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The Intertwined Effects of Hydrographic Barriers, Palaeoclimate and Life History on Genetic Structure of Marine Populations: A Case Study of Two Marine Invertebrates

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Abstract

Marine organisms with pelagic planktonic larval stages have high dispersal potential, yet their ranges are restricted by present and historical geographic and oceanographic features. Hydrogeographic features, such as straits and water current patterns, as well as paleoclimate can affect population connectivity, genetic differentiation, and ultimately speciation, but species distributions are also affected by life history characteristics. This study evaluates the effect of the Turkish Straits System (TSS) on the genetic differentiation of two benthic invertebrates with pelagic larvae, the Mediterranean mussel, *Mytilus galloprovincialis*, and the rock-pool prawn, *Palaemon elegans*, using mitochondrial and nuclear markers (microsatellites for *M. galloprovincialis* and Histone H3 gene for *P. elegans*). For both species, the mitochondrial DNA analyses separated the Black Sea and the Eastern Mediterranean (Aegean and Levantine seas) populations into two clusters. In contrast, in both species the nuclear data indicated no differentiation between Black Sea and Eastern Mediterranean populations. The results suggest that for both species, some individuals of Black Sea origin moved south, most likely through the transport of their pelagic larvae *via* the surface currents of the TSS. However, for the most part, individuals of Mediterranean origin (from Aegean and Levantine seas) were not able to successfully migrate in the opposite direction, though the small differences in the frequencies of migration in this direction indicate the effects of the life history characteristics of the two species on genetic structure.

Keywords: *Mytilus galloprovincialis*; *Palaemon elegans*; mtDNA; microsatellites; Turkish Straits System; Black Sea; Mediterranean Sea.

Introduction

Evidence suggests that, although sympatric speciation does occur (Coyne & Orr, 2004; Bolnick & Fitzpatrick, 2007), the primary mechanism responsible for the origin of species is genetic differentiation due to allopatric isolation of populations by geographic barriers (Mayr & Ashlock, 1991). Compared to terrestrial landscapes, such geographic barriers to gene flow in the marine environment are far less obvious, and some marine species show panmixia over wide geographical ranges due to long-distance dispersal capabilities of eggs, larvae or adults that can be transported by currents (Palumbi, 1994; Ward *et al*. 1994; Cunningham & Collins, 1998). Interestingly, some marine species show greater population genetic differentiation than expected based on their dispersal capabilities alone, due to reasons such as habitat discontinuity, isolation-by-distance, biogeographic barriers in their distribution area and anthropogenic transport (Palumbi, 1994; Riginos & Nachman, 2001; Pascual *et al*., 2017).

Straits (e.g., Cook, Tsugaru, Danish, Gibraltar) have been shown to act as allopatric barriers restricting gene flow for multiple groups of marine species, including fishes (Nilsson *et al*., 2001; Teacher *et al*., 2011; Briggs & Bowen, 2012), intertidal endemic limpets (Goldstien *et al*., 2006), and sea stars (Ayers & Waters, 2005), among others (Pascual *et al*., 2017). However, in the sea, as on land, there are other determinants of geographically-based genetic structure than just geophysical barriers, including paleoclimate (Dahlgren *et al*., 2000; Néraudeau *et al*., 2001; Huyse *et al*., 2004), life history characteristics of species (e.g., larval type and duration) (Riginos & Victor, 2001; Galarza *et al*., 2009; Riginos *et al*., 2011; Pascual *et al*., 2017), and/or habitat specificity (Edgar, 2000; Coulson, 2006; Waters *et al*., 2007; Ayre *et al*., 2009). Examination of the genetic diversity and divergence of

organisms around straits can shed light on how these different factors affect vicariant speciation in marine systems. Studies of multiple organisms around the Gibraltar Strait, which connects the Atlantic and Mediterranean basins, have shown that physical barriers, life history traits, and paleoclimate fluctuations have influenced population connectivity and intraspecific evolution across the Atlantic-Mediterranean transition zone (Patarnello *et al*., 2007; Pascual *et al*., 2017). While the Gibraltar Strait has been extensively studied, there are few studies investigating the evolutionary mechanisms that drive intraspecific speciation in the Eastern Mediterranean and the Black Sea, especially through the Turkish Straits System (TSS), the junction zone between these two basins (Kalkan *et al*., 2016).

The TSS (formed by the Dardanelles, the Sea of Marmara and the Bosphorus Strait) connects Black, Marmara and Mediterranean seas through the Bosphorus Strait and the Dardanelles, and it acts as a transitional zone between these three different marine basins (Fig. 1). Though not investigated thoroughly in terms of its molecular ecology and evolution (see review by Kalkan *et al*., 2016), the TSS is a unique ecosystem with each segment having different biological, physiographical and hydrological characteristics (Oguz *et al*., 1990; Unluata *et al*., 1990; Besiktepe *et al*., 1994; Ozturk & Ozturk, 1996; Oguz & Ozturk, 2011). The TSS has a well-defined two-layer stratification and two-layer current system driven by the density and sea-level differences between the Mediterranean and Black Sea. The brackish Black Sea waters (~18‰) flow to the Aegean Sea as the upper layer (the southward flow), and dense saline Mediterranean-origin waters (~38.5‰) flow toward the Black Sea as the lower layer (the northward flow); a sharp density interface (pycnocline) occurs at a depth of roughly 25 m (Unluata *et al*., 1990, Besiktepe *et al*., 1994). A strong pycnocline considerably restricts vertical water exchange between these two layers (Besiktepe *et al*., 1994), which affects the biotic characteristics of the two layers. The upper layer is mostly colonized by biota of Black Sea origin while the lower layer is mostly colonized by biota of Mediterranean origin (Ozturk & Ozturk, 1996).

In the case of the Sea of Marmara, the pycnocline-based stratification is known to separate two distinct zooplankton communities in the upper and lower layers (Mutlu, 2005; Yilmaz, 2008). For planktonic invertebrate larvae, restricted exchange across the pycnocline may limit geographic dispersal as well (see references in (Stancyk & Feller, 1986; Young, 1995), see also (Gallager *et al*., 1996; Dobretsov & Miron, 2001). The strong two-layered current regime through the TSS may be responsible for the asymmetrical dispersal of planktonic larvae between marine basins. The north-to-south surface current acts as a biological corridor to bring Black Sea species toward the Sea of Marmara and Aegean Sea; yet, it prevents species with Aegean or Sea of Marmara distributions from dispersing from south to north (Ozturk & Ozturk, 1996; Kalkan & Bilgin, 2016; Kalkan *et al*., 2016).

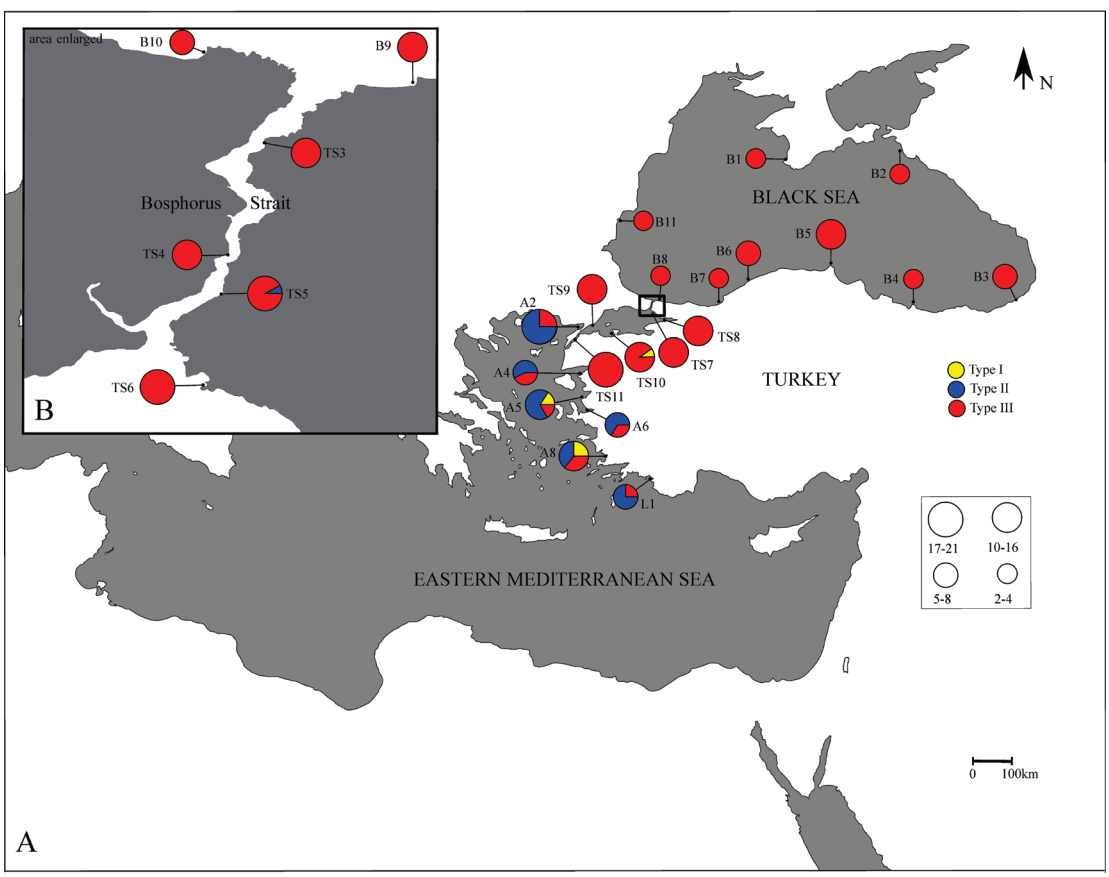


Fig. 1: *Mytilus galloprovincialis* mtDNA clade frequencies for collection sites (a) along the coasts of Turkey, and (b) within and adjacent to the Bosphorus Strait. Circle sizes are proportional to sample sizes (Map was created from template obtained from d-maps.com and edited in InkScape v0.91).

Another important factor determining the present distributions of species in and around the TSS is the turbulent geological history of this region during the glacial and interglacial periods when the Black Sea periodically lost and re-established contact with the Aegean Sea. The present TSS connections were established ~10 kya when Black Sea waters flooded the Marmara basin (Aksu *et al*., 2002; Cagatay *et al*., 2002; Hiscott *et al*., 2007). The first Mediterranean water penetration into the Black Sea occurred ~ 8.5 kya (Major, 2002; Ryan *et al*., 2003; Major *et al*., 2006) and persistent Black Sea outflow wakened temporarily (Balabanov, 2006; Hiscott *et al*., 2007). The second influx of Mediterranean waters started at ~ 8 kya, which was probably the initiation of permanent two-way flow in the Bosphorus Strait (Aksu *et al*., 2002; Kaminski *et al*., 2002; Major, 2002; Hiscott *et al*., 2007). Finally, the modern two-way flow system was established ~ 7 kya (Aksu *et al*., 2002; Hiscott *et al*., 2007).

TSS provides an excellent system to investigate the effects of geography, geological history and life-history on genetic structure and distribution of genetic diversity. To do this, we picked two common marine invertebrate species in the Mediterranean and Black seas, the Mediterranean mussel, *Mytilus galloprovincialis,* and the rockpool prawn, *Palaemon elegans* (Paterno *et al*., 2019; Udekem, 1999, respectively). The distribution of *P. elegans* extends to the Aral and Caspian seas on the east and to the Baltic Sea in the west (Zenkevich, 1963; Grabowski, 2006). *M. galloprovincialis,* on the other hand, is distributed throughout the Mediterranean and along the Atlantic coasts of southern Europe and North Africa (Gosling, 1984, McDonald *et al*., 1991; Gosling, 1992). It has also been introduced to the Sea of Japan, Southern California, and Puget Sound (McDonald *et al*., 1991; Suchanek *et al*., 1997).

Both species have planktonic larval stages. Fertilization of *M. galloprovincialis*'s eggs and larval development occur in the water column; therefore, its dispersal occurs during its planktonic larval stage which can take 16–30 days (Bierne *et al*., 2002; Pascual *et al*., 2017). In addition, empirical estimates related to dispersal distances of mussels showed that larvae can typically disperse 20-50 km (Hilbish & Koehn, 1985; Gilg & Hilbish, 2003), which depends on the velocity of the currents. For *P. elegans*, the complete larval development takes place in the water column with 6-9 zoeal stages based on environmental conditions (Fincham, 1977), and its planktonic larval duration (PLD) can also last more than four weeks (Pascual *et al*., 2017). Hence, both species have similar PLDs, which implies that their larval dispersal would be affected by the current system of the TSS in a similar manner. However, as adult *M. galloprovincialis* is a sessile benthic; whereas, *P. elegans* can walk and swim along the seafloor (vagile benthic), which might have differential effects on the genetic differentiation patterns of each species (Pascual *et al*., 2017).

Previous genetic studies for both species show differentiation between the Black Sea and the Mediterranean (Ladoukakis *et al*., 2002; Kalkan *et al*., 2011). In *M. galloprovincialis*, Ladoukakis *et al*. (2002) found genetic differentiation across a large geographic area between the populations from the Mediterranean (Adriatic, Ionian, and southern, middle and northern Aegean) and northern Black Sea (Sevastopol, Ukraine) using RFLPs. At a more local level, Kalkan *et al*. (2011) used mitochondrial DNA and microsatellites to test whether the Bosphorus Strait is a hydrographic barrier restricting gene flow for *M. galloprovincialis* between the Black Sea and the Sea of Marmara. Those data indicated that the Bosphorus Strait itself is not the primary cause of genetic differentiation observed between northern Black Sea and northern Aegean populations. In another study from the western and central Adriatic and the Black seas, the overall genetic structure of *M. galloprovincialis* was investigated applying the 2b RAD approach (Paterno *et al*., 2019). Besides genetic heterogeneity in the Adriatic for *M. galloprovincialis*, they found a marked difference in genetic structure between the Mediterranean and the Black Sea. The authors concluded that the narrow straits (Bosphorus and Dardanelles) and the existence of the environmental barriers (temperature, salinity) are the probable causes limiting gene flow between the two seas.

In a similar study on *P. elegans*, Reuschel *et al*. (2010) found three different haplogroups (referred to as Type I, II and III) from mtDNA sequences over a larger geographic range. Specimens belonging to Type I were from the Atlantic and Alboran Sea in the Mediterranean, Type II exclusively from the Mediterranean Sea and Type III from the Baltic, Caspian and Black seas, as well as the Mediterranean. Surprisingly, Type III COI sequences were more than 50 mutational steps (p=~8%) away from sequences in the Type I and Type II haplogroups. In view of this striking finding, Reuschel *et al*. (2010) proposed that the Type III haplogroup could represent a cryptic species formed due to an isolation event dating to the Messinian Salinity Crisis (~5.96 mya). Next, Kalkan *et al*. (2013) found two genetically distinct COI haplogroups, probably shaped by the two-way current regime of the TSS described above, supporting the presence of two potential cryptic taxa within *P. elegans*, as suggested by Reuschel *et al*. (2010). More recently, Deli *et al*. (2018b) examined the population genetic and phylogeographic structure in Type II and Type III haplogroups in *P. elegans* throughout the range of the species, however they provided no definitive explanations with regards to the different phylogeographic patterns observed in and around the TSS.

However, as only few samples from the TSS were included in these previous investigations, it was not possible to understand how the TSS itself affected the observed genetic differentiation. The TSS, the only water way providing a connection of Black Sea with Mediterranean, might indeed play a significant role on the connection of populations on both sides of the System.

In this study, we examine mtDNA and nuclear data to test the hypothesis that in each species the formation of two distinct mtDNA haplogroups was due to the Quaternary glacial–interglacial fluctuations (~2.58 mya-present), specifically the intermittent connection between the Black Sea and Mediterranean. There are studies showing the genetic diversity of multiple species were shaped by

the climatic fluctuations of the Pleistocene (e.g., Magoulas *et al*., 1996; Major *et al*., 2006, Luttikhuizen *et al*., 2008; Yebra *et al*., 2011; Boissin *et al*., 2016). We also expect that the different life history characteristics of these species might have affected their dispersal abilities following the opening of the TSS, resulting in some differences in contemporary patterns of genetic diversity and connectivity. As discussed above, while *P. elegans* retains the ability to move as adults (vagile benthic), *M. galloprovincialis* has a sessile adult stage (sessile benthic / very low motility), so its dispersal ability is limited to relatively passive transport during the pelagic larval stage due to their different life strategies. Therefore, we expect to find a greater frequency of *P. elegans* of Aegean origin in the TSS, when compared to *M. galloprovincialis*, due to the former's higher ability to have dispersed against prevailing currents of the TSS in its adult stage.

Materials and Methods

Sampling and DNA extraction

Samples of both species were collected in shallow waters (0-15 m) by free and SCUBA diving; the mussel samples were collected directly by hand, and prawn samples with a scoop net. A total of 242 *Mytilus galloprovincialis* specimens collected from 26 locations were evaluated (Table 1, Fig. 1). Of these, 96 specimens were collected previously from eight locations around the Bosphorus Strait and northern Sea of Marmara by Kalkan *et al*. (2011). The additional 146 specimens were collected from 18 new sampling locations, including nine sites in the Black Sea, two sites in the Sea of Marmara, one site in the Dardanelles, five sites in the Aegean Sea, and one site in the Levantine Sea. For *Palaemon elegans*, a total of

Table 1. Collection details for *Mytilus galloprovincialis* and *Palaemon elegans* samples included in this study.

Collection Site (Site codes; Map ref.)	Country	Sea / Region	Geographic Coordinates	Sample Size (N)		
				Mytilus	Palaemon	Microsats
Sevastopol (B1)	Ukraine	Black Sea	44°36'58.79"N 33°30'40.41"E	5	-	10
Krasnodar Krai: Malyy Utrish (B2)	Russia	Black Sea	44°42'29.50"N 37°27'18.89"E	4	-	9
Hopa: Kemalpaşa Harbor (B3)	Turkey	Black Sea	41°28'55.27"N 41°31'12.09"E	7	6	10
Ordu: Perşembe (B4)	Turkey	Black Sea	41°08'00.39"N 37°40'52.03"E	3	3	10
Sinop: Karakum (B5)	Turkey	Black Sea	42°00'57.92"N 35°10'56.89"E	13	6	11
Kastamonu: Gideros Cove (B6)	Turkey	Black Sea	41°51'35.62"N 32°51'25.13"E	6	17	10
Düzce: Akçakoca (B7)	Turkey	Black Sea	41°05'23.65"N 31°07'20.22"E	2	16	9
İstanbul: Şile (B8)	Turkey	Black Sea	41°10'48.26"N 29°36'42.67"E	2	-	10
İstanbul: Riva (B9)	Turkey	Black Sea	41°14'01.72"N 29°13'36.13"E	11	-	20*
İstanbul: Kilyos (B10)	Turkey	Black Sea	41°15'06.19"N 29°02'18.46"E	5	-	22*
Varna (B11)	Bulgaria	Black Sea	43°12'17.43"N 27°55'59.94"E	2	-	10
Istanbul: Hamsi Limani (TS1)	Turkey	Sea of Marmara, Bosphorus	41°12'27.06"N 29°06'20.09"E	-	7	-
Istanbul: Büyük Liman (TS2)	Turkey	Sea of Marmara, Bosphorus	41°12'18.38"N 29°06'09.08"E	-	11	-
Istanbul: Anadolu Kavagi (TS3)	Turkey	Sea of Marmara, Bosphorus	41°10'22.75"N 29°05'18.14"E	13	-	18*
Istanbul: Rumeli Hisari (TS4)	Turkey	Sea of Marmara, Bosphorus	41°05'16.60"N 29°03'24.95"E	11	-	21*
Istanbul: Kuzguncuk (TS5)	Turkey	Sea of Marmara, Bosphorus	41°02'19.32"N 29°01'56.98"E	16	-	19*
Istanbul: Kalamiş (TS6)	Turkey	Sea of Marmara	40°58'38.36"N 29°02'14.16"E	17	-	23*

Continued

Table 3 continued

Collection Site (Site codes; Map ref.)	Country	Sea / Region	Geographic Coordinates	Sample Size (N)		
				Mytilus	Palaemon	Microsats
Istanbul: Burgaz Island (TS7)	Turkey	Sea of Marmara	40°52'41.27"N 29°03'09.74"E	14	17	25*
Kocaeli: Derince (TS8)	Turkey	Sea of Marmara	40°44'57.49"N 29°48'44.24"E	12	-	10
Tekirdağ: Mürefte (TS9)	Turkey	Sea of Marmara	40°40'34.82"N 27°15'52.88"E	13	-	22*
Balikesir: Erdek (TS10)	Turkey	Sea of Marmara	40°23'45.20"N 27°47'23.93"E	10	-	10
Çanakkale (TS11)	Turkey	Sea of Marmara, Dardanelles	40°09'08.30"N 26°24'18.14"E	16	5	8
Edirne: Enez (A1)	Turkey	Aegean Sea	40°41'45.48"N 26°03'16.60"E	-	25	-
Edirne: Ibrice Harbor (A2)	Turkey	Aegean Sea	40°36'07.51"N 26°32'30.69"E	21	-	10
Gökçeada: Kaleköy Harbor (A3)	Turkey	Aegean Sea	40°13'54.53"N 25°53'39.18"E	-	24	-
Balikesir: Ayvalik (A4)	Turkey	Aegean Sea	39°18'47.85"N 26°41'16.49"E	8	5	10
Izmir: Foça (A5)	Turkey	Aegean Sea	38°40'01.43"N 26°44'44.84"E	6	-	9
Izmir: Bayrakli (A6)	Turkey	Aegean Sea	38°27'41.95"N 27°09'47.77"E	8	-	10
Izmir: Çeşme (A7)	Turkey	Aegean Sea	38°20'12.77"N 26°23'18.96"E	-	1	-
Bodrum: Güllük (A8)	Turkey	Aegean Sea	37°14'15.32"N 27°35'43.25"E	13	-	9
Bodrum: Turgutreis (A9)	Turkey	Aegean Sea	37°00'20.28"N 27°15'23.41"E	-	1	-
Datça: Palamutbükü (A10)	Turkey	Aegean Sea	36°40'09.97"N 27°30'09.62"E	-	5	-
Fethiye: Karagözler (L1)	Turkey	Levantine Sea	36°38'25.32"N 29°06'03.12"E	4	8	10
Antalya: Kaş (L2)	Turkey	Levantine Sea	36°11'48.18"N 29°38'39.41"E	-	13	-
Antalya: Demre (L3)	Turkey	Levantine Sea	36°11'45.65"N 29°50'56.79"E	-	2	-
Mersin: Taşucu (L4)	Turkey	Levantine Sea	36°16'25.78"N 33°48'55.10"E	-	11	-
	Total Individuals	242	183	345		

* data from Kalkan *et al*. (2011)

183 samples were collected from 19 sampling sites, representing five sites in the Black Sea, four sites in the TSS, six sites in the Aegean Sea, and four sites in the Levantine Sea (Fig. 2, circles). An additional 93 samples from Deli *et al*. (2018b) (Fig. 2, squares) were also included in the analyses. Table 1 shows the details for collection sites and sample sizes for each species. Total genomic DNA was extracted using a Roche High Pure PCR Template Preparation Kit (Indianapolis, USA) following the instructions of the supplier, and DNA extracts were subsequently stored at -20°C.

Laboratory Procedures

i) Sequencing

Out of the 266 COIII sequences, 24 were removed from the phylogeographic analyses, as they represented the C-genome. Of the remaining 242 *M. galloprovincialis* COIII sequences included in the analyses, 146 sequences were newly generated and 96 sequences were from the Bosphorus Strait that had been previously analyzed by Kalkan *et al*. (2011). A partial fragment of the mtD-

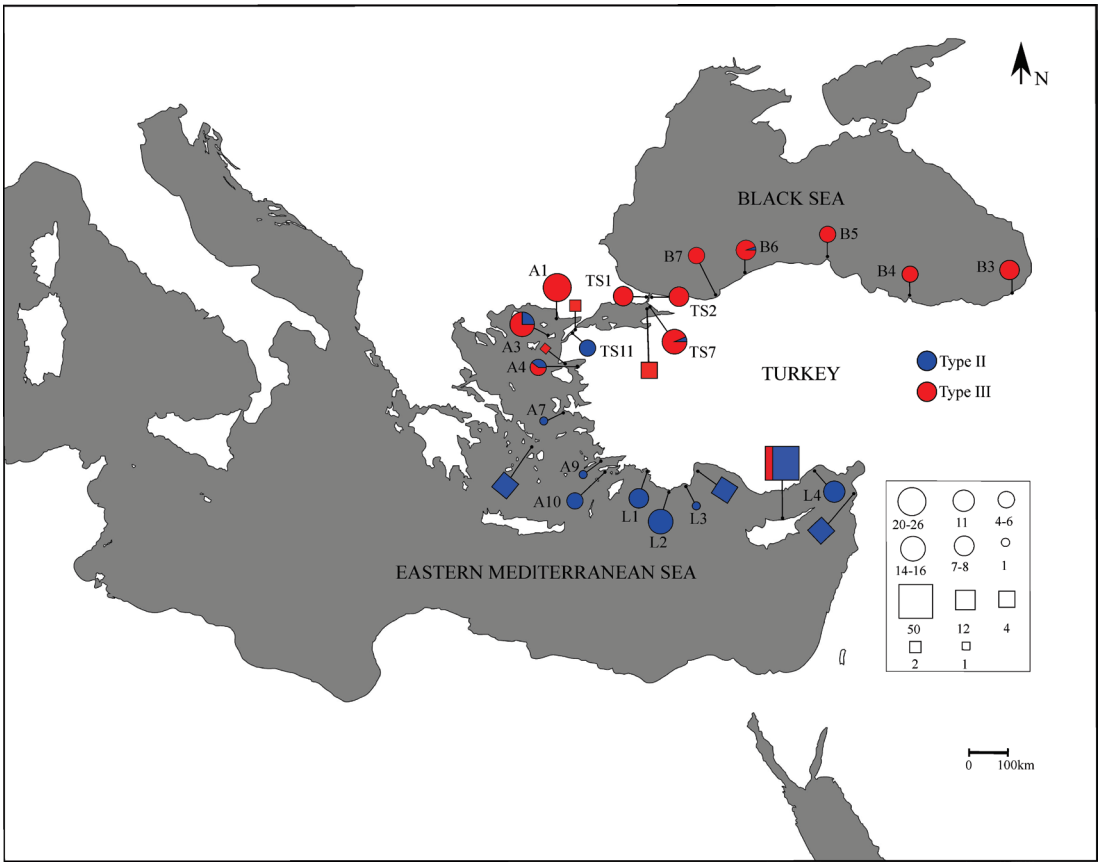


Fig. 2: Palaemon elegans mtDNA clade frequencies for populations along the coasts of Turkey from collection sites in this study (circles) and Deli et al. 2018b (squares). Circle and square sizes are proportional to sample sizes (Map was created from template obtained from d-maps.com and edited in InkScape v0.91).

NA COIII gene (813bp) was amplified with the primers 5'-TATGTACCAGGTCCAAGTCCGTG-3' and 5'-ATGCTCTTCTTGAATATAAGCGTAC-3', using the conditions described in Saavedra *et al*. (1997) and following the PCR protocol previously described in Kalkan *et al*. (2011). For *P. elegans* a 290 bp fragment of the mitochondrial CO1 gene was sequenced for 183 individuals using the primers LCO1490 and HC02198 and the protocol described in Bilgin *et al*. (2015). In addition, for *P. elegans*, a 257 fragment of the nuclear histone gene H3 was amplified for 91 individuals using the primer pair H3F-H3R following the protocol by Colgan *et al*. (1998).

The amplified products were purified using the Roche High Pure PCR Product Purification Kit (Indianapolis, USA) and were subsequently sequenced commercially at Macrogen Inc., South Korea. The resultant chromatograms were manually edited with the software Sequencher v. 4.8 (http://www.genecodes.com) and aligned using ClustalX (Thompson *et al*., 1997).

ii) Microsatellites

Five microsatellite primer pairs (Mgμ2, Mgμ3, Mgμ4, Mgμ6 and Mgμ181) (Presa *et al*., 2002) were amplified for 175 *M. galloprovincialis* individuals from 18 sampling locations (Table 1). These data were combined with data from 170 individuals published by Kalkan *et al*. (2011) for a total of 345 individuals. The five microsatellite loci were PCR amplified in individual 25μL reactions, containing 1μL of genomic DNA, 2.5μL 10X high fidelity buffer, 2μL of $MgCl_2$ (25mM), 0.25μL of dNTPs (10mM each), 0.75μL of each primer (10μM) and 0.1 units of Taq polymerase, following the protocol described by Presa *et al*. (2002). The PCR products were sent to Macrogen Inc., South Korea for automated sizing of the fragments. Allele data were subsequently processed with the software Peak Scanner V.1.0 (Applied Biosystems) and scored manually.

Data Analyses

i) Sequencing

In the mussel family Mytilidae mitochondrial DNA can be transmitted both maternally and paternally; a mechanism referred to as doubly uniparental inheritance (DUI) (Saavedra *et al*., 1997). In mussels there are three types of mitochondrial genomes (F, M and C) (Zouros & Foltz, 1984; Skibinski *et al*., 1994); while F type mitochondrial DNA is transmitted maternally to offspring of both sexes (F genome), the M and C types are transmitted paternally to male offspring only (Ladoukakis *et al*., 2002; Venetis *et al*., 2007). To restrict the *M. galloprovincialis* mtDNA analyses to F-genome sequences only, sequences representing the M-genome (AY363687), C-genome (DQ469133, DQ445474, DQ445468) and F-genome

(DQ403170, DQ445471, DQ445477, AF063260–AF063265) were retrieved from GenBank and compared with sequences from our samples to exclude M or C-genome sequences from further phylogeographic analyses.

For *P. elegans* mtDNA 10 CO1 sequences (DQ882102–DQ882105, HE573175–HE573177, JQ306029–JQ306031) were added to the dataset from GenBank (from Poland, Portugal, Baltic Sea, and the UK), to compare with the 183 sequenced individuals from Turkey. Haplotype networks were constructed with the median-joining method using Network version 4.6.1.0 (Bandelt *et al*., 1999). In addition, neutrality tests, including Tajima's D (Tajima, 1989), Fu's Fs (Fu, 1997) and Ramos-Onsins and Rozas' R_2 (Ramos-Onsins & Rozas, 2002) were performed along with mismatch analyses (Harpending, 1994) using DnaSP v. 5.0 (Librado & Rozas, 2009) to detect selection and/or recent demographic expansions in either species.

Past population demography of *P. elegans* was reconstructed using extended Bayesian skyline plots (EBSPs) as implemented in BEAST v.2.4.8 (Bouckaert *et al*., 2014). Parameters for the analyses included application of strict molecular clocks based on an estimated mitochondrial clock rate of 1.4% per million years for the CO1 gene in the shrimp family Alpheidae (Knowlton & Weigt, 1998). In BEAST, the prior on substitution model parameter was selected as general time reversible, and 500 million iterations were used as the chain length, with the first 10% of the iterations discarded as burn-in. The software Mega X (Kumar *et al*., 2018) was used to pick the best model of evolution for our data. TRACER v.1.6. (Rambaut *et al*., 2014) was used to ascertain that the ESS values were over 200. The median and the 95% central posterior density intervals of the demographic estimate of EBSPs were visualized with the scripts provided with the BEAST package, using R 3.5.1. (https://www.R-project.org).

ii) Microsatellites

MICRO-CHECKER (Van Oosterhout *et al*., 2004) was used to identify potential genotyping errors due to null alleles, large allelic dropout, and scoring errors due to stuttering. Microsatellite diversity within groups was estimated as the number of alleles per locus (Na), observed heterozygosity (Ho) and expected heterozygosity (He) using the program GENALEX v.6.5 (Peakall & Smouse, 2006, 2012). The same program was used to estimate the significance of any deviations from Hardy-Weinberg equilibrium (HWE). A correction was not made for null alleles. Linkage disequilibrium was tested for each locus-population combination using the web version of GENEPOP 4.0.10 (Raymond & Rousset, 1995; Rousset, 2008), which employs a Markov chain method following the algorithm of Guo & Thompson (1992). Arlequin v. 3.11 was used to carry out F_{ST}- and R_{ST}-based analyses of molecular variance (AMOVA) to assess the levels of population differentiation over all loci (Excoffier *et al*. 1992). Pairwise Φ_{st} and R_{ST} values were scaled multidimensionally (MDS), using the software XLstat 7.5.2 (Addinsoft, 2004) in order to evaluate the similarities or differences between populations.

Genetic structure among populations was evaluated using a clustering technique in the software STRUCTURE v. 2.3 (Pritchard *et al*., 2000) applying the no-admixture model as the potential population origin of each sampling site was not taken into account in the analysis. Burn-in and MCMC (Monte Carlo Markov chain) lengths of 50,000 and 250,000 were used, respectively. Twelve runs were carried out for the entire data set for each value of K (number of clusters) from 1 to 20. Likelihoods provided for each K were transformed into probabilities (Pritchard *et al*., 2000), and the most probable value of K was used as a point estimate. In many cases, once the optimal K was reached, likelihoods for larger Ks plateaued and the variance among runs increased (Pritchard *et al*., 2000). Thus, following the recommendations by Evanno *et al*. (2005), we used the ΔK measure that has been proposed to provide a better estimate of the true K. The k-test (Reich and Goldstein 1998) was used to test for signatures of population expansion using microsatellites. The test was implemented using the software kgtests (Bilgin, 2007).

Results

Haplotype networks and demographic history

For *Mytilus galloprovincialis* mtDNA, a total of 266 COIII sequences were obtained, but 24 of these were identified as C-genome sequences and were excluded from further analyses (See materials and methods section for a brief discussion on the F, C and M genomes). No M-genome haplotypes were identified in our samples. In 242 F-genome sequences analyzed, a total of 48 unique haplotypes were detected. Multiple parameters of genetic diversity for *M. galloprovincialis* are given in Supplementary Table S1.

The *M. galloprovincialis* COIII network contains three main haplogroups (Fig. 3) referred to as Types I, II and III). Type II and Type III haplogroups are both characterized by star-like genealogies. In addition, Type III contains an extra branch, leading to a second smaller star-like sub-network. Type III is separated from the Type II and Type I sequences by at least 19 and 20 mutations (~2.4%), respectively. The most common Type III haplotype (H1) is considered to be the putative most ancestral haplotype and is connected to numerous lower frequency haplotypes by one to nine mutations. H1 was found in all Black Sea and TSS sampling sites (in about 58% of individuals sampled) but was not found in the Aegean sampling sites except in (site A2, Ibrice Harbor; one individual) and the Gulf of Izmir (site A6, Izmir: Bayrakli; one individual). While the Type III haplogroup was primarily made up of haplotypes (including H1 and haplotypes that are only one or two mutational steps from H1) found in the Black Sea and TSS (174 of 189 individuals), individuals with Type III haplotypes, which were more

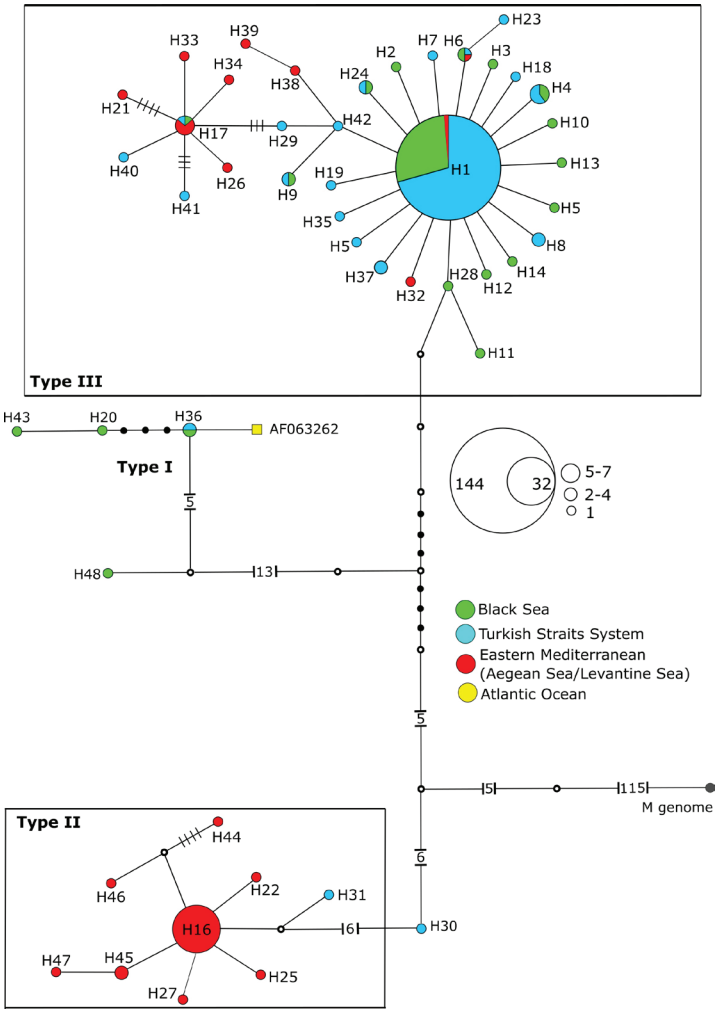


Fig. 3: *Mytilus galloprovincialis* haplotype network based on COIII sequences. The diameter of each circle is proportional to the number of individuals with the haplotype; shaded partitions inside the circles represent the composition of the haplotype found in each region. Small white circles are missing haplotypes, and each line and the transverse lines represent a single mutational change.

distantly related to H1 (e.g., H17 and its closely related haplotypes), were found at five sites in the Eastern Mediterranean Sea (Aegean and Levantine Sea coasts). In contrast, Type II haplotypes were found at all sampling sites in the Aegean, but none was found in the Black Sea, and only two Type II individuals were found from sites in the TSS (Istanbul: Kuzguncuk, Bosphorus Strait, TS5). Five individuals, grouped with Atlantic *M. galloprovincialis* haplotype (accession number AF063262), were included in Type I and were separated from the Type II and Type III haplogroups by at least 29 and 20 mutations, respectively. Four of these were sampled from the Aegean Sea (A1, A5 and A8), and one from the TSS (TS10) (see Supplementary Table S2 for the number of samples belonging to each type [I, II, III] at each collection site).

For *Palaemon elegans* mtDNA, 40 unique CO1 haplotypes were found in a total of 193 sequences (including 10 GenBank sequences). Multiple parameters of genetic diversity for *P. elegans* are given in Supplementary Table S3. Again, two main haplogroups, corresponding to Types II and III of Reuschel *et al*. (2010), were recovered for the CO1 gene region in the haplotype network (Fig. 4). Types II and III were separated from each other by at least 18 mutations (6.2%). This difference corresponds to a divergence time of approximately 5.52 mya (±0.53 mya=1 s.d.), based on the mitochondrial clock rate of 1.4% per million years for shrimp of the family Alpheidae (Knowlton & Weigt, 1998). The most common and ancestral Type II haplotype (H18) was found in 41 of the 66 samples with Type II haplotypes. The most common and ancestral Type III haplotype (H1) was found in 61 of the 117 specimens with Type III haplotypes. While Type II was found primarily in Aegean/Levantine Sea specimens (59 of 66), Type III was found in individuals from the Black Sea, the TSS and the northern Aegean (see Supplementary Table S4 for the number of samples belonging to each type [II, III] at each collection site).

For both species, mismatch analyses of Type II and Type III mtDNA haplogroups showed unimodal distributions of pairwise differences, indicative of population expansions (Fig. 5). These findings were corroborated by Tajima's D, Fu's Fs and Ramos-Onsins and Rozas' R_2 values, whose generally significant values for the different populations and types indicated population expansion (Supplementary Table S1, S3 and S5). In *P. elegans*, the extended Bayesian Skyline plots (EBSP) for Type II showed an expansion pattern starting around 120 kya (Fig. 6b). In contrast, for Type III haplotypes, the pattern

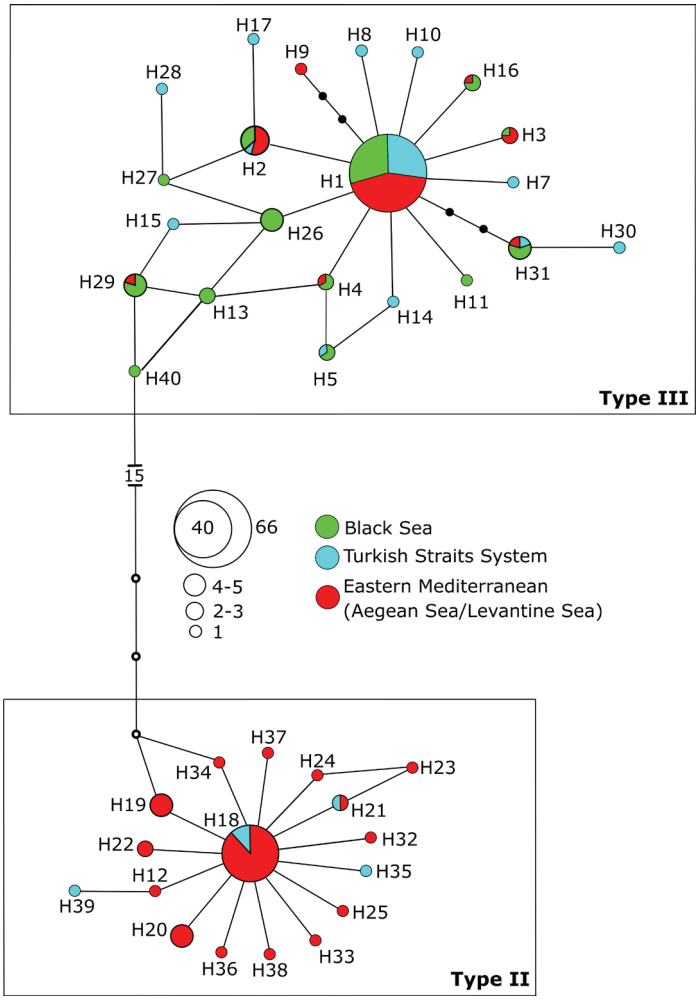


Fig. 4: *Palaemon elegans* haplotype network based on COI sequences. The diameter of each circle is proportional to the number of individuals with the haplotype; shaded partitions inside the circles represent composition of the haplotype from each region. Small white circles are missing haplotypes, and each line is a single mutational change.

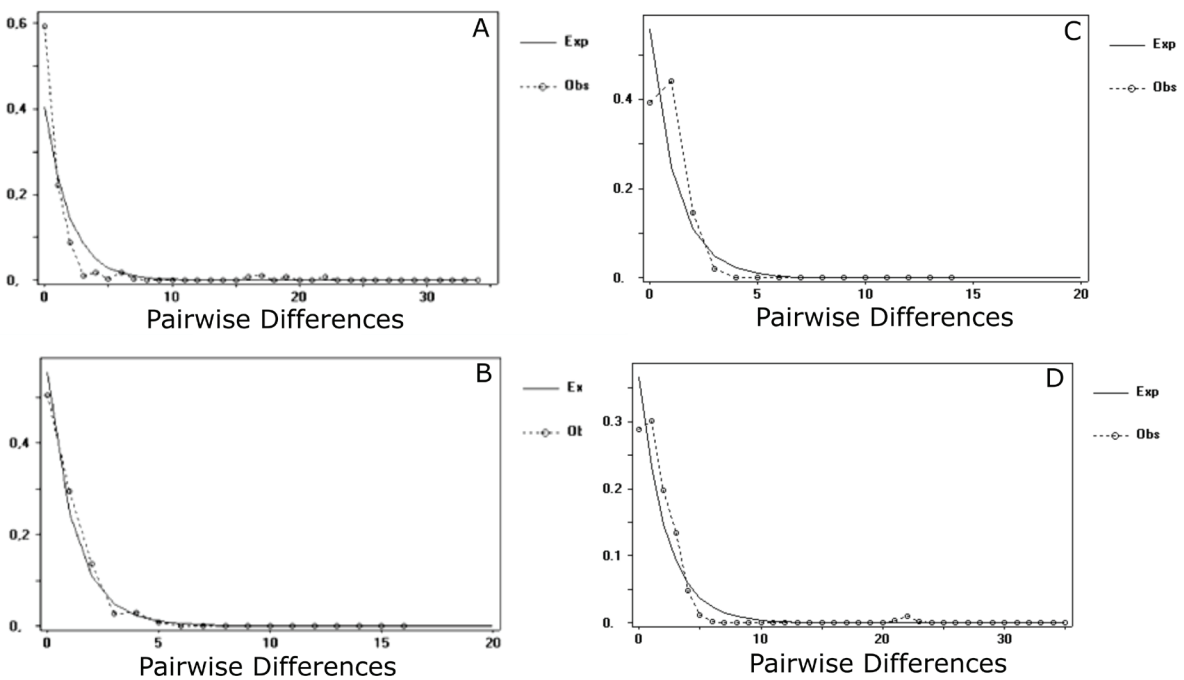


Fig. 5: Mismatch distribution and τ values obtained from *Mytilus galloprovincialis* COIII gene sequence data for Type II (a) and for Type III (b), and *Palaemon elegans* COI gene sequence data for Type II (c) and for Type III (d). Lines with empty circles represent the observed distribution, and black lines represent the expected distribution under a sudden expansion model.

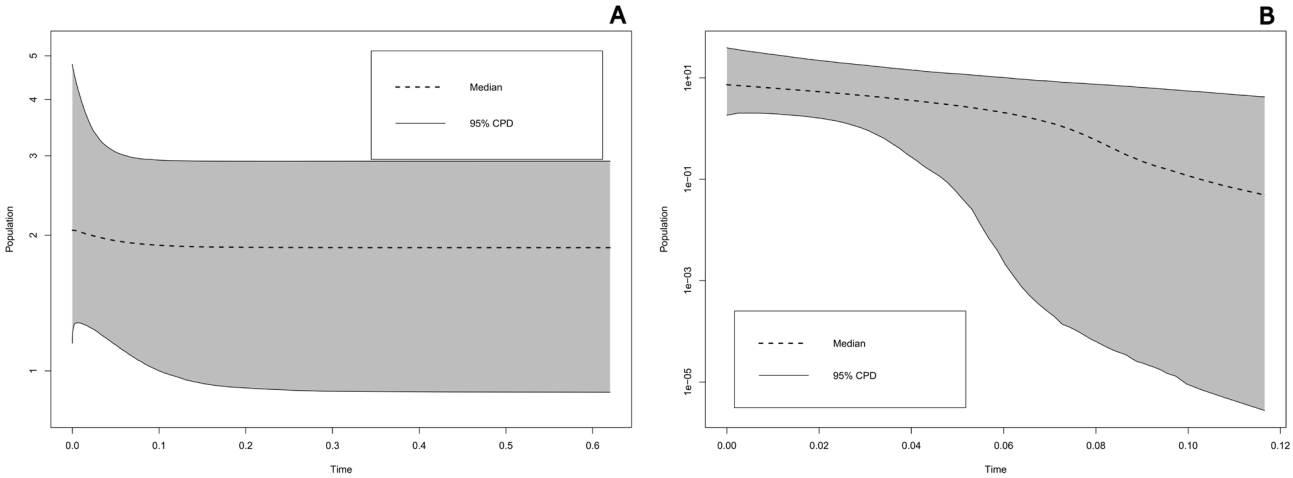


Fig. 6 (a-b): Extended Bayesian skyline plots for Type III (a) and Type II (b) in *Palaemon elegans*. Time is in millions of years.

indicates a common ancestral haplotype traced to around 600 kya, with a constant population size until around 120 kya, after which an expansion starts (Fig. 6a).

For *P. elegans*, a nuclear DNA haplotype network was constructed using sequences of the nuclear H3 region. Twenty-one unique haplotypes were detected in sequences from 91 individuals, and no double peaks, potentially suggestive of heterozygous individuals, were observed in the sequencing chromatogram results. Contrary to the mtDNA haplogroups that showed a clear differentiation, there was no support for any obvious genetic or geographic differentiation in the H3 data set (Fig. 7). Rather, two main haplotypes, a common haplotype (Pel/H5; 46 individuals) and a less common haplotype (Pel/H8; 11 individuals), were connected in a network to other haplotypes, with mutational distances of one bp for the most part.

Microsatellite structure in M. galloprovincialis

Five microsatellite loci previously used to evaluate population genetic structure of *Mytilus galloprovincialis* around the Bosphorus Strait (Kalkan *et al*., 2011) were used to genotype a total of 345 mussels from 18 sampling locations along the coast of Turkey. All loci deviated significantly from Hardy-Weinberg equilibrium due to heterozygote deficiency (Chi-square test, P<0.001); observed heterozygosities (0.149 to 0.496) were significantly lower than expected heterozygosities (0.627 to 0.923). According to MICRO-CHECKER genotype analyses, the validity of excessive homozygote size classes and high level of heterozygote deficiencies found at each locus may be the result of null alleles, as there was no evidence for large allele dropout. In addition, alleles of one repeat unit difference may result from PCR or sizing artifacts that resulted in stuttering at two loci (Mgμ4 and Mgμ181). Except for two pairs of loci (Mgμ2-Mgμ3 and

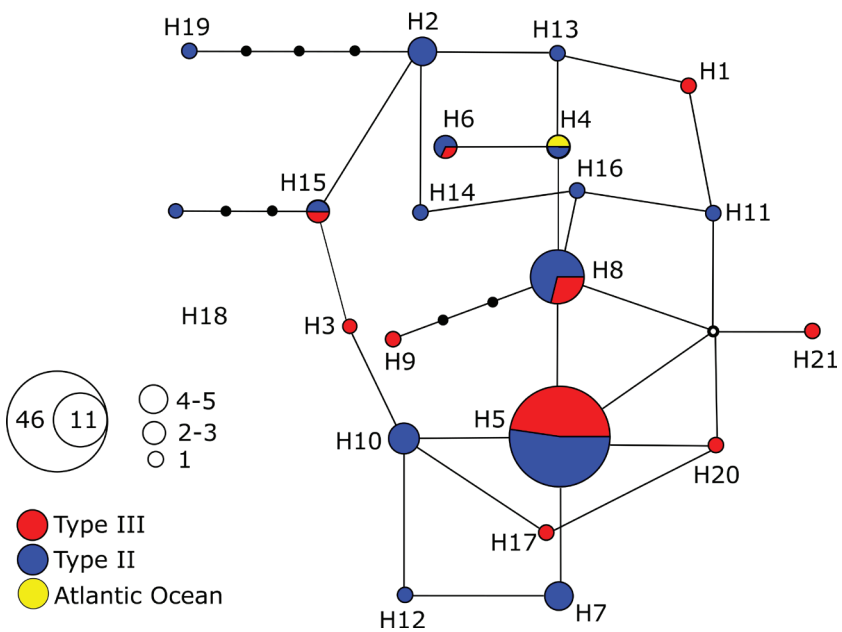


Fig. 7: *Palaemon elegans* haplotype network based on H3 gene sequences. The diameter of each circle is proportional to the number of individuals with the haplotype; shaded partitions inside the circles represent occurrence of the haplotype from each region. Small white circles are missing haplotypes and each line is a single mutational change.

Mgμ181-Mgμ6), no linkage disequilibrium was detected among the five loci after Bonferroni correction (Fisher's Exact Test, P>0.13) (Rice, 1989).

Allelic diversities of the five *M. galloprovincialis* microsatellite loci were similar in terms of the number of alleles per locus across all sampling sites (N_a = 6–10) and the effective number of alleles (N_e = 3.3–5.7). For the subsequent analyses, the microsatellite data set was analyzed for allelic diversity as two separate population groups based on the two haplogroups observed in mtDNA (Type II and Type III). Type III samples, found primarily in the Black Sea and TSS, were found to have a higher overall allelic diversity (in terms of N_a, N_e and number of private alleles) than Type II samples, found primarily in the Aegean and Levantine seas (Table 2).

Based on AMOVA using all loci, the highest percentage of variation was found within populations (84%). The percentage of variation among populations was 16%. Multilocus estimates of overall R_{ST} and F_{ST} values were 0.15 and 0.12, respectively. Pairwise R_{ST} and F_{ST} values were estimated among all populations and are reported in the Supplementary Table S6. Within the different water bodies (the Black Sea, the TSS and the Eastern Mediterranean Sea) some of R_{ST} estimates were significant (15 out of 60 between the Black Sea and the Mediterranean, 13 out of 54 between the TSS and the Mediterranean and 20 out of 90 between the TSS and the Black Sea), and majority of significant values were higher than 0.25 (Supplementary Table S6). In addition, about half of the F_{ST} values between the Black Sea and the Mediterranean (28 out of 60), and between the Black Sea and the TSS (44 out of 90), and between the TSS and the Mediterranean (28 out of 54) were found to be significant, and majority of these were higher than 0.20. In summary, a fair number of the pairwise microsatellite comparisons between the populations were significant, indicating local differentiation. In addition, while the non-metric multidimensional scaling (MDS) of pairwise mitochondrial Φ_{ST} values showed a differentiation between the Aegean and other samples, the non-metric MDS of nuclear R_{ST} and F_{ST} values did not indicate any differentiation among populations (Fig. 8).

Clustering analysis of all 345 samples conducted in STRUCTURE indicated the most likely number of populations (K) as three; however, there was no obvious correlation between the STRUCTURE-defined populations and the grouping of samples by location or Type II and III mtDNA haplogroups (Fig. 9). The STRUCTURE analysis also showed some local differentiation among individual sampling sites, specifically B1, B2, TS6, TS9, and A1 (Fig. 9). The *k*-tests for microsatellite loci supported evidence of population expansion for samples overall (P=0.026), and for samples with Type III haplotypes (P=0.026), but not for samples with Type II haplotypes (P=0.1693).

Discussion

Black Sea origin of Type III haplotypes and unidirectional dispersal

For both species we have examined here, *Mytilus galloprovincialis* and *Palaemon elegans*, mtDNA data indicate two distinct haplogroups with similar geographic distribution patterns. In both cases, one haplogroup (Type III) is found all along the coasts of Turkey, including the Black Sea and the TSS, and the other haplogroup (Type II) has a more restricted southern distribution, absent from the Black Sea and the TSS. The phylogeographic patterns in the mtDNA data for both species suggest that Black Sea and Eastern Mediterranean (Aegean and Le-

Table 2. Levels of genetic variability at five *Mytilus galloprovincialis* microsatellite loci in Type II and Type III populations. Sample size (N); number of alleles (Na); number of effective alleles (Ne); expected heterozygosity (He); observed heterozygosity (Ho).

Locus	Mgμ 2	Mgμ 3	Mgμ 4	Mgμ 6	Mgμ 181	Mean
Type II						
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
Type III						
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

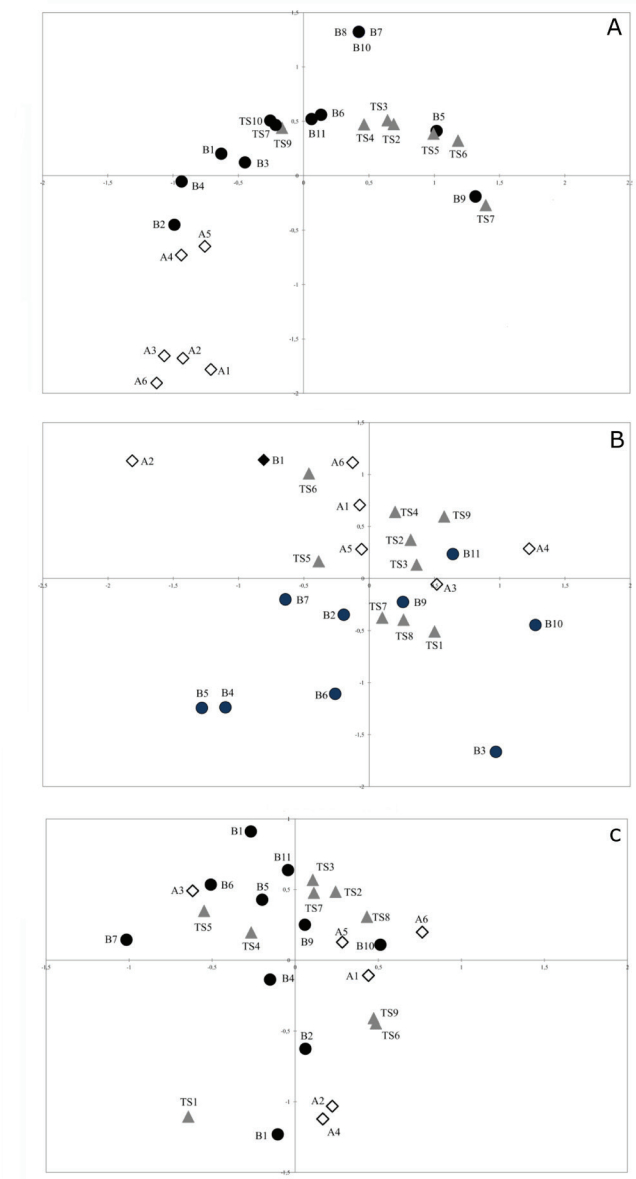


Fig. 8: Non-metric multidimensional scaling of *Mytilus galloprovincialis* pairwise F_{ST} values. (a) mtDNA data (stress=0.044), (b) microsatellite data-R_{ST} based (stress=0.140), (c) microsatellite data- F_{ST} based (stress=0.103).

vantine seas) populations of *M. galloprovincialis* and *P. elegans* were differentiated during the previous ice ages, and that these populations remained mostly isolated from each other until the connection of the Black Sea and the Mediterranean was finally established around 8 kya, after which the populations gained the opportunity for limited unidirectional dispersal from north to south.

Paleoclimatic events, particularly cycles of warming and cooling, have caused changes in temperature, salinity, connectivity and circulation conditions of the marine environment. These effects can be extreme for inland seas, such as the Black Sea, whose conditions may change dramatically when connections to larger bodies of water are lost or gained (Huyse *et al*., 2004). Climate variations in the Quaternary glacial-interglacial periods have resulted in population subdivisions, population declines and local extinctions in the Black Sea (Akse *et al*., 2002; Hewitt, 2004; Boissin *et al*., 2011, 2016). The intermittent connection between the Black Sea basin and the Mediterranean has shaped genetic diversity and diversification of numerous species during the Pleistocene climate shifts (Boissin *et al*., 2016; Paterno *et al*., 2019).

Our estimate of divergence time between types II and III of *P. elegans* (approximately 5.52 mya) falls within the Messinian Salinity Crisis that took place between 5.96–5.32 ±0.02 mya (Krijgsrnan *et al*., 1999), in the late Pliocene/Messinian. This divergence time estimate is more recent than that of 6.85 mya calculated by Reuschel *et al*. (2010) based on relatively few *P. elegans* samples from the eastern Mediterranean and the Black Sea. Reuschel *et al*. (2010) had also suggested that the Type III individuals found in the Black Sea were of Mediterranean origin. In addition, they propose that the occurrence of a cryptic species within this complex (Type III), probably resulting from an isolation event during the Messinian Crisis.

However, with increased sampling in the east, we have constructed a more complete haplotype network, providing evidence of a more recent split between Type

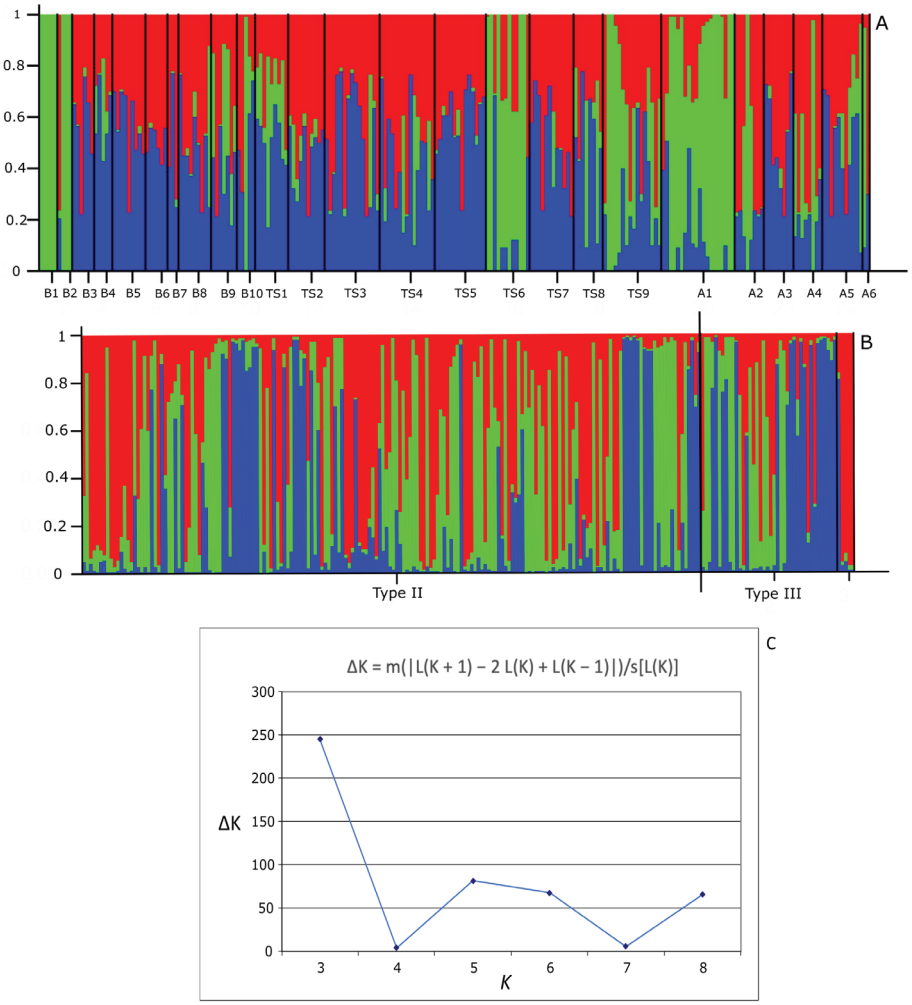


Fig. 9: STRUCTURE clustering results, based on microsatellites, for *Mytilus galloprovincialis* at K=3. The probability of individuals belonging to (a) each sampling site, (b) Type II or Type III for each individual. (c) K calculated as K = mIL"(K)I s[L(K)]. The modal value of this distribution is the true K or the uppermost level of structure, here three clusters.

II and Type III, and a Black Sea origin for the Type III haplogroup. Deli *et al*. (2018b) also hypothesized that Type III might have spread from the Black Sea where they have been subjected the salinity fluctuations due to repeated isolations from the Mediterranean during the glacial and interglacial periods of the Pleistocene, and our results based on a detailed sampling regime along Turkey's Black Sea coast supports Deli *et al*. (2018b)'s conclusions.

Based on the EBSPs, we see onset of expansion for Types II & III that date to around 120 kya. This time interval falls within the Riss-Würm interglacial period that dates to around 125 kya (Panin & Popescu, 2007). The warming up of the sea temperature during this interglacial could have resulted in population size increases for *P. elegans*, as seen in other marine species including the echinoderm *Cucumaria frondosa* (expansion onset c.a. 120 kya (So *et al*., 2011)), red seaweed *Palmaria palmata* (expansion onset at 128 kya (Maggs *et al*., 2008)), and polycheate tubeworm *Pectinaria auricoma* (expansion onset at 149 kya (Jolly *et al*., 2006)). This pattern illustrates the importance of population expansions predating the last glacial maximum (LGM) in shaping the demography of present-day marine species and communities (Hoarau *et al*., 2007).

Following the inundation of the Black Sea by the melting ice from Scandinavia and Russia regions after the LGM (~10 kya), water began to outflow from the Black Sea to the Mediterranean (Aksu *et al*., 2002; Hiscott *et al*., 2007). This outflow ultimately resulted in the opening of connections between the Aegean Sea and the Sea of Marmara (the Dardanelles Strait) and between the Black Sea and the Mediterranean (the Bosphorus Strait) (Aksu *et al*., 2002; Hiscott *et al*., 2007). Following this event, ancestral Type III *M. galloprovincialis* and *P. elegans* originating in the Black Sea were able to disperse into the Aegean, and ultimately to the rest of the greater Mediterranean (as reported by Deli *et al*. (2018b)). Transport of *P. elegans* to the Baltic Sea, as observed by Reuschel *et al*. (2010) could potentially have occurred through the brackish river systems that run through continental Europe, which can explain the absence of Type III *P. elegans* elsewhere in the Atlantic.

The lack of Type II individuals of either species in the Black Sea, and their low occurrence in the TSS, indicates that dispersal through the open TSS occurred in one direction only, most likely with buoyant planktonic larvae transported by the north-to-south moving surface current

of the TSS from the Black Sea to the Aegean Sea. Hence, although Type III individuals of both species were able to colonize the Aegean Sea from the Black Sea, Type II individuals, for the most part (see below for a discussion on the effects of differences in larval life history characteristics between *M. galloprovincialis* and *P. elegans*) were not able to colonize the TSS and the Black Sea in the reverse direction. According to the current population genetic structure of these species, the primary hydrographic barrier to dispersal appears to coincide with the Dardanelles.

Similar Black Sea origin scenarios have been hypothesized for the Mediterranean green crab, *Carcinus aestuarii* (Deli *et al*., 2018a) and the marbled crab *Pachygrapsus marmaratus* (Cetin *et al*., 2015). The idea of a Black Sea refugium was also proposed by Luttikhuizen *et al*. (2008) for the common shrimp *Crangon crangon*, where they suggest that the species could have persisted during the sea's history of dramatic fluctuating conditions (Svitoch *et al*., 2000) in salinities as low as <7‰; in the case of *M. galloprovincialis* and *P. elegans*, they have been shown to survive in salinities as low as 11‰ (Hamer *et al*., 2008) and 0.6‰ (Janas *et al*., 2013), respectively. Paterno *et al*. (2019) also explained the genetic differentiation found at *M. galloprovincialis* populations between the Black Sea and the Mediterranean being due to isolation of the Black Sea during the post glacial period. Boissin *et al*. (2016) supported the idea that the Black Sea might have acted as a potential refuge that could have preserved the ancestral genetic diversity until the connection between the Black Sea and the Mediterranean was established about 8000 years ago. With the results of this study, the case-studies and evidence for the phenomenon of out-of-Black-Sea dispersal of populations isolated during the glacial periods are mounting.

Life history characteristics of *M. galloprovincialis* and *P. elegans* also seem to influence the distribution of their genetic diversity. Although they both have similar planktonic larval duration (approx. 4-6 weeks), their mobility levels during their adult stages are different (Pascual *et al*., 2017). In particular, the degree of mobility of their adult stage likely determines the degree to which Type II individuals from the Aegean Sea were able to colonize within the TSS. For *M. galloprovincialis*, whose adult stage is categorized as sessile benthic, only two Type II individuals (out of 122; 1.64%) were found in the TSS. For *P. elegans*, whose adult stage is vagile benthic, six Type II individuals (out of 46 – including six samples of TSS origin from Deli *et al*. (2017); 13.04%) were found in the TSS. With its mobile adult stage, it would have been easier for *P. elegans* individuals to disperse against the surface current over time, likely resulting in a higher relative distribution of Type II *P. elegans* than *M. galloprovincialis* in the TSS.

It should also be noted that the mtDNA of six individuals of *M. galloprovincialis* from the study area clustered with Atlantic haplotypes (Type I). Five of these individuals were collected from three different Aegean sampling sites (A1, A5 and A8) and the last one was from the TSS (Erdek, TS10). Because the Mediterranean Sea has sustained extensive maritime activity, many organisms can be easily transported via ballast water and hull fouling (Knapp, 1993; Wachsmann, 1998; Minchin *et al*., 2002). Therefore, these six individuals that grouped with the Atlantic haplotypes could have been transported by the ballasts of ships or as spat attached to the introduced mussels.

Cytonuclear Discordance

In both *M. galloprovincialis* and *P. elegans*, Type II and Type III individuals are easily distinguished with mtDNA sequence data, but these differences are not reflected in the nuclear DNA data. Using nuclear markers, microsatellites for *M. galloprovincialis* and H3 sequences for *P. elegans*, there was no significant nuclear genetic differentiation between mtDNA haplogroups (Type II and Type III). For both species, the discordance between nuclear DNA and mtDNA could be due to the latter being mostly haploid and maternally inherited (Hoarau *et al*., 2004), and its effective population size [Ne(mt)] being four times smaller than that of the nuclear loci (Hefti-Gautschi *et al*., 2009). The smaller effective population size affects the rate of genetic drift and lineage sorting, which generally occur at an accelerated rate in mtDNA (Funk & Omland, 2003) resulting in higher levels of population genetic differentiation for mtDNA loci compared to nuclear DNA loci.

Another potential contributor to the observed cytonuclear discordance is secondary contact of historically isolated populations. If populations or taxa have been geographically isolated for a long period of time, resulting in genetic differentiation, if they come back into contact and hybridize, they can display patterns of biogeographic mitochondrial-nuclear discordance (Toews & Brelsford, 2012). During the period of isolation, divergent mutations accumulate in both their mitochondrial and nuclear DNA due to selection and drift (Toews & Brelsford, 2018). During secondary contact, if reproductive isolation is not complete, the previously isolated populations will interbreed and form hybrid zones. Therefore, discrepancy between bi-parentally inherited nuclear DNA and maternally inherited mitochondrial DNA can arise as nuclear introgression and recombination homogenizes nuclear gene pools, while mtDNA haplotypes are passed faithfully from one generation to the next (Avise, 2000; Tarnowska *et al*., 2010). In our study, the lack of genetic differentiation found in the nuclear microsatellite loci is likely due to the populations (representing types II and III) not having established complete reproductive isolation. Our subsequent analysis of a diagnostic non-repetitive nuclear region (Inoue *et al*., 1995) (results available upon request), which showed no base differences between individuals belonging to types II and III corroborated that all analyzed individuals belonged to *M. galloprovincialis*. The lack of morphological divergence between Types II and III in *P. elegans* (Reuschel *et al*., 2010) also supports this notion of a single species or a cryptic species complex. Hence, the current evidence suggests that, for

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

As a final note, specifically focusing on the microsatellite data for *M. galloprovincialis,* we see extensive heterozygote deficiency at all loci. Heterozygote deficiency could be directly generated by (1) inbreeding, causing high proportion of homozygotes across all loci, (2) Wahlund effect, (3) selection against heterozygotes and (4) null alleles (Castric *et al*., 2002; Hoarau *et al*., 2004; Van Oosterhout *et al*., 2004). In addition, sudden increases in population sizes can result in homozygote excess (Watterson, 1986). There are a number of investigations concerning the heterozygote deficiency in marine organisms (e.g., Waldman & McKinnon, 1993; Zouros & Foltz, 1994; Raymond *et al*., 1997), and heterozygote deficiencies relative to Hardy-Weinberg expectations in bivalve populations have been commonly reported (Passamonti *et al*., 1999, Huvet *et al*., 2000; Laudien *et al*., 2003). Here we present yet another case of such heterozygote deficiency in a bivalve.

Conclusion

This study shows how geographic and hydrographic barriers, Paleoclimatic events and life history characteristics act together to shape the genetic make-up of populations of marine species. The predominant determinants of the intraspecific patterns of distribution of genetic diversity in both *P. elegans* and *M. galloprovincialis* were Paleoclimatic events leading to allopatric isolation during the ice ages. The Black and the Mediterranean seas had an intermittent connection during the Pleistocene climatic fluctuations (Magoulas *et al*., 1996; Boissin *et al*., 2016; Paterno *et al*., 2019). Therefore, many marine species have a chance to expand and colonize via their pelagic larvae or their own mobility capacity between the different basins. In both species, ancestral populations were isolated and differentiated from each other during the Pleistocene, resulting in the formation of mtDNA haplogroup types II and III. The Type III haplogroups in both species were likely able to colonize the Sea of Marmara and the Aegean Sea after the establishment of the connection between these water bodies at the end of the last ice age, with the pelagic larvae of both species being transported by the surface currents of the TSS. The gene flow was one-way for the most part, however, as the TSS did not allow the dispersal of the planktonic larvae of the Type II individuals northwards. Therefore, the TSS, due to its peculiar hydrographic features, acts as a unidirectional barrier to gene flow. This is where we see idiosyncrasies due to the different life histories of the two species. Individuals with Type II haplotypes of the Mediterranean origin in *P. elegans*, likely due to their vagile benthic adult stages, have been able to recolonize the TSS at a higher frequency than the sessile *M. galloprovincialis*. Although non-sessile, the dispersal range of *P. elegans* is limited; hence its movement from south to north into the Black Sea would have happened in a piecemeal fashion, which might have taken as long as 120 Ky based on the extended Bayesian skyline plot results.

From a molecular taxonomic perspective, the mitochondrial DNA differentiation was not reflected in the nuclear genome, however, suggesting that the isolation during the ice ages did not result in the formation of reproductive barriers. 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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Supplementary Data

The following supplementary information is available online for the article:

Table S1. Parameters of genetic diversity for *M. galloprovincialis*. N (number of samples), Nh (number of haplotypes), Nps

(number of polymorphic sites), h (haplotype diversity), ∏ (nucleotide diversity), D (Tajima's D), Fs (Fu's Fs), R_2 (Ramos-Onsins & Rozas' R_2). The significant (P <0.05) D, Fs and R_2 values are given in bold.

Table S2. The total number of individuals and number of samples belonging to each type (I, II, III) at each collection site for *M. galloprovincialis*.

Table S3. Parameters of genetic diversity for *P. elegans*. N (number of samples), Nh (number of haplotypes), Nps (number of polymorphic sites), h (haplotype diversity), ∏ (nucleotide diversity), D (Tajima's D), Fs (Fu's Fs), R_2 (Ramos-Onsins & Rozas' R_2). The significant (P <0.05) D, Fs and R_2 values are given in bold.

Table S4. The total number of individuals and number of samples belonging to each type (II, III) at each collection site for *P. elegans*.

Table S5. Neutrality tests for different populations and types of *Mytilus galloprovincialis* and *Palaemon elegans* based on COIII and CO1 sequences, respectively. D, Tajima's D; Fs, Fu's Fs; R2, Ramos- Onsins and Rozas's R_2. The significant (P <0.05) D, Fs and R_2 values are given in bold.

Table S6. Above diagonal: Pairwise F_{ST} values between populations of *Mytilus galloprovincialis* for five microsatellite loci. Below diagonal: Pairwise R_{ST} values between populations of *Mytilus galloprovincialis* for five microsatellite loci. Significant P values (P<0.05) are indicated in italics.