	-	-	-
TS2	17	2	1	0.118 (0.101)	0.00036 (0.00031)	-1.16387	-0.748	**0.2353**
TS3	7	1	0	0	0	-	-	-
TS10	3	1	-	-	-	-	-	-
TS14	3	1	-	-	-	-	-	-
A1	3	2	-	-	-	-	-	-
A2	12	1	0	0	0	-	-	-
A3	20	1	0	0	0	-	-	-
A7	20	1	0	0	0	-	-	-
A8	1	1	-	-	-	-	-	-
A10	9	1	0	0	0	-	-	-
A11	7	2	1	0.286 (0.196)	0.00087 (0.00060)	-1.00623	-0.095	0.3499
M2	16	2	1	0.125 (0.106)	0.00038 (0.00032)	-1.16221	-0.700	0.2421
M4	4	1	0	0	0	-	-	-
Total	129	6	5	0.076 (0.032)	0.00023 (0.00010)	**-1.87189**	**-9.980**	**0.0386**
Black Sea	7	2	1	0.286 (0.196)	0.00087 (0.00060)	-1.00623	-0.095	0.3499
Turkish Straits System	30	2	1	0.069 (0.063)	0.00021 (0.00019)	-1.14923	-1.183	0.1825
Mediterranean Sea	92	4	3	0.065 (0.036)	0.00020 (0.00011)	-1.62177	**-5.516**	0.0595

3.3.2. Phylogenetic trees - (Phylogeny of the haplotypes)

Fourty-two CO1 and four 16S sequences were retrieved from GenBank for *Pachygrapsus marmoratus* and used in the phylogenetic analyses (Appendix 1). Two main haplogroups were observed in the maximum-likelihood and neighbor-joining trees for the mitochondrial CO1 gene with low bootstrap values, 51% and 43%, respectively. Both trees of the CO1 showed a main separation into two main clades (Group A and Group B) (Figure 3.15, 3.16). No separation was observed in phylogenetic trees of the 16S dataset (Figure 3.17, 3.18).

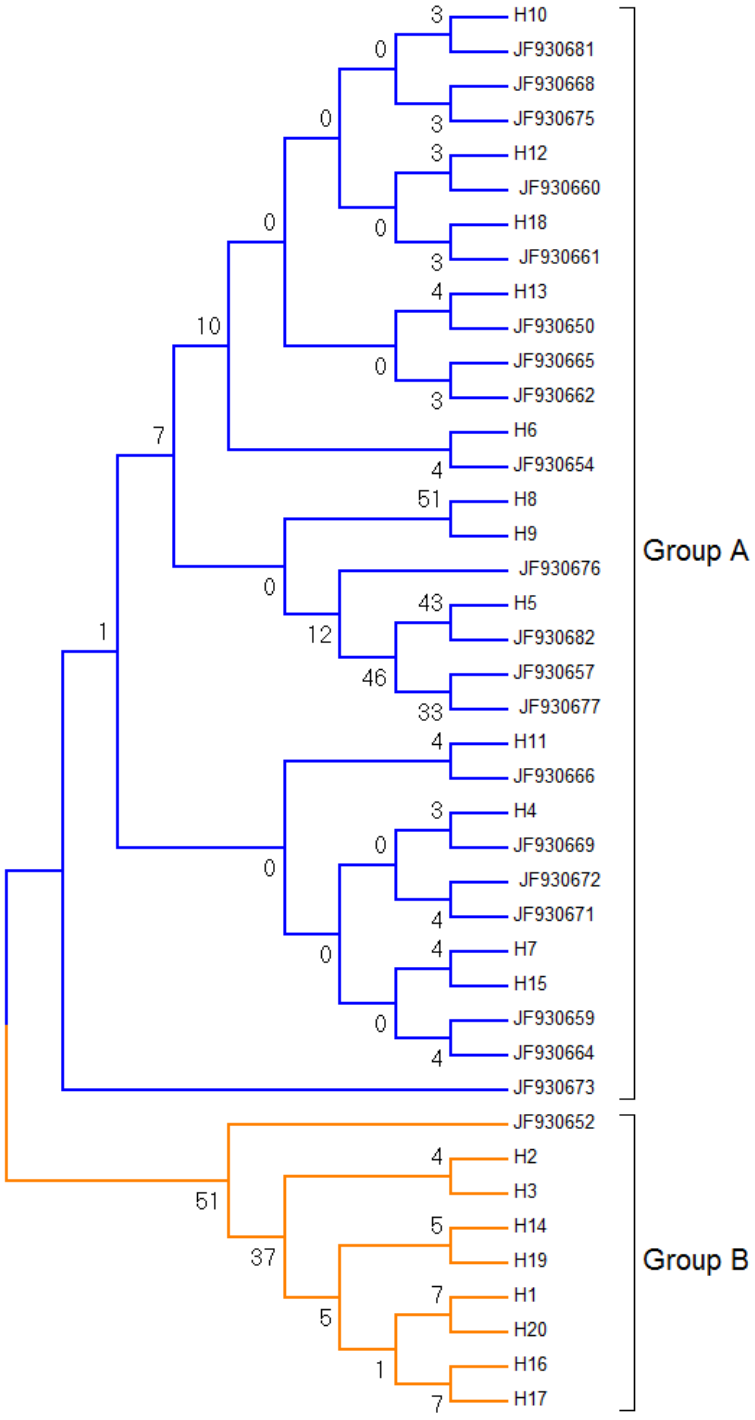


Figure 3.15. Maximum-likelihood tree of 20 haplotypes belonging to CO1 dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. The numbers corresponds to the bootstrap values of maximum-likelihood tree.

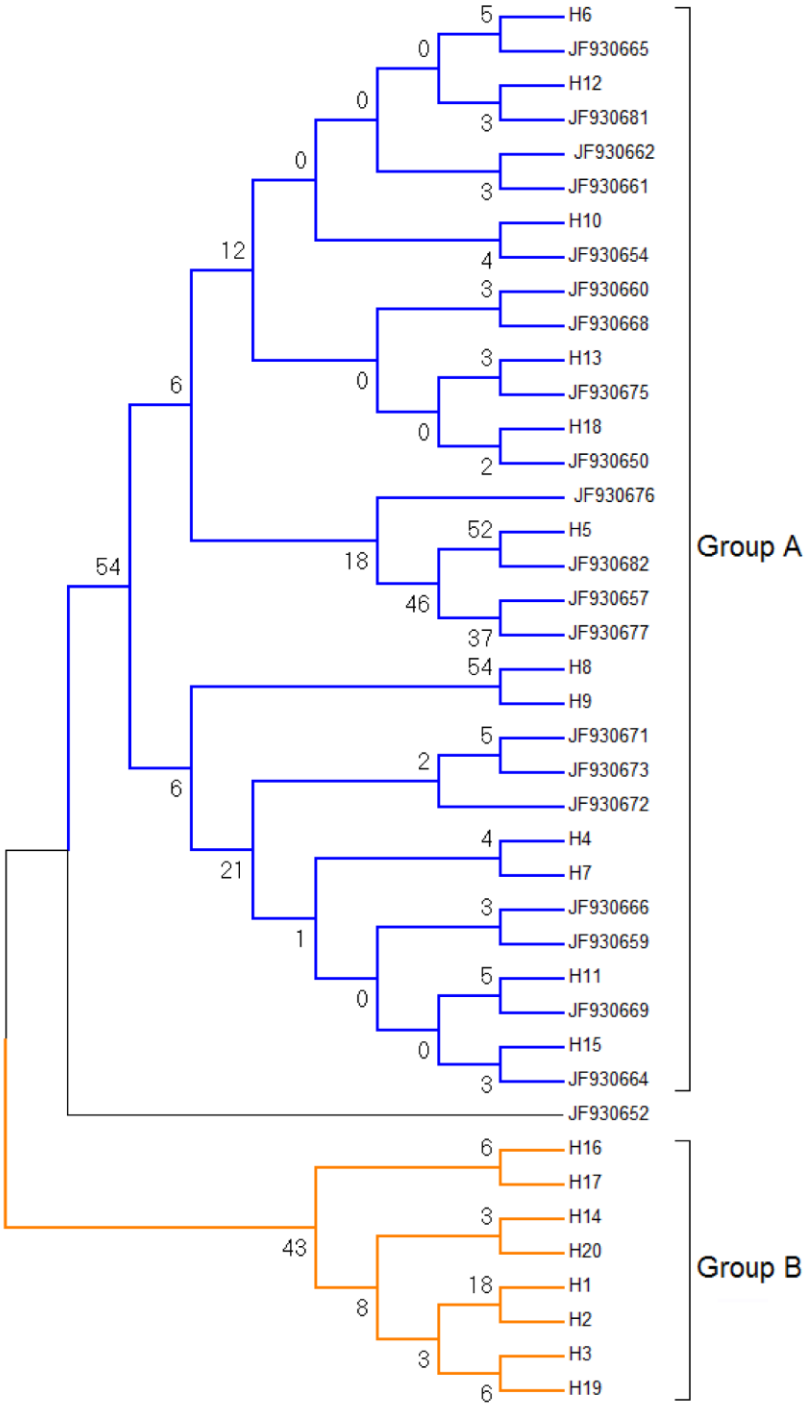


Figure 3.16. Neighbour-joining tree of 20 haplotypes belonging to CO1 dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. The numbers corresponds to the bootstrap values of neighbour-joining tree.

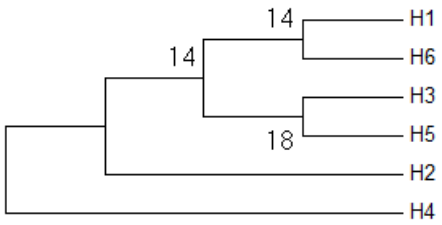


Figure 3.17. Maximum-likelihood tree of six haplotypes belonging to 16S dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. The numbers corresponds to the bootstrap values of maximum-likelihood tree.

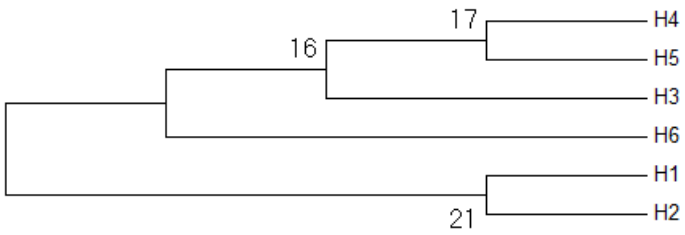


Figure 3.18. Neighbour-joining tree of six haplotypes belonging to 16S dataset from the Black Sea, Turkish Straits System and the Mediterranean Sea. The numbers corresponds to the bootstrap values of neighbour-joining tree.

3.3.3. Haplotype Network

The haplotype network of the CO1 dataset displayed a star-like shape in the center, surrounded by several rare haplotypes. The two main clades in the tree (Group A and Group B) can be seen to be differentiated geographically (Figure 3.19). These main haplotypes differ from each other by one or two mutations. Sequences belonging to 51 (out of 129) specimens were grouped in Group B. Out of these, 44 shared a common haplotype, which represented the ancestral haplotype (H1). Group A included 61 specimens and 54 out of that represented the other ancestral haplotype (H4). While all specimens of Group A except three specimens were from the Mediterranean Sea, Group B was observed in the Black Sea, the TSS and the Mediterranean Sea and the frequencies of the Black Sea, the TSS and the Mediterranean were ~16% (8 out of 51), 59% (30 out of 51) and ~25% (13 out

of 51), respectively (Figure 3.20). The geographical distribution of the clades can be seen in Figure 3.20.

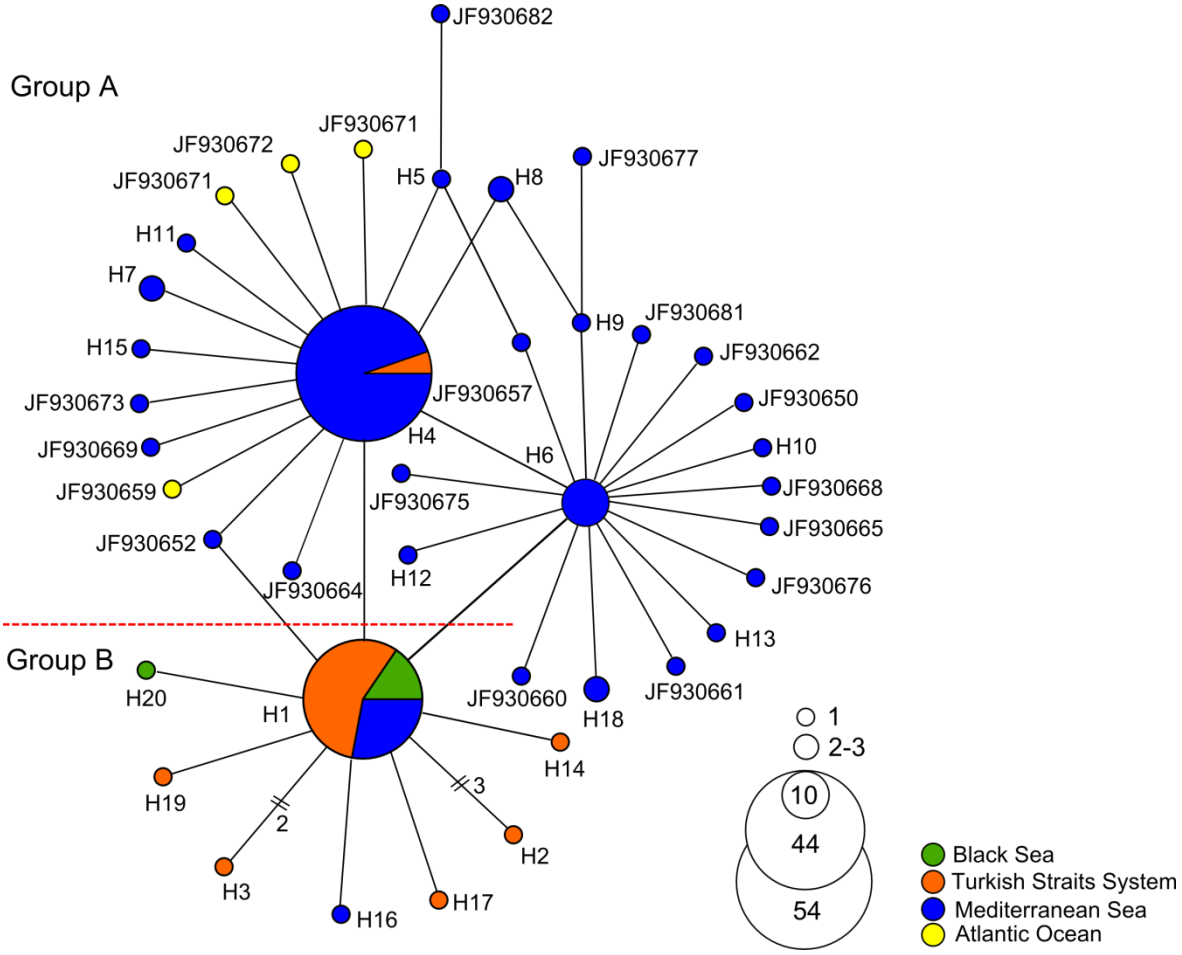


Figure 3.19. The haplotype network based on the cytochrome c oxidase subunit I (CO1) sequences of *Pachygrapsus marmoratus*. The partitions inside a circle represent the frequency of the haplotype for each population and its diameter is proportional to the frequency of the haplotype. Each line represents a single mutational change. The dashed-line shows the separation of the two groups.

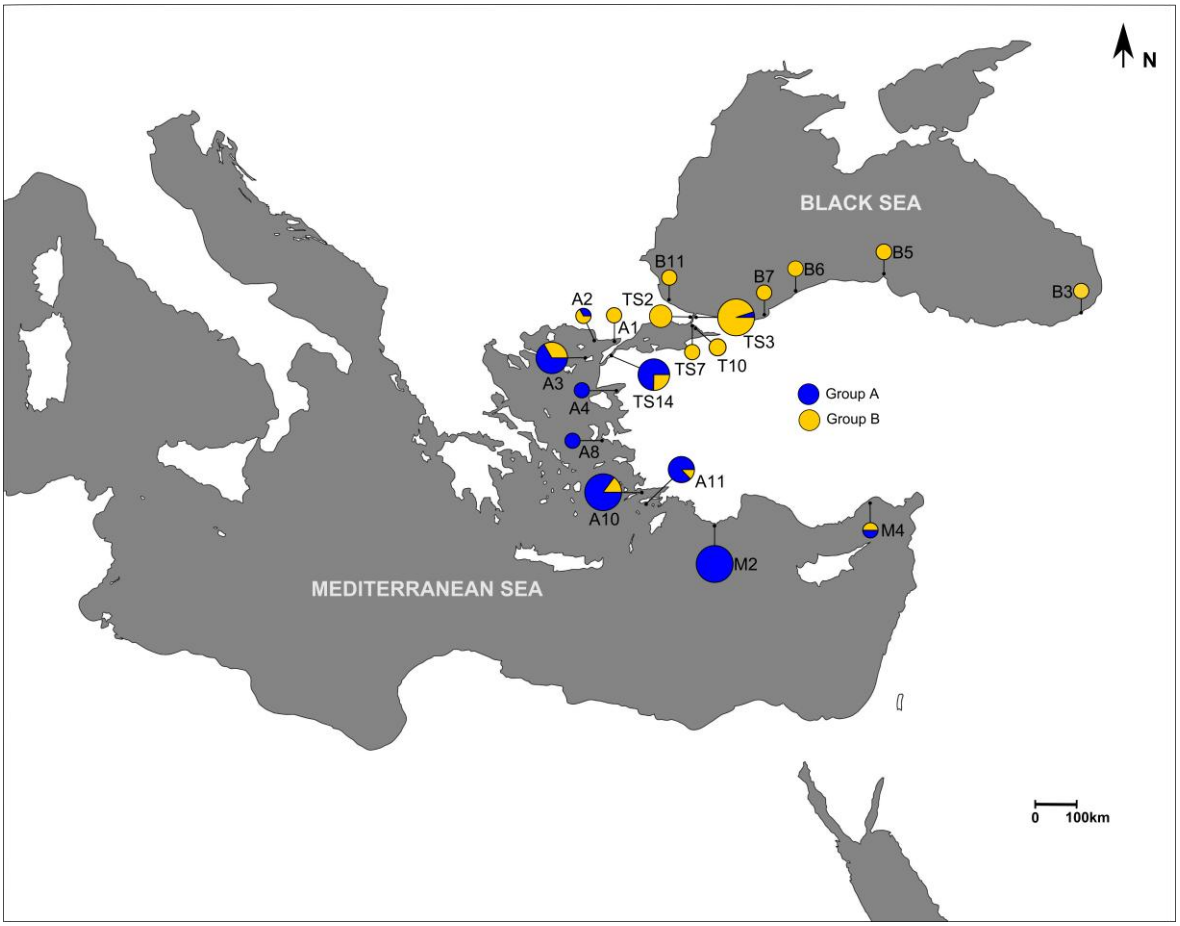


Figure 3.20. The CO1 clade frequencies of Group A and Group B.

Haplotype network of 16S data set represented one main haplotype (H1), connected to five less-frequent haplotypes by steps of one mutation (Figure 3.21). H1 was found in the Black Sea, the TSS and the Mediterranean Sea with the frequencies of 6%, 22% and 72%, respectively. No separation of groups was detected with respect to different water bodies.

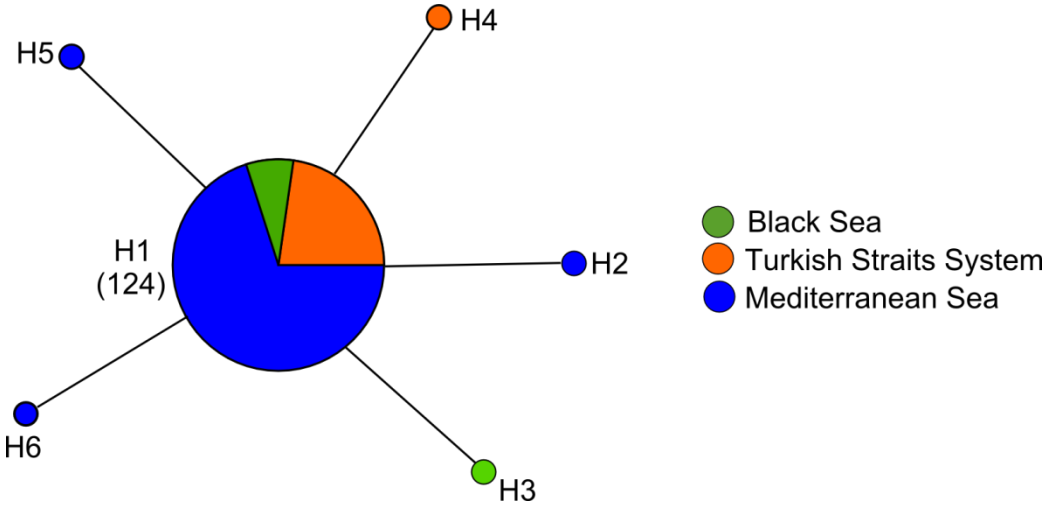


Figure 3.21. The haplotype network based on the 16S rRNA sequences of *Pachygrapsus marmoratus*. The partitions inside a circle represent the frequency of the haplotype for each population and its diameter is proportional to the frequency of the haplotype. Each line represents a single mutational change.

3.3.4. Population Structure

The AMOVA for mitochondrial CO1 showed population differentiation among the Black Sea, the Turkish Straits System and the Mediterranean Sea (Φ_{st}= 0.51). 46.04% of the total variance was found to be due to the genetic differentiation among three basins and 4.66% and 49.30% were due to variance being distributed among populations within types and within populations, respectively. Pairwise Φ_{st} values were found to be significant between the TSS and the Mediterranean, and were higher than 0.30 (22 out of 45). Nine pairwise Φ_{st} values out of 45 were found to be significant and higher than 0.40 between the Black Sea and the Mediterranean Sea. Pairwise Φ_{st} values between sampling sites are given in Table 3.20.

The AMOVA for 16S rRNA showed a relative lack of population differentiation among the Black Sea, the TSS and the Mediterranean Sea (Φ_{st}= 0.12). The molecular variance was found to be 1.81% among three basins, 10.08% among populations between basins and 88.10% within populations. The pairwise Φ_{st} values are given in Table 3.21, and none of the values were significantly greater than zero.

Table 3.20. Pairwise Φ_{st} values of the CO1 dataset among the Black Sea, the Turkish Straits System and the Mediterranean Sea. Significant P values ($P < 0.05$) are indicated in bold.

		Black Sea					**Turkish Straits System**					**Mediterranean Sea**								
		B3	B5	B6	B7	B11	TS2	TS3	TS7	TS10	TS14	A1	A2	A3	A7	A8	A10	A11	M2	M4
Black Sea	B3	0																		
	B5	0	0																	
	B6	0	0	0																
	B7	-1	-1	0	0															
	B11	0	0	0	0	0														
Turkish Straits System	TS2	-1	-1	-0.33	0.18	-0.33	0													
	TS3	0	0	0	0.47	0	-0.10	0												
	TS7	0	0	0	0	0	-0.33	0	0											
	TS10	-1	-1	-0.20	-0.11	-0.20	**0.35**	0.19	-0.20	0										
	TS14	-0.11	-0.11	0.25	0.27	0.25	**0.50**	**0.50**	0.25	0.24	0									
Mediterranean Sea	A1	1	1	1	0.60	1	0.76	1	1	0.09	-0.67	0								
	A2	-1	-1	-0.20	-0.03	-0.20	0.09	0.19	-0.20	0	-0.14	0	0							
	A3	-0.14	-0.14	0.14	0.24	0.14	**0.36**	**0.30**	0.14	**0.31**	-0.12	-0.30	-0.13	0						
	A7	0.65	0.65	**0.69**	**0.71**	**0.69**	**0.71**	**0.74**	0.69	**0.68**	0.15	-0.94	**0.42**	**0.20**	0					
	A8	1	1	1	0.80	1	**0.78**	1	**1**	0.42	-0.08	0	0.37	0.05	-0.30	0				
	A10	0.27	0.27	**0.40**	0.46	**0.40**	**0.51**	**0.49**	0.40	**0.50**	-0.09	-0.37	0.07	-0.01	**0.14**	-0.01	0			
	A11	0.58	0.58	**0.66**	0.65	0.66	**0.67**	**0.74**	**0.66**	**0.56**	-0.05	-0.81	0.25	0.04	-0.03	-0.22	-0.02	0		
	M2	0.60	0.60	**0.65**	**0.67**	**0.65**	**0.69**	**0.70**	**0.65**	**0.65**	0.08	-0.79	**0.37**	**0.15**	0.01	-0.23	**0.07**	-0.05	0	
	M4	-0.3	-0.33	0.11	0.18	0.11	**0.34**	0.39	**0.11**	0.17	-0.20	-0.33	-0.33	-0.15	0.24	0.11	-0.04	0.04	0.19	0

Table 3.21. Pairwise Φ_{st} values of the 16S dataset among the Black Sea, the Turkish straits System and the Mediterranean Sea. Significant P values ($P < 0.05$) are indicated in bold.

		Black Sea				**Turkish Straits System**				**Mediterranean Sea**								
		B3	B5	B6	B11	TS2	TS3	TS10	TS14	A1	A2	A3	A7	A8	A10	A11	M2	M4
Black Sea	B3	0																
	B5	0	0															
	B6	0	0	0														
	B11	0	-1	0	0													
Turkish Straits System	TS2	-0.33	-1	-0.33	0.55	0												
	TS3	0	0	0	0.59	-0.06	0											
	TS10	0	0	0	0.25	-0.19	0	0										
	TS14	0	0	0	0.25	-0.19	0	0	0									
Mediterranean Sea	A1	-0.20	-1	-0.20	0.05	0.31	0.30	0	0	0								
	A2	0	0	0	0.73	-0.02	0	0	0	0.47	0							
	A3	0	0	0	0.83	0.01	0	0	0	0.61	0	0						
	A7	0	0	0	0.83	0.01	0	0	0	0.61	0	0	0					
	A8	0	0	0	-1	-1	0	0	0	-1	0	0	0	0				
	A10	0	0	0	0.66	-0.04	0	0	0	0.38	0	0	0	0	0			
	A11	-0.31	-1	-0.31	0.27	0.04	0	-0.17	-0.17	0.10	0.08	0.17	0.17	-1	0.04	0		
	M2	-0.33	-1	-0.33	0.53	0	-0.06	-0.19	-0.19	0.29	-0.02	0.01	0.01	-1	-0.04	0.04	0	
	M4	0	0	0	0.38	-0.14	0	0	0	0.11	0	0	0	0	0	-0.10	-1.34	0

3.3.5. Neutrality Tests and Mismatch Analyses

Three neutrality tests (Tajima's D, Fu's Fs, and Ramos-Onsins and Rozas's R_2) and mismatch distribution analysis were used to reconstruct the population demographic history for the populations of the Black Sea, the TSS and the Mediterranean Sea, separately and also for the entire population. For CO1, the analyses of the two groups (Group A and B) separately and the entire data set showed unimodal distributions of pairwise differences, indicative of population expansion (Figure 3.22 a-c). However, the three neutrality tests were significant for the entire population only (Table 3.20). In addition, the whole data set for 16S rRNA result showed unimodal distributions of pairwise differences, indicative of population expansion (Figure 3.23). In this gene, all three neutrality tests were found to be significant for the entire population, and only Fu's Fs was found to be significantly negative for the Mediterranean population.

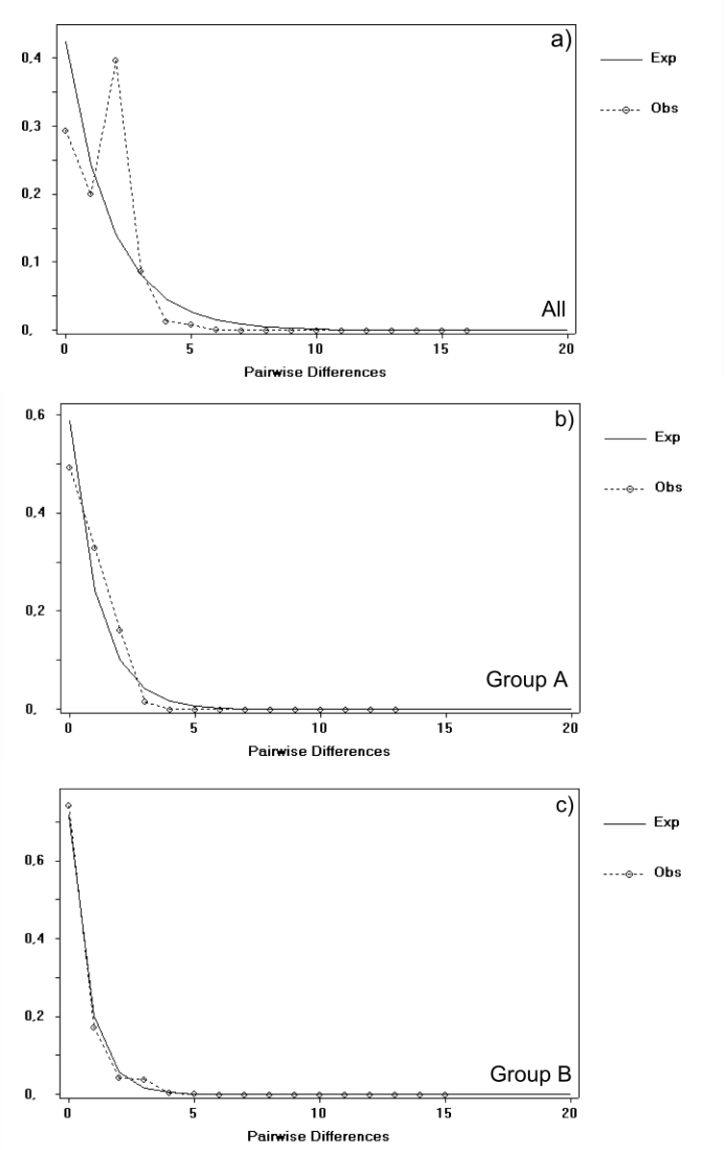


Figure 3.22. (a-c) Mismatch distribution obtained from CO1 sequence data for the entire population, Group A and Group B populations. The lines with empty circles represent the observed distribution and the black lines represent the expected distribution under a sudden expansion model.

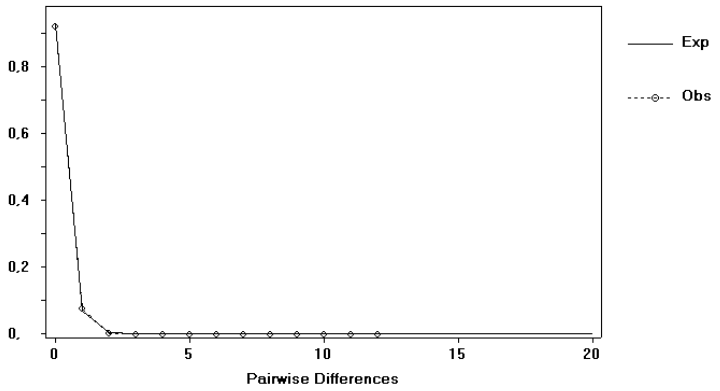


Figure 3.23. Mismatch distribution obtained from 16S sequence data for the entire population. The lines with empty circles represent the observed distribution and the black lines represent the expected distribution under a sudden expansion model.

3.3.6. Discussion on Mitochondrial Phylogeography and Dispersal Patterns

Fratini et al. (2008) showed that the populations of *Pachygrapsus marmoratus* exposed to heavy metal stress were genetically less variable than non-exposed ones. The population genetic structure of *P. marmoratus* along the Portuguese coast was investigated in another study and local genetic heterogeneity was detected in Praia das Avencas (Silva et al., 2009). These two studies evaluated the population differentiation of *P. marmoratus* populations at a local scale. On the other hand, Fratini et al. (2011) investigated the difference between western Mediterranean and eastern Atlantic populations of *P. marmoratus* using both mitochondrial and nuclear DNA markers at a larger geographic scale. They recorded high genetic similarity between western Mediterranean and eastern Atlantic populations of *P. marmoratus* based on the mtDNA dataset. According to their results, the Gibraltar Strait or the Almeria Oran Front did not play a barrier role for gene flow in *P. marmoratus*. However, they did detect a certain degree of genetic heterogeneity at a local scale for Portuguese (Silva et al., 2009) and Tuscan (Fratini et al., 2011) populations of *P. marmoratus* using the same set of microsatellite markers.

In the present study, we focused on the population structure of eastern Mediterranean and Black Sea populations of *P. marmoratus*. The 16S rRNA gene showed very low variability and no genetic heterogeneity in *P. marmoratus* populations. On the other hand,

two main haplogroups, differentiated from each other by two mutation steps, were detected using CO1 data set. This separation was supported by both the haplotype network and phylogenetic trees. The star-like shape of the network and the separation of two haplogroups by few mutations support the shallow genetic differentiation between the two groups. However, the most important point is that while Group A comprises the haplotypes from the Mediterranean and Atlantic Ocean, Group B includes the specimens from all basins (the Black Sea, the TSS and the Mediterranean). Three specimens representing the TSS were included in Group A. The slight differences that define groups A and B are likely to be due to the separation of the Black Sea and the Aegean before their connection was last established through TSS approximately 7000 years ago (Zaitsev and Mamaev, 1997). This structure, seen also in *Mytilus galloprovincialis* and *Palaemon elegans* populations, again, shows us the semi-permeable character of the TSS. While the system let the haplotypes disperse towards the Mediterranean through the Black Sea, it let only limited introduction of the Mediterranean haplotypes in the reverse direction. Significant and high pairwise Φ values especially between the TSS and the Mediterranean Sea supports the differentiation among these different basins.

4. SUMMARY AND CONCLUSIVE REMARKS

The detection of population differentiation in the marine realm is not straightforward due to the high dispersal capabilities of eggs, larvae and adults (Palumbi 1994; Ward et al. 1994). In addition, geographic barriers, considered to comprise one of the main mechanisms of speciation are far less obvious in the marine environment (Cunningham and Collins, 1998; Palumbi, 1994; Ward et al., 1994). However, all these generalizations may not be valid in every situation. Many studies have shown that high dispersal capabilities of marine organisms do not necessarily translate into high levels of realized gene flow (see Patarnello et al. 2007). Multiple studies investigated the effects of marine straits on gene flow from all around the world including the Danish Straits (Nilsson et al. 2001, Teacher et al. 2011), the Cook Strait (Goldstien et al. 2006), the Tsugaru Strait (Briggs & Bowen 2012), and the Gibraltar Strait (Patarnello et al. 2007). Considering topographical features in general and straits in particular, the general pattern expected to be observed, categorically speaking, is that the feature should either be a barrier to gene flow, or not.

In this study, we investigated the role of a unique marine ecosystem, the two-layered current regime of the Turkish Straits System, on the genetic structure of *Mytilus galloprovincialis* Lamark, 1819, *Palaemon elegans* Rathke, 1837 and *Pachygrapsus marmoratus* (Fabricius, 1787) populations. The system, consisting of the Bosphorus Strait, the Sea of Marmara and the Dardanelles, is the only connection between the Black Sea and the Mediterranean.

4.1. *Mytilus galloprovincilalis*

Previous research showed that the genetic differentiation of the Aegean and northern Black Sea (Ukraine) populations of *Mytilus galloprovincialis* was significant. Focusing on the differentiation between the northern Aegean and the Black Sea, and sampling around the Bosphorus Strait, Kalkan et al. (2011) used mitochondrial DNA and microsatellites to test whether the strait restricted gene flow locally, and was a barrier that caused the previously observed differentiation between northern Black Sea and northern Aegean. This

work showed that the Bosphorus Strait did not present a hydrographic barrier limiting the gene flow between the Black Sea and the Sea of Marmara.

Here, we extended the sampling locations from Kalkan et al. (2011) to the Black Sea, the Dardanelles and the Aegean to understand the effect of the TSS on gene flow and pinpoint where the phylogeographic break reported by Ladoukakis et al. (2002) took place. We used a geographically intense sampling strategy, collecting mussels from 26 sites along the Turkish coast, Bulgaria, Ukraine and Russia. Two different halplogroups; one (Clade A) predominantly was found in the Black Sea-TSS and the other (Clade B) almost exclusively in the Aegean using the mitochondrial data. However, microsatellites do not detect this differentiation between the common (clade A) and the Aegean (clade B) populations of *M. galloprovincialis*. Our results indicate a scenario where populations of the mussels were differentiated during the last glaciation, resulting in two divergent mtDNA clades in the Black Sea and the Aegean. After the connection between the Black Sea and the Aegean was re-established, the Black Sea populations colonized the Sea of Marmara and the Aegean; whereas the differentiated Aegean populations did not colonize the Sea of Marmara and the Black Sea in the reverse direction.

4.2. *Palaemon elegans*

Reuschel et al. (2010) revealed a complex population structure in *Palaemon elegans* and clearly distinct genetic lineages using 16S rRNA and COI mitochondrial genes with samples from Northeastern Atlantic, Baltic Sea, entire Mediterranean, Black Sea and Caspian Sea. Their defined haplogroups of Type II and Type III match our groups II and I, respectively. In this study, Group I with samples distributed relatively evenly includes specimens from all sampling sites. On the other hand, Group II consists mostly of samples from the Mediterranean, with only a few from the Black Sea and the TSS. It is likely that due to the two-layered currents of the TSS flowing in opposite directions (from the Black Sea to the Aegean at the top layer, and vice versa in the bottom), Group I was able to disperse from the Black Sea to the Mediterranean relatively easily after the last ice age. On the other hand, group II, originating in the Mediterranean was not able establish itself as effectively in the Black Sea (which has brackish water characteristics). Type III in

Reuschel et al. (2010) which correspond to our Group I also seems to prefer brackish water habitats. Reuschel et al. (2010) suggested that these different genetic types may correspond to separate cryptic species. However, we detected fewer differences between two groups and further amalysis are needed to clarify this argument.

4.3. *Pachygrapsus marmoratus*

Population genetic structure of *Pachygrapsus marmoratus* has been recently investigated throughout the western Mediterranean Sea and the eastern Atlantic Ocean and it has been suggested that there is a recent and weak genetic differentiation between European Atlantic and some Mediterranean populations of *P. marmoratus* (Fratini et al., 2012). Here, we tested the hypothesis that the Turkish Straits System may act as a phylogeographic break having a potential to separate the Black Sea and the Mediterranean populations by restricting the gene flow between them and we did not detect distinct genetic differentiation among the different geographic basins. However, the dispersal pattern of the haplotypes from the Black Sea, the TSS and the Mediterranean give us the signs of the important role of the Turkish Straits System on gene flow of marine invertebrates. For the future work, extending the sampling sites and increasing the sample size especially along the Black Sea, as well as investigating nuclear regions will be useful for better understanding the population structure of *P. marmoratus* and effects of the TSS on the gene flow among its populations.

Here I present a special case where the Turkish Straits System (TSS) acts as a barrier to gene flow from the Mediterranean to the Black Sea, but not so in the other direction. Though relatively rare, this is not the first time such a phenomenon has been recorded. For instance, Nilsson et al. (2001) showed that the Swedish west-coast salmon populations differed from the geographically close southern Baltic ones because of the absence of inward and limited outward gene flow through the Danish Straits. This study provides another example to such a special mechanism of genetic differentiation in the marine realm.

Biogeographically speaking, as mentioned previously, Ozturk & Ozturk (1996) had characterized the TSS as being a corridor, a barrier or an acclimatization zone for different species. This study shows that actually TSS can be a barrier and a corridor at the same time. Though relatively few in number, previous genetics studies of marine species in and around the TSS did not exhibit this pattern (e.g. Peijnenburg & Pierrot-Bults 2004, Peijnenburg et al. 2006, Viaud-Martínez et al. 2007). This was mainly because the focus of these studies were the extent of differentiation between the Black Sea and the Mediterranean in a broad sense, without a dense sampling methodology in and around the TSS. Hence the fine scale sampling intensity that achieved in this study is unprecedented for TSS, and considering the genetics studies on straits in general, provides a robust sampling framework for investigating their effect on genetic differentiation.

The data obtained in this study showed that the TSS played a semi-permeable role for the populations of *Mytilus galloprovincialis*, *Palaemon elegans* and *Pachygrapsus marmoratus*. Further studies investigating the role of TSS on the gene flow in other species, with a dense sampling strategy as employed in this one will help to make broader generalizations on the effects of this system in shaping evolutionary history of other marine organisms in the region.

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APPENDIX A: ACCESSION NUMBERS OF THE SEQUENCES RETRIEVED FROM GENBANK

Table A.1. Accession numbers of the sequences belonging to *Mytilus galloprovincialis*, *Palaemon elegans* and *Pachygrapsus marmoratus*.

Mytilus galloprovincialis	**CO1**	**16S**	**COIII**	**Location**
DQ445471			+	Black Sea
DQ445477			+	Black Sea
DQ445474			+	Black Sea
DQ445468			+	Black Sea
DQ403170			+	Black Sea
DQ469133			+	Greece
AY363687			+	Greece
AF063260			+	Spain
AF063264			+	Spain
AF063290			+	Spain
AF063262			+	Spain
AF063265			+	Spain
AF063261			+	Spain
AF063263			+	Spain
Palaemon elegans				
DQ882104	+			Poland
DQ882102	+			Poland
DQ882105	+			Poland
DQ882103	+			Poland
HE573177	+			Type III
HE573176	+			Type II
HE573175	+			Type I
JQ306029	+			UK
JQ306030	+			UK
JQ306031	+			UK
JQ306093	+			Portugal
JQ306028	+			UK
EU868696		+		?
HE573178		+		Type I
JQ042298		+		Helgoland-Germany
DQ079729		+		?
Pachygrapsus marmoratus				
JF930656	+			Italy, Corsica, Spain
JQ306089	+			Portugal
JQ306090	+			Portugal
JF930663	+			Corsica
JF930670	+			Italy, Spain, Portugal, Fuerteventura
JF930651	+			Italy, Corsica, Spain, Morocco, Portugal, Fuerteventura
JQ306087	+			Portugal
JQ306088	+			Portugal
JF930672	+			Spain, Fuerteventura

Table A.1. Accession numbers of the sequences belonging to *Mytilus galloprovincialis*, *Palaemon elegans* and *Pachygrapsus marmoratus* (cont.)

Pachygrapsus marmoratus	**CO1**	**16S**	**COIII**	**Location**
JF930676	+			Italy
JF930658	+			Italy, Morocco
JF930621	+			Italy
JF930679	+			Italy
JF930655	+			Italy, Corsica, Spain, Morocco
JF930653	+			Italy, Corsica, Spain, Morocco, Fuerteventura
JF930657	+			Italy, Corsica
JF930660	+			Italy
JF930673	+			Italy
JF930652	+			Italy
JF930668	+			Spain
JF930654	+			Italy, Corsica, Spain, Morocco, Portugal, Fuerteventura
JF930677	+			Italy
JF930675	+			Italy
JF930650	+			Italy, Spain
JF930667	+			Spain
JF930669	+			Spain
JF930666	+			Morocco
JF930674	+			Italy
JF930659	+			Italy
JF930664	+			Corsica
JF930680	+			Italy
JF930665	+			Italy
JF930678	+			Italy
JF930662	+			Italy
JF930671	+			Spain, Fuerteventura
JF930681	+			Italy
JF930682	+			Italy
JF930661	+			Italy
FR871307		+		Spain
DQ079728		+		?
AY919094		+		Spain
AM946022		+		France

APPENDIX B: THE SAMPLING SITES

Table B.1. The codes, names and coordinates of the sampling sites

	Codes	Sampling Sites	Coordinates
Black Sea	B1	Sevastopol	44°36'58.79"N 33°30'40.41"E
	B2	Novorossiysk	44°42'29.50"N 37°27'18.89"E
	B3	Kemalpaşa	41°28'55.27"N 41°31'12.09"E
	B4	Ordu	41° 8'0.17"N 37°40'53.87"E
	B5	Sinop	42° 0'57.92"N 35°10'56.89"E
	B6	Gideros	41°51'35.62"N 32°51'25.13"E
	B7	Akcakoca	41° 5'23.65"N 31° 7'20.22"E
	B8	Şile	41°10'48.26"N 29°36'42.67"E
	B9	Riva	41°14'1.78"N 29°13'36.45"E
	B10	Varna	43°12'17.43"N 27°55'59.94"E
	B11	Karaburun	41°20'36.16"N 28°41'12.00"E
	B12	Kilyos	41°15'6.19"N 29°2'18.46"E
Turkish Straits System	TS1	Poyrazköy	41° 12'7.06"N 29° 7'36.97"E
	TS2	Hamsi Limanı	41°12'18.82"N 29° 6'12.38"E
	TS3	Büyük Liman	41°10'51.80"N 29°6'22.21"E
	TS4	Anadolu Kavağı	41°10'22.75"N 29° 5'18.14"E
	TS5	Rumeli Hisari	41° 5'16.60"N 29° 3'24.95"E
	TS6	Kuzguncuk	41° 2'19.32"N 29° 1'56.98"E
	TS7	Moda	40° 58'46.76"N 29° 1'31.59"E
	TS8	Kalamış	40°58'38.36"N 29° 2'14.16"E
	TS9	Burgazada	40°52'41.27"N 29° 3'9.74"E
	TS10	Heybeliada	40°52'11.57"N 29° 5'17.56"E
	TS11	İzmit Körfezi	40°44'57.49"N 29°48'44.24"E
	TS12	Mürefte	40°40'35.36"N 27°15'53.60"E
	TS13	Erdek	40°23'45.20"N 27°47'23.93"E
	TS14	Çanakkale	40° 9'8.30"N 26°24'18.14"E
Mediterranean Sea	A1	Saros	40°36'7.51"N 26°32'30.69"E
	A2	Enez	40°41'45.48"N 26° 3'16.60"E
	A3	Gökçeada	40°13'54.53"N 25°53'39.18"E
	A4	Ayvalık	39°18'47.85"N 26°41'16.49"E
	A5	Foça	38°40'1.43"N 26°44'44.84"E
	A6	İzmir	38°27'41.95"N 27° 9'47.77"E
	A7	Urla	38°21'51.04"N 26°46'16.66"E
	A8	Çeşme	38°20'12.64"N 26°23'19.13"E
	A9	Güllük	37°14'15.32"N 27°35'43.25"E
	A10	Turgutreis	37° 0'20.28"N 27°15'23.41"E
	A11	Palamutbükü	36°40'9.97"N 27°30'9.62"E
	M1	Fethiye	36°38'25.32"N 29° 6'3.12"E
	M2	Kaş	36°11'48.18"N 29°38'39.41"E
	M3	Kekova	36°11'45.65"N 29°50'56.79"E
	M4	Taşucu	36°16'25.78"N 33°48'55.10"E

APPENDIX C: A LIST OF HAPLOTYPE SEQUENCES

Haplotype sequences of *Mytilus galloprovincialis* (COIII)

H1

GTATGAGTCTGGTTTGCATATTATTGAGAACTTTTAGATGGTGACGCGATTTAATTCGTGAAGG
AGACATTGGGCTTCACACTCGTTTTGTGATCAAAAGATTTCGAGATGGCGTTGCCTTGTTTATTC
TGTCTGAAGTGATATTTTTCTTCACTTTTTTCTGGACTTTTTTCCATAATGCTCTAAGGCCTTCGT
GTGAGTTAGGAATACGGTGGCCTCCTCCTGGAATTCGTACACCAAACCCGTCATCTACTAGTCT
GTTTGAGACAGGTCTTCTAATTAGAAGAGGGTTGTTTGTAACTCAAGCCCATAAGAGGATGCGC
TTGAAGGATTACGATGTAGGGCCATTCATCGGTTTAGTGGTGACAATCGTATGCGGGACCGTGT
TTTTTTTGGTACAACTGCGGGAATATTATTGAAACTCCTACACTATTGCAGATAGGGTTTATGGT
AGGGTTTTCTATTTACTAACTGGATTCCATGGGATACATGTTGTCGTAGGGACTATTTGGCTAAC
GGTAAGGTTAGTTCGACTATGACGCGGGGAGTTTTCTAGTCAACGACACTTTGGGT

H2

GTATGAGTCTGGTTTGCATATTATTGAGAACTTTTAGATGGTGACGCGATTTAATTCGTGAAGG
AGACATTGGGCTTCACACTCGTTTTGTGATCAAAAGATTTCGAGATGGCGTTGCCTTGTTTATTC
TGTCTGAAGTGATATTTTTCTTCACTTTTTTCTGGACTTTTTTCCATAATGCTCTAAGGCCTTCGT
GTGAGTTAGGAATACGGTGGCCTCCTCCTGGAATTCGTACACCAAACCCGTCATCTACTAGTCT
GTTTGAGACAGGTCTTCTAATTAGAAGAGGGTTGTTTGTAACTCAAGCTCATAAGAGGATGCGC
TTGAAGGATTACGATGTAGGGCCATTCATCGGTTTAGTGGTGACAATCGTATGCGGGACCGTGT
TTTTTTTGGTACAACTGCGGGAATATTATTGAAACTCCTACACTATTGCAGATAGGGTTTATGGT
AGGGTTTTCTATTTACTAACTGGATTCCATGGGATACATGTTGTCGTAGGGACTATTTGGCTAAC
GGTAAGGTTAGTTCGACTATGACGCGGGGAGTTTTCTAGTCAACGACACTTTGGGT

H3

GTATAAGTCTGGTTTGCATATTATTGAGAACTTTTAGATGGTGACGCGATTTAATTCGTGAAGG
AGACATTGGGCTTCACACTCGTTTTGTGATCAAAAGATTTCGAGATGGCGTTGCCTTGTTTATTC
TGTCTGAAGTGATATTTTTCTTCACTTTTTTCTGGACTTTTTTCCATAATGCTCTAAGGCCTTCGT
GTGAGTTAGGAATACGGTGGCCTCCTCCTGGAATTCGTACACCAAACCCGTCATCTACTAGTCT
GTTTGAGACAGGTCTTCTAATTAGAAGAGGGTTGTTTGTAACTCAAGCCCATAAGAGGATGCGC
TTGAAGGATTACGATGTAGGGCCATTCATCGGTTTAGTGGTGACAATCGTATGCGGGACCGTGT
TTTTTTTGGTACAACTGCGGGAATATTATTGAAACTCCTACACTATTGCAGATAGGGTTTATGGT
AGGGTTTTCTATTTACTAACTGGATTCCATGGGATACATGTTGTCGTAGGGACTATTTGGCTAAC
GGTAAGGTTAGTTCGACTATGACGCGGGGAGTTTTCTAGTCAACGACACTTTGGGT

H4

GTATGAGTCTGGTTTGCATACTATTGAGAACTTTTAGATGGTGACGCGATTTAATTCGTGAAGG
AGACATTGGGCTTCACACTCGTTTTGTGATCAAAAGATTTCGAGATGGCGTTGCCTTGTTTATTC
TGTCTGAAGTGATATTTTTCTTCACTTTTTTCTGGACTTTTTTCCATAATGCTCTAAGGCCTTCGT
GTGAGTTAGGAATACGGTGGCCTCCTCCTGGAATTCGTACACCAAACCCGTCATCTACTAGTCT
GTTTGAGACAGGTCTTCTAATTAGAAGAGGGTTGTTTGTAACTCAAGCCCATAAGAGGATGCGC
TTGAAGGATTACGATGTAGGGCCATTCATCGGTTTAGTGGTGACAATCGTATGCGGGACCGTGT
TTTTTTTGGTACAACTGCGGGAATATTATTGAAACTCCTACACTATTGCAGATAGGGTTTATGGT
AGGGTTTTCTATTTACTAACTGGATTCCATGGGATACATGTTGTCGTAGGGACTATTTGGCTAAC
GGTAAGGTTAGTTCGACTATGACGCGGGGAGTTTTCTAGTCAACGACACTTTGGGT

H5

GTATGAGTTTGGTTTGCATATTATTGAGAACTTTTAGATGGTGACGCGATTTAATTCGTGAAGGA
GACATTGGGCTTCACACTCGTTTTGTGATCAAAAGATTTCGAGATGGCGTTGCCTTGTTTATTCT
GTCTGAAGTGATATTTTTCTTCACTTTTTTCTGGACTTTTTTCCATAATGCTCTAAGGCCTTCGTG
TGAGTTAGGAATACGGTGGCCTCCTCCTGGAATTCGTACACCAAACCCGTCATCTACTAGTCTG
TTTGAGACAGGTCTTCTAATTAGAAGAGGGTTGTTTGTAACTCAAGCCCATAAGAGGATGCGCT
TGAAGGATTACGATGTAGGGCCATTCATCGGTTTAGTGGTGACAATCGTATGCGGGACCGTGTT
TTTTTTGGTACAACTGCGGGAATATTATTGAAACTCCTACACTATTGCAGATAGGGTTTATGGTA
GGGTTTTCTATTTACTAACTGGATTCCATGGGATACATGTTGTCGTAGGGACTATTTGGCTAACG
GTAAGGTTAGTTCGACTATGACGCGGGGAGTTTTCTAGTCAACGACACTTTGGGT

H6

GTATGAGTCTGGTTTGCATATTATTGAGAACTTTTAGATGGTGACGCGATTTAATTCGTGAAGG
AGACATTGGGCTTCACACTCGTTTTGTGATCAAAAGATTTCGAGATGGCGTTGCCTTGTTTATTC
TGTCTGAAGTGATATTTTTCTTCACTTTTTTCTGGACTTTTTTCCATAATGCTCTAAGGCCTTCGT
GTGAGTTAGGAATACGGTGGCCTCCTCCTGGAATTCGTACACCAAACCCGTCATCTACTAGTCT
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H7

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H8

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H9

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H13

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H22

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H23

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H24

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H25

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H26

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H27

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H28

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H29

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H31

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H33

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H34

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H35

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H36

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H37

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H38

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H39

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H42

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H50

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H51

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H52

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H53

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H54

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H55

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H56

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H57

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Haplotype sequences of *Palaemon elegans* (CO1)

H1

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H3

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H5

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H9

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H10

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H11

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H12

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H13

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H16

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H23

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H24

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H33
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H50
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TTT-ACC TCCTTCC-TTAACTCTACTTCTTT-CTAGAGGAAT GGTTGAAAGG GGAGTAGGAA CAG
GATGAACTGTT TA CCC TCCTCTAGCAAAGAGGATTAGGACA TG CTGGC GCTTC TGTAGATC
TTGGTA TTTTCTCCCTT CATTTAGC AGGAATC TCTTCCATCCTAGGGG CAGTTAACTTTATTAC
TACTGTAATCAATATA CGAGCTCCAGGTATAA CAATGGATCGAACTCCTCTTTTCGTGTGAG C
T GT TTTTCTAACAGCTATTCTTCTTTTACTATCCTTA
H51
AT-TAATGCTA-GGAGCCCCT-GATATGGCTTT-TCC-ACGAATAAA-TAATATAAGGTTTT-GACT T
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GGATGAA CTGTT TACCC TCC TCTAGCGA-GAGGATTAGGACATGCTGGCGCTTCT GTAGA T C
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H52
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H53
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A GC TGTTTTTCTAACGGCTATCCTTCTTTTGCTATCCTTA
H54
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H55
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G G TATTTTCTCCCTTCATTTAGCAGGAATCTCTTCCATCCTAGGAGCAGTTAACTTTATTACTAC
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H56
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TGG TATTTTCTCCCTTCATTTAG CA GG AATCTCTTCCATCCT AGGAGCAGTTAAC TTTA TT AC
T ACT GTAATCA ATATACGAG CTCCAGGTATAACAATGGATCGAACTCCTCTTTTCGTTTGAG C
TGTTT TTCTAACGG CTATCCTTCTTTTACTATCCTTA

H57
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H58
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H59
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H60
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H61
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H62
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H63
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H64
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H65
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G C TGTTTTTCTAACGGCTATCCTTCTTTTACTATCCTTA
H66
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H67
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H68
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H69
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H70
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H71
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H72
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H73
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H74
ATGTAATGCTA-GGAGCCCCT-GATATGGCTTT-TCC-ACGAATAAA-CAATATAAGGTTTT-GA CT T TT-ACC CCCT TCC-TTAACTCTCCTTCTTT-CTAGAGGGATG GTTGAAAGGGGA GTGG GAAC A GGA TGAACTGTTTACC CTC CTCT AGCAA-GAGGATTAGGACATGCTGGCGCT TCTGT AGA T C TTGGTATTTTCTCCCT TCATTTAG CAGGAA TCTCTTCCATC CTAG GAGCAGTTAACTTTAT T A CTAC TGTAATCAATATACGAGCTCCAGG TATAACAATAG ATCGAACTCCTC TTTTCG TATGG G C TGTTTTTCTAACAGCTATTCTTCTTTTACTATCCTTA
H75
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H76
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H77
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H78
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Haplotype sequences of *Palaemon elegans* (16s)

H1
AATTTAATAAGGGGACGATAAGACCCTATAAAACTTTATGCAC-A-A-TATGTCTTGTC-TTTAAA TTATATCTAAATATTGGTTGGT-TAAAAGTGCATTCCGTTGG GGAGACGTT GATATAAACT GT AACTGT-CTTATTAAACAGA-ATAATTATTATT ATAATTTGATCC TTCTTTGTGGAT AAAAA GA A TA A GTTACTTTAGGG ATAACAGC GTAA TTTTCT CAGAG AGTTC TTATC GAAG AGAA T A GT TGC
H2
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H3
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H4
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H5
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H6
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H7
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H8
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H9
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H10
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H11
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H12
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H13

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H14

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H15

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H16

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H17

AATTTAATAAGGGGACGATAAGACCCTGTAAAACTTTATGCACG--A-CATGTCTTGTC-TTTAA A TTATATGTAAATATTGGTTGGT-TAAAAGTGCATTCCGTTGGGGAGACGTTGATATAAGCTGTAA CTGTT-TTATTAAATAGA-ATAATTATTATTATAATTTGATCCTTCTTTGTGGATAAAAAGAATAA GTTACTTTAGGGATAACAGCGTAATTTTCTCAGAGAGTTCTTATCGAAGAGAATAG TTGC

H18

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H19

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H20

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H21

AATTTAATAAGGGGACGATAAGACCCTATAAAACTTTATGCACG--A-CATGTCTTGTC-TTTAA A TTATATCTAAATATTGGTTGGT-TAAAAGTGCATTCCGTTGGGGAGACGTTGATATAAGCT GTAA CTGT-CTTATTAAATAGA-ATAATTGTT ATTATAATTTGA TCCTTCTTTGTGGATAAAAA GAA TA AGTTACTTT AGGGATAACA GCGTAATTTTCTCAGAG AGTTCTTATCGAAGAGAATA GTTGC

H22

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GC

H23

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H24

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H25

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H26

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H27

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H28

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TGC

H29

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TTGC

Haplotype sequences of *Pachygrapsus marmoratus* (CO1)

H1

GGTAAGGAAAGCAAGAGAAGGATAGCAGTAATAAAGACTGCTCAAACAAACAATGGTATTTGG
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H2

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H3

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H4

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Haplotype sequences of *Pachygrapsus marmoratus* (16S)

H1
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H6

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