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Genetic diversity of marbled goby populations in the Anatolian coasts of the north-eastern Mediterranean

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Abstract

The demographic histories, genetic relationships and population structure of sedentary fish *Pomatoschistus marmoratus* (Risso, 1810), which was sampled from the north-eastern basin of the Mediterranean Sea (including the Turkish coasts of the Black Sea, Aegean Sea, Levantine Sea and Sea of Marmara), were investigated by mitochondrial cytochrome c oxidase subunit I (652 bp) and cytochrome b (526 bp) regions. It was found that the population groups had high haplotype diversity while the nucleotide diversity was quite low for both gene regions. Phylogeographic analyses of the haplotypes indicated that the Levantine population (LEV) were genetically different from other populations. Also, the gene flow between LEV and the other populations was very limited. The results of the analyses of neutrality and mismatch distributions that were applied to the population groups were evaluated as a whole. It was determined that the haplogroup that represents the Black Sea and Sea of Marmara populations (BLAMAR) was stable, but the Levantine population (LEV) was under the sudden demographic expansion model following the population bottleneck. The genetic variance indices indicated sudden demographic expansion following population contraction. This was supported by star-shaped haplotype networks. The reason for this limited gene flow and differentiation between the Levantine population (LEV) and the others was linked with wind-driven offshore transport of the larvae and surface currents in these subbasins. The timing of the differentiation, demographic histories of populations associated with geological and palaeo-climatic events and current ecological conditions were discussed.

Original Article

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Introduction

The semi-enclosed Mediterranean Sea underwent substantial changes due to its evolutionary history throughout the Tertiary period. Combined effects of the complex geological events, and palaeo-climatic history of the Mediterranean Sea, play a key role in shaping speciation and population structuring (Patarnello *et al.*, 2007). Recent research, which describes genetic differentiation in some Mediterranean species, has indicated that habitats, biological and oceanographic conditions have a great impact on population structures, in addition to climatic changes and geological events (Avise, 2000; Lemaire *et al.*, 2005; Maggio *et al.*, 2009; Mejri *et al.*, 2011).

The gobiid fishes represent one of the most speciose groups in the Mediterranean Sea (Miller, 1986). Among the Eastern Atlantic and Mediterranean gobiid fishes, the paraphyletic genus *Pomatoschistus* is one of the dominant gobiid group, consisting of 14 mainly marine species (Eschmeyer *et al.*, 2019). Research on *Pomatoschistus* species and populations has revealed that there is a high genetic differentiation between both the western and eastern Mediterranean Sea and Atlantic–Mediterranean populations of these species (Gysels *et al.*, 2004a, 2004b; Larmuseau *et al.*, 2009; Mejri *et al.*, 2009, 2011; Boissin *et al.*, 2011; Tougard *et al.*, 2014).

The marbled goby *Pomatoschistus marmoratus* (Risso, 1810) is a small benthic fish inhabiting near-shore sandy habitats and is widespread throughout the eastern Atlantic (western Spain and south-western coasts of France), Mediterranean, Black Sea, Azov Sea and Suez Canal (Miller, 1986). They prefer spending their entire life cycle in shallow coastal regions, including lagoons with differing salinity values (Miller, 1986; Mazzoldi & Rasotto, 2001; Rigal *et al.*, 2008; Gonzalez-Wangüemert & Vergara-Chen, 2014) and have a pelagic larval duration of 40–50 days under laboratory conditions (Locatello *et al.*, 2017). The habitat preference of *P. marmoratus* is very close to the shoreline (generally 0–3 m), which is unstable and most affected by palaeo-climatic events. In addition, *P. marmoratus* exhibits benthic eggs and adults are known to be poor swimmers and habitat dependent, which makes this species quite convenient for researching genetic relationships between populations.

It is known that sand goby populations, which are important components of coastal biodiversity with their key roles in the food chain, are subject to many different selection pressures in the historical process of the Mediterranean Sea. Therefore, geographic variations between populations were emphasized (Gysels *et al.*, 2004*a*, 2004*b*; Huyse *et al.*, 2004; Larmuseau *et al.*, 2009; Mejri *et al.*, 2009, 2011; Boissin *et al.*, 2011; Tougard *et al.*, 2014). The present study aims to obtain information about the evolutionary mechanisms and historical processes

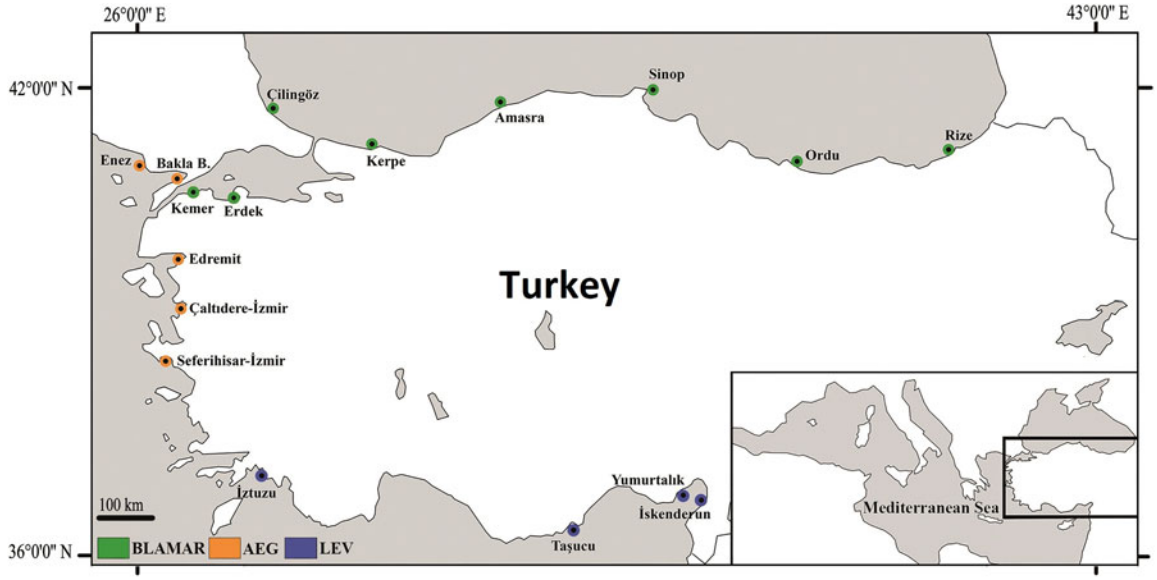

Fig. 1. Sampling locations of *P. marmoratus*. Each colour represents locations from different Seas. LEV, Levantine Sea; AEG, Aegean Sea; BLAMAR, Black Sea & Sea of Marmara.

that affected the *P. marmaratus* populations in the sub-basins in the north-eastern Mediterranean Sea by identifying intra- and inter-population variation, possible isolations and gene flow.

Materials and methods

Sampling

A total of 326 *P. marmoratus* specimens were sampled from 16 localities in the north-eastern Mediterranean (Turkish coasts of the Black Sea, Aegean Sea, Levantine Sea and Sea of Marmara) (Figure 1). Samplings and underwater observations were carried out in different seasons during a 3-year period from 2015–2018 with replications until 30 individuals were sampled from each sub-basin (Table 1). The specimens were caught from coastal shorelines at 0–3 m depth using hand nets in both scuba and free diving. All specimens were euthanized via an overdose anaesthetic (quinaldine). Pectoral fin clips of the specimens were fixed in 96% ethanol for genetic analysis.

DNA extraction, amplification and sequencing

Total genomic DNA was isolated from the fin clips using the PureLink Genomic DNA mini kit (Invitrogen) and GeneJET Genomic DNA Purification Kit (Thermo Scientific). Fragments of mitochondrial DNA (mtDNA) were PCR-amplified with universal mitochondrial cytochrome *c* oxidase subunit I (COI) FishF1 and FishF2 primers, which are described in Ward *et al.* (2005) and specific primers PomCB1F and GobCB2R designed for the amplification of the mitochondrial cytochrome *b* (cyt *b*) described in Tougard *et al.* (2014). The PCR thermal profile consisted of an initial step of 2 min at 95°C followed by 35 cycles of 30 s at 94°C, 30 s at 54°C, and 1 min at 72°C, followed in turn by 10 min at 72°C for COI, and DNA denaturation at 94°C for 5 min, followed by 35 cycles including a denaturation at 94°C for 45 s, an annealing at 50°C for 1 min, an extension at 72°C for 2 min, and a final extension at 72°C for 10 min for cyt *b*. Sequences produced by a private company (Macrogen Inc., Seoul, South Korea) were obtained for both strands to confirm polymorphic sites. They were aligned using MEGA v.7.0 (Kumar *et al.*, 2016).

Population structure and phylogeographic analyses

Sequences were aligned using CLUSTAL W (Thompson *et al.*, 1994) and manually edited in BIOEDIT 7.2.5. (Hall, 1999). Polymorphic sites (s), haplotype diversity (*h*), nucleotide diversity (π), and the genetic structures of the locations were estimated using DnaSP v.5.0 (Librado & Rozas, 2009). The analysis of molecular variance (ϕ_{st}) and estimates of the gene flow (N_em) (Hudson *et al.*, 1992) were calculated using Arlequin 3.5 (Excoffier & Lischer, 2010), and *P* values were obtained by permutating data 1000 times.

The phylogeographic analyses for haplotypes were conducted on each separate gene, using MEGA. *Pomatoschistus microps* (GenBank accession numbers KM077855 and HF969830) was used to root the tree. The best-fit models of nucleotide substitution for COI and cyt *b* were calculated by the Akaike and Bayesian Information Criteria (AIC and BIC) approaches. The model with the lowest BIC and AIC scores are considered to describe the substitution pattern the best. Maximum likelihood (ML) analysis was performed with the software package MEGA, using the Kimura two parameter (K2P) distance model and Tamura–Nei model for COI and cyt *b* analyses, respectively, which were chosen after running the 'Model Selection' tool in MEGA. A bootstrap test with 1000 replicates was performed to verify the robustness of the tree. A median-joining haplotype network was generated through PopART (Leigh & Bryant, 2015).

The samples which were clustered in a haplogroup, belonging to the same region, were subsequently used for analyses by means of a hierarchical analysis of molecular variance AMOVA (Excoffier *et al.*, 1992) using the software Arlequin 3.5 (Excoffier & Lischer, 2010). The historical demographic expansions were examined by the *D* test of Tajima, F_s test of Fu and Ramos-Onsins and Roza's R_2 (Tajima, 1989; Fu, 1997; Ramos-Onsins & Rozas, 2002). While significant negative *D* and F_s statistics can be interpreted as a sudden population expansion after a bottleneck event, positive values indicate either balancing selection or demographic stability. The R_2 test, suitable for small sample sizes, is expected to produce lower values under a recent severe population growth scenario (Ramos-Onsins & Rozas, 2002). Historical demographic expansions were also investigated by examination of frequency distributions of pairwise differences between sequences (mismatch distribution) (Rogers &

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Table 1. Geographic locations of the *P. marmoratus* sampling sites, codes and number of samples

Sub-basin/Sampling Site	Code	Number of samples
Southern Black Sea		
West		52
Kerpe	KRP	
Amasra	AMS	
Cilingoz	CLN	
Central		48
Sinop	SNP	
Ordu	ORD	
East		33
Rize	RZ	
Sea of Marmara		40
Erdek	ERD	
Kemer	KMR	
Eastern Aegean Sea		
North		47
Bakla B.	SRZ	
Enez	ENZ	
Edremit	EDR	
Central		40
Izmir (Caltidere)	IZM	
Northern Levantine Sea		
West		6
Iztuzu	IZT	
Central		30
Tasucu	TSC	
East		30
Yumurtalık	YUM	

Harpending, 1992). While the multimodal distributions indicate demographic stability, a unimodal distribution is consistent with sudden expansion (Slatkin & Hudson, 1991). Observed and expected distributions were compared with Harpending's (1994) sum of squared deviations (SSD), and with the raggedness index (r) which were implemented in Arlequin 3.5. Due to this, the non-significant values of SSD indicate that the data do not deviate from that expected under the model of expansion. Besides this, the populations in demographic equilibrium, i.e. stable populations, have a large raggedness index and the populations that underwent recent expansion have small raggedness indices (Harpending, 1994).

The time since possible population expansion (t) was calculated separately for COI and cyt b through the equation $\tau = 2ut$ (Rogers & Harpending, 1992), with u being the mutation rate of the sequence, calculated by $u = 2\mu\kappa$, where μ is the mutation rate per nucleotide and κ is the number of nucleotides. A mutation rate of 2–4% per nucleotide and per million years (Myr) was used for COI analysis (Brown *et al.*, 1979; Mejri *et al.*, 2009, 2011) and 1–2% was used for cyt b analysis (Gysels *et al.*, 2004*a*, 2004*b*). To calculate the number of generations since expansion, we used the generation time of two years for *P. marmoratus* (Miller, 1986).

Table 2. Genetic diversity of the COI and cyt b sequences of *P. marmoratus*

Locality	BLAMAR	AEG	LEV
COI			
N	130	84	66
N_h	30	26	24
h	0.762 ± 0.035	0.819 ± 0.039	0.889 ± 0.025
π	0.002 ± 0.0001	0.003 ± 0.0003	0.004 ± 0.0004
Cyt b			
N	127	32	62
N_h	21	1	12
h	0.802 ± 0.028	0	0.401 ± 0.080
π	0.005 ± 0.0002	0	0.001 ± 0.0005

Number of analysed sequences (N); number of haplotypes (N_h); Haplotype diversity (h); Nucleotide diversity (π). LEV (Levantine Sea), AEG (Aegean Sea), BLAMAR (Black Sea & Sea of Marmara).

Results

The abundance and prevalence of the Levantine Sea populations were found to be very low although the samplings were carried out in different seasons (with replications). Besides, these populations were observed only during the autumn (during the period of recruitment to stock). It should be noted that, during this period, we could observe just juvenile specimens, while adult individuals were very rarely observed. We think that the majority of individuals up to adult length are predated by lessepsians fishes in the area. However, it was found that the Aegean, Marmara and the Black Sea populations reached high abundance with uniform size classes if suitable habitats were found.

Genetic diversity

A 652 and 526 bp fragment of COI and cyt b gene were obtained for the 280 and 221 specimens of the marbled goby respectively, which were collected from the four sub-basins in the north-eastern Mediterranean Sea. The sequences were deposited in the GenBank under the accession numbers MT181761–MT181840 and MT181989–MT182021 for COI and cyt b, respecitvely. The COI sequences revealed 80 haplotypes, produced by the 105 variable sites, of which 69 were parsimony informative. A total of 74 variable sites and 59 parsimony informative sites produced 33 haplotypes for cyt b (Supplementary Table S1). All populations represented high values of haplotype diversity and low values for nucleotide diversity for each gene. The mean haplotype diversity (h) of the haplogroups varied from 0.762–0.889 and the mean nucleotide diversity (π) of the haplogroups varied from 0.002–0.004 for COI. The mean haplotype diversity (h) of the haplogroups varied from 0.401–0.802 and the mean nucleotide diversity (π) of the haplogroups varied from 0.001–0.005 for cyt b (Table 2).

Population structure and phylogeographic analyses

Phylogeographic analysis of the haplotypes, performed on COI, revealed a phylogeographic division between analysed regions (Figure 2). Due to this, three highly divergent haplogroups from different seas were detected: (1) Haplotypes of the Sea of Marmara and the Black Sea grouped and generated BLAMAR haplogroup; (2) Aegean Sea haplogroup (AEG) and (3) Levantine Sea haplogroup (LEV) (Figure 2). Cyt b sequences showed lower resolution among populations and two haplogroups

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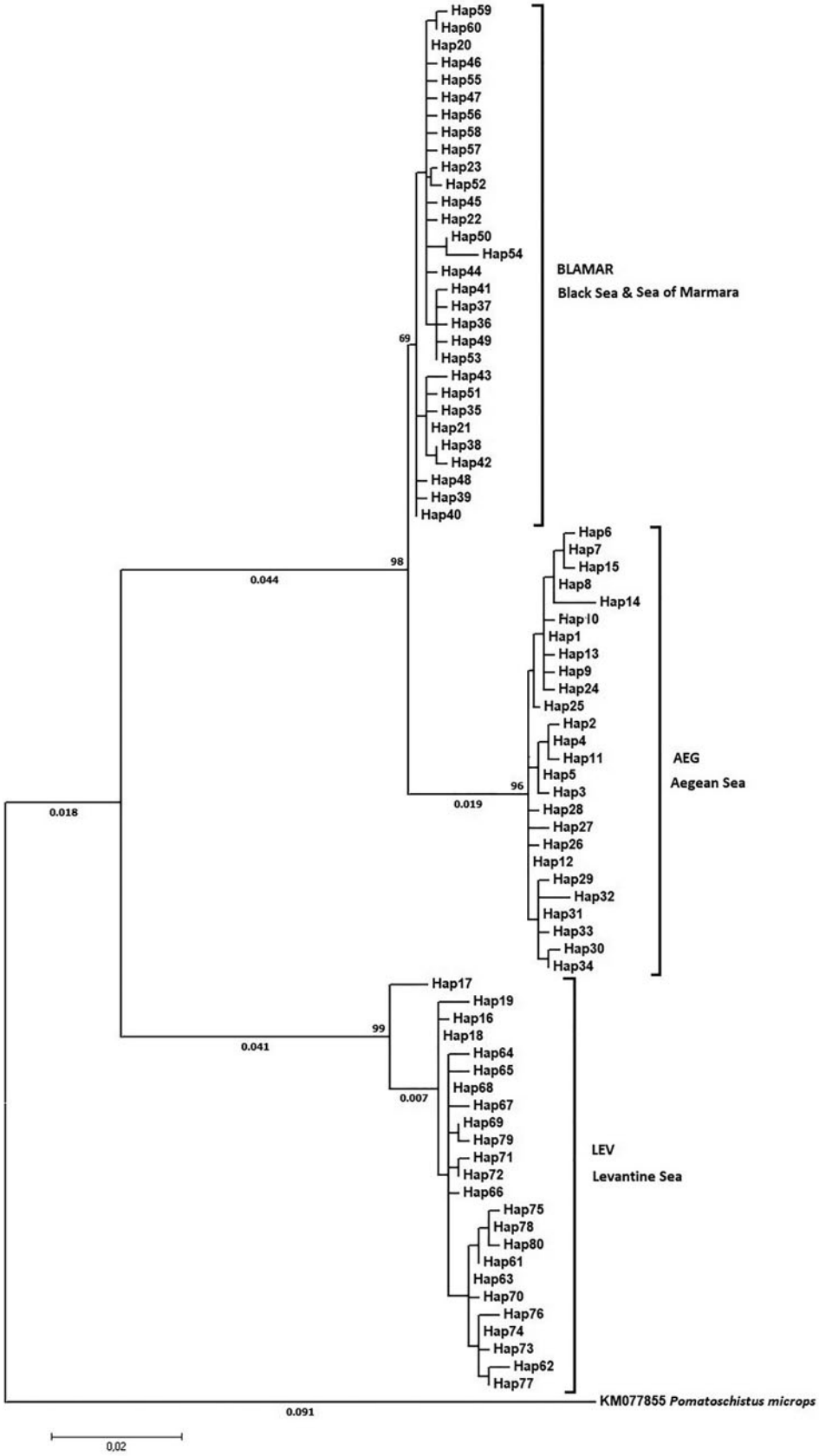

Fig. 2. ML tree constructed from COI sequences based on K2P model with *P. microps* (KM077855) as outgroup. Numbers at nodes are for ML bootstrap percentages (≥50%). The tree is drawn to scale, with branch lengths (under the branches) measured in the number of substitutions per site.

were detected: (1) Haplotypes of the Black Sea, Sea of Marmara and Aegean Sea grouped and generated one haplogroup AEGBLAMAR; (2) Levantine Sea haplogroup (LEV) (Figure 3).

The estimated differentiation times of the haplogroups (LEV/AEG/BLAMAR) from each other were calculated. According to the analysis of the LEV and AEGBLAMAR haplogroups, based on the cyt *b* gene, the estimated differentiation times were 19.3–9.65 myr BP. The estimated differentiation times of both AEG-LEV and LEV-BLAMAR populations were found to be 6.95–3.48 and 5.85–2.98 myr BP and 0.68–1.35 myr BP for the AEG-BLAMAR haplogroups, respectively, based on the COI gene, which is thought to evolve faster and accumulate mutations.

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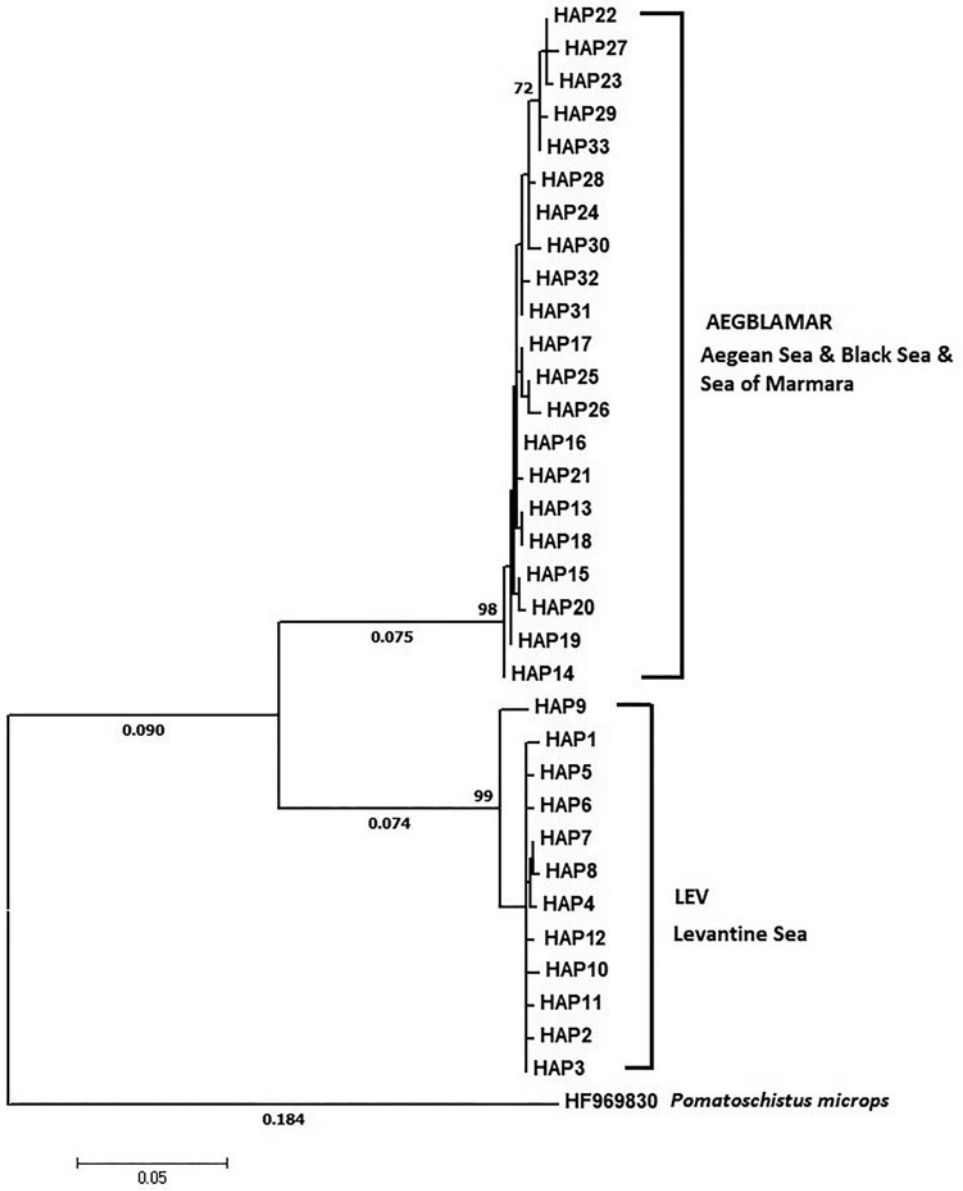

Fig. 3. ML tree constructed from cyt *b* sequences based on Tamura–Nei model with *P. microps* (HF969830) as outgroup. Numbers at nodes are for ML bootstrap percentages (≥50%). The tree is drawn to scale, with branch lengths (under the branches) measured in the number of substitutions per site.

The most recent differentiation (1.35–0.68 myr BP) was observed between BLAMAR and AEG groups (Table 3).

AMOVA revealed that most of the total molecular variance (93.40%) was attributed to regional differences among groups. In addition, 2.14% was apportioned among the population within the group, and 4.46% was found to be the individuals within the population for COI (Table 4). Two alternative groupings were tested with AMOVA for cyt *b* sequences to find the best fit for our data: I. Clustering samples according to three haplogroup results, [BLAMAR] *vs* [AEG] *vs* [LEV]; and II. Clustering the samples according to two haplogroup results, [AEGBLAMAR] *vs* [LEV]. The grouping of sites which maximizes $^{\phi}_{ct}$ was assumed to be the most probable geographic subdivision. Due to this, the first grouping showed the most probable geographic subdivision and AMOVA revealed that the total molecular variance (97.19%) can be found in regional differences among groups, while 1.83% was found among the population within the group and 0.99% individuals within the population for cyt *b* (Table 4).

Evolutionary relationships among haplotype sequences were also analysed by median-joining networks for both genes, and these networks also supported the existence of three haplogroups (BLAMAR, AEG and LEV) for COI, and two haplogroups (AEGBLAMAR and LEV) for cyt *b* which were in agreement with ML trees in the north-eastern basin of the Mediterranean Sea (Figure 4A, B). The haplotype networks (Figure 4) resulted in a star-like shape, suggesting past demographic expansions for LEV (for both genes) and AEG haplogroups (Slatkin & Hudson, 1991), which were also consistent with mismatch analysis (Figure 5). The demographic history of the clades was inferred from neutrality tests (Fu's F_s, Tajima's D statistics and Ramos-Onsins and Rozas R_2) and mismatch distributions (sum of squared deviations SSD and raggedness index r) (Figure 5). A unimodal distribution was observed for LEV clade suggesting sudden expansion for both COI and cyt *b* analyses but results for BLAMAR clade were more confusing. While the results of Ramos-Onsins and Rozas R_2, SSD and r analyses and a multimodal distribution indicated stable populations for BLAMAR clade, negative values of Fu's F_s and Tajima's D statistics were observed for this clade. This inconsistency between mismatch distributions and neutrality test was explained by statistical power of the tests in previous studies (Ramos-Onsins & Rozas, 2002; Tougard *et al.*, 2014). Due to this, BLAMAR clade is accepted as stable according to the results of Ramos-Onsins and Rozas R_2, SSD and r analyses and rejecting the population expansion model for both COI and cyt *b*. AEG clade produced significantly negative Fu's F_s and Tajima's D statistics and provided a unimodal

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Table 3. Estimated differentiation times of the haplogroups based on a molecular clock of between 2% (slow/below diagonal) and 4% (fast/above diagonal) for COI gene and 1% (slow/below diagonal) and 2% (fast/above diagonal) for cyt *b* gene, respectively

	LEV	AEG	BLAMAR
COI			
LEV	–	3.48	2.98
AEG	6.95	–	0.68
BLAMAR	5.85	1.35	–
	LEV	AEGBLAMAR	
Cyt *b*			
LEV	–	9.65	
AEGBLAMAR	19.3	–	

Table 4. AMOVA results for COI and cyt *b* sequences of *P. marmoratus*

	Source of variation	% of variation	ϕ-statistics
COI	Among groups	93.40	$\phi_{ct} = 0.934^*$
	Among populations within groups	2.14	$\phi_{sc} = 0.955^*$
	Within populations	4.46	$\phi_{st} = 0.324^*$
Cyt *b*	Among groups	97.19	$\phi_{ct} = 0.971^*$
	Among populations within groups	1.83	$\phi_{sc} = 0.649^*$
	Within populations	0.99	$\phi_{st} = 0.990^*$

Group 1 = BLAMAR (Black Sea and Sea of Marmara), Group 2 = AEG (Aegean Sea), Group 3 = LEV (Levantine Sea). *$P < 0.05$.

pairwise mismatch analysis for the COI gene, which indicates population expansion. In addition to all these, cyt *b* analysis did not detect any polymorphism for AEG clade. Due to this, the estimates regarding timing since expansion based on COI analysis ranged from 0.194–0.097 myr BP and 0.236–0.118 myr BP for LEV and AEG clades, respectively, while it ranged from 475–237 BP for LEV clade in cyt *b* analysis (Figure 5).

Gene flow N_em estimates and pairwise genetic distances between the clades of *P. marmoratus* were given in Table 5. The gene flow between the LEV and the other clades (BLAMAR and AEG) were found to be remarkably low, which is congruent with ϕ-statistics and indicated small number of migrants per generation ($N_em < 0.03$). Pairwise genetic distances indicated that the LEV clade was also significantly differentiated, which also supported the limited gene flow between LEV and other clades (Table 5).

Discussion

The understanding of population structure and the factors which affect it in the marine environment is important for conservation strategies for habitats, species and other conservation measures (Kelly & Palumbi, 2010). Different mechanisms may cause genetic differentiation between populations, such as vicariance processes caused by palaeo-climatic and geological events, oceanographic currents, habitat discontinuities, local adaptation, larval behaviour, dispersal capabilities of larvae and adults and even reproductive strategy (Marques *et al.*, 2006; Giovannotti *et al.*, 2009; Earl *et al.*, 2010; Riginos *et al.*, 2011; Schunter *et al.*, 2011; Gonzalez-Wangüemert & Perez-Ruzafa, 2012; Hirase *et al.*, 2012; Durand *et al.*, 2013; Portnoy *et al.*, 2013).

The modern Mediterranean Sea and the Indo-West Pacific Region evolved independently from each other when the Arabian and Anatolian plates collided (Rögl, 1999; Seidenkrantz *et al.*, 2000). The Mediterranean–Atlantic connection became constricted at the end of the Miocene after the isolation of Paratethys from the Mediterranean. A consequence of this was the desiccation of the Mediterranean, in an event known as the Messinian Salinity Crisis (MSC), which took place about 6.14–5.96 myr (Hsü *et al.*, 1973; Krijgsman *et al.*, 1999). The present distribution of *P. marmoratus* in the Eastern Mediterranean is thought to be the result of the isolation of Paratethys from the Mediterranean and MSC (Miller, 1990; Huyse *et al.*, 2004).

Estimated differentiation times (19.3–9.65 myr BP) of the LEV and AEGBLAMAR populations based on the cyt *b* gene coincide with the period in which Paratethys was first isolated from the Mediterranean Sea and, according to the results of COI analyses which are thought to evolve faster and accumulate mutations, the estimated differentiation time of both AEG-LEV and LEV-BLAMAR populations (6.95–3.48 and 5.85–2.98 myr BP, respectively) coincide with the opening of the Gibraltar Strait after MSC.

The impact of the salinity crisis and cyclic glacial ages with significant decreases in sea levels, which affected the origin of biodiversity in the Mediterranean Sea basin, have a significant impact on the populations of the gobies (Stefanni & Thorley, 2003; Gysels *et al.*, 2004*a*, 2004*b*; Huyse *et al.*, 2004; Larmuseau *et al.*, 2009; Mejri *et al.*, 2009, 2011; Boissin *et al.*, 2011). The sudden demographic expansion of populations with low genetic diversity and re-colonization of sand gobies have been described in many studies as the impact of the last glacial period which ended about 10 kyr BP (Hewitt, 1996, 1999, 2000; Taberlet *et al.*, 1998; Stefanni & Thorley, 2003; Gysels *et al.*, 2004*a*, 2004*b*; Huyse *et al.*, 2004). The sea level of Anatolian coasts had substantial changes during the last glacial period as did the whole Mediterranean basin (Lambeck, 1995; Özdoğan, 1997; Lambeck & Purcell, 2005; Gökaşan *et al.*, 2010). It is known that the sea level was at least 100–165 m lower in general during the last glacial maximum (van Andel & Shackleton, 1982; Lambeck & Purcell, 2005) and the borders of the territorial areas extending into the Aegean Sea were wider because of the sea level drops (Lambeck, 1995; Özdoğan, 1997; Lambeck & Purcell, 2005). The lacustrine conditions occurred intermittently both in the Sea of Marmara and the Black Sea, but analysis of the deep sea cores indicates that the Sea of Marmara had similar conditions to that of the present Black Sea during the last glacial period (Meriç, 1990; Özdoğan, 1997). The level of the Sea of Marmara was ~45–50 m above the level of the Aegean Sea both during the last glacial period and also possibly the previous glacial period (Aksu *et al.*, 1999). The Turkish Straits System (Dardanelles and Bosphorus) acted as rivers carrying meltwater from northern Europe into the Black Sea, and then through the Marmara Sea to the Aegean Sea and the eastern Mediterranean Sea during the glacial to interglacial transitions (Stanley & Blanpied, 1980; Aksu *et al.*, 1999). These interactions between the Black Sea, Sea of Marmara and the Aegean Sea could prevent populations becoming completely isolated but also could restrict an effective gene flow. Due to this, the estimated differentiation time of BLAMAR and AEG groups (1.35–0.68 myr BP) can be linked with sea level changes in the last glacial period. Our results also underlined that demographic analyses as well as high haplotype diversity indicate a recent and rapid population divergence during the last glacial period due to τ values of the LEV and AEG groups (0.194–0.097 myr and 0.236–0.118 myr, respectively).

The potential distribution of planktonic larvae and eggs and the lack of geographic barriers among populations contribute to

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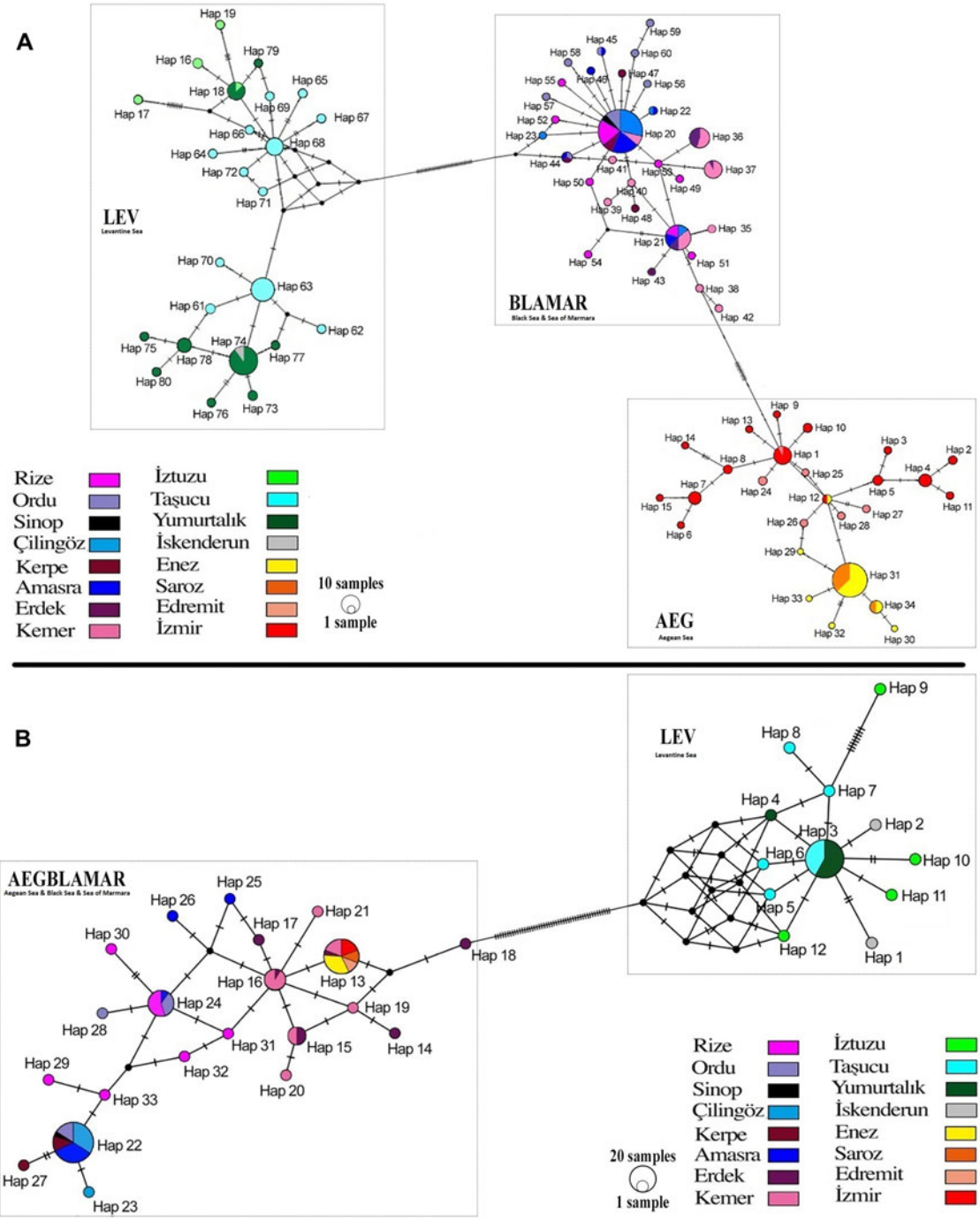

Fig. 4. Median joining network (A) for COI and (B) for cyt *b* genes. The network is connecting all sequences through putative mutational step and separating the three major clades for COI and two major clades for cyt *b* congruent with the ML tree subdivision. The number of samples is reflected by the size of the circles and each line segment between haplotypes represents a single mutation. Inferred intermediate haplotypes that were not sampled are shown as black dots.

continuous gene flow and low genetic differentiation in marine ecosystems (McCusker & Bentzen, 2010; Wang *et al.*, 2011). The continuous gene flow between populations was detected in various studies that were conducted in the sub-basins of the Eastern Mediterranean, although there were biotic and abiotic factors that resulted in the populations' differentiations (Bektaş & Beldüz, 2008; Keskin & Atar, 2012; Turan *et al.*, 2016; Bektaş *et al.*, 2018). However, many studies have shown that intra-specific differences in many Mediterranean species are associated with the present-day physical barriers that may affect gene flow (Borsa *et al.*, 1997; Mejri *et al.*, 2009, 2011). Analyses of both gene regions indicated that the population groups had high haplotype diversity, while the nucleotide diversity was quite low in the present study. Moreover, the Levantine population is genetically different from all other populations and gene flow was determined to be very limited. When the results of the analyses of the neutrality and mismatch distribution that were applied to the haplogroups are evaluated as a whole, it was found that the haplogroup covering the Black Sea and Sea of Marmara was in equilibrium. However, the haplogroup covering the Levantine Sea coasts was under the sudden demographic expansion model following a population bottleneck, which was in agreement with a star-shaped haplotype network.

It was concluded that the limited gene exchange between the Levantine population and other populations is still affected by the prevailing hydrographic conditions. Bakun & Agostini (2001) reported that the eastern Aegean and north-western Levantine shores (approximately the Teke peninsula) are influenced by strong winds blowing from the land to the sea, and

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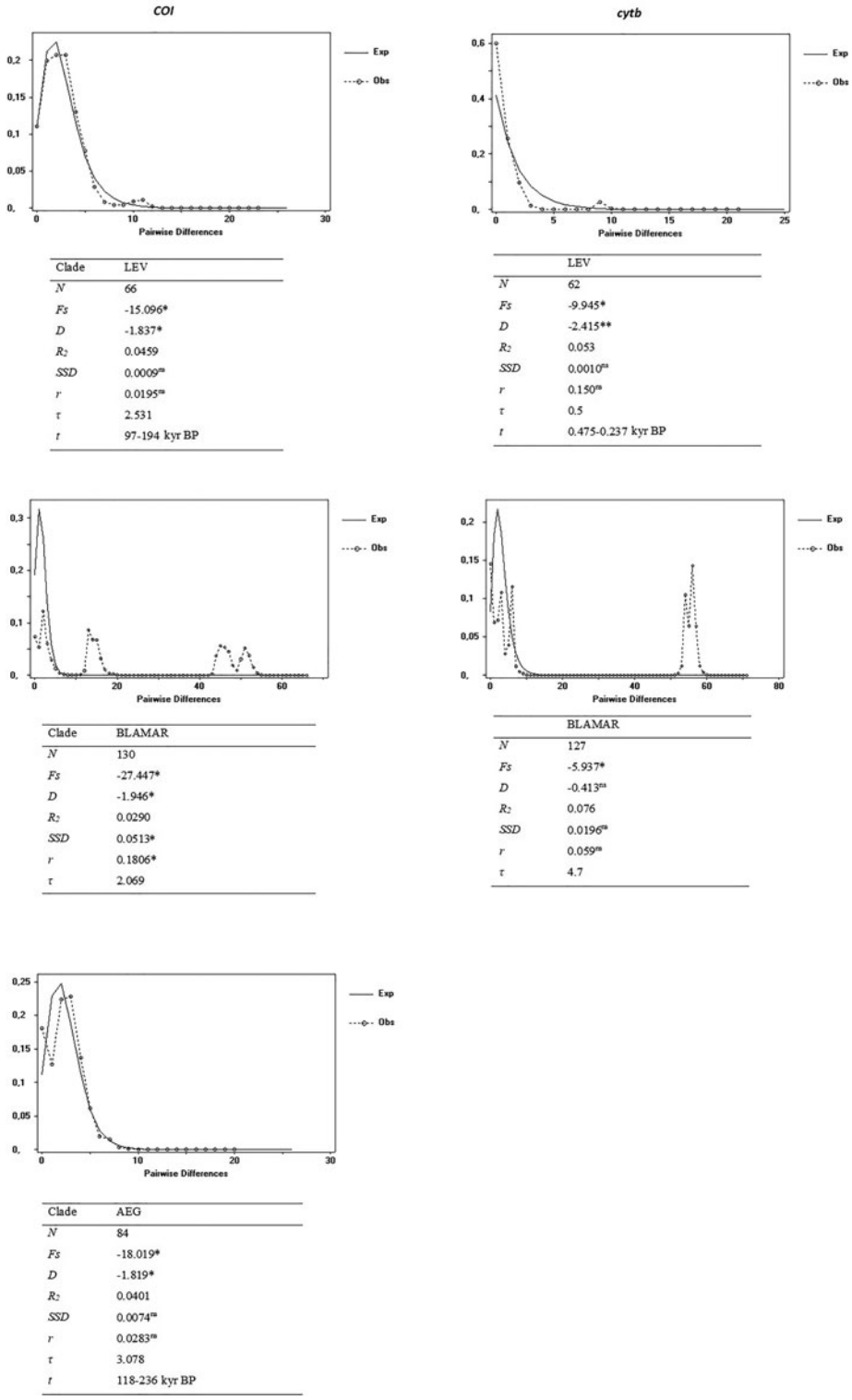

Clade	LEV
N	66
Fs	-15.096*
D	-1.837*
R_2	0.0459
SSD	0.0009^{ns}
r	0.0195^{ns}
τ	2.531
t	97-194 kyr BP

	LEV
N	62
Fs	-9.945*
D	-2.415**
R_2	0.053
SSD	0.0010^{ns}
r	0.150^{ns}
τ	0.5
t	0.475-0.237 kyr BP

Clade	BLAMAR
N	130
Fs	-27.447*
D	-1.946*
R_2	0.0290
SSD	0.0513*
r	0.1806*
τ	2.069

	BLAMAR
N	127
Fs	-5.937*
D	-0.413^{ns}
R_2	0.076
SSD	0.0196^{ns}
r	0.059^{ns}
τ	4.7

Clade	AEG
N	84
Fs	-18.019*
D	-1.819*
R_2	0.0401
SSD	0.0074^{ns}
r	0.0283^{ns}
τ	3.078
t	118-236 kyr BP

Fig. 5. Mismatch distribution analysis for COI and cyt *b* genes with neutrality tests and demographic indices for each *Pomatoschistus marmoratus* clade. Fu's test of neutrality (F_s), Tajima's *D* neutrality test (*D*), statistics of Ramos-Onsins and Rozas (R_2), sum of squared deviations (SSD), raggedness index (*r*), the time since possible population expansion (*t*). The time since possible population expansion (*t*) was expressed in 10^{-3} years before present (kyr BP) (*$P < 0.05$, **$P < 0.01$, not significant ns). LEV, Levantine Sea; AEG, Aegean Sea; BLAMAR, Black Sea & Sea of Marmara.

consequently the coastal surface water movements are directed towards the open sea by the Ekman transport and the Coriolis force. Researchers have stated that this transport is further strengthened in spring and summer (this corresponds to the spawning season and larval stage of *P. marmoratus*; Mazzoldi & Rasotto, 2001) and consequently strong upwelling areas are formed in the Aegean and north-western Levantine coasts. This situation has also been observed by authors during fieldwork. For these reasons, it is concluded that the limited gene flow between the Levantine population and other populations is affected by the transport from the coast to the open sea on the eastern Aegean Sea and the northern Levantine Sea.

The entire life cycle, including the reproduction of *P. marmoratus*, takes place in shallow coastal habitats (generally 0–3 m) (Miller, 1986) and this transport is thought to limit the gene flow by preventing the chance of survival in deep-water habitats during the transition phase of the larva to benthic form. Drost *et al.* (2015) observed a similar situation in the gobies distributed in the South African coast. The researchers stated that the prevailing winds on the coasts of South Africa are directed offshore, and the larvae move away from the coastal waters due to wind-driven upwelling and Ekman transport. This situation limits the gene flow by decreasing the rate of recruitment success. Besides this, the offshore wind-driven surface flows and Ekman transport

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Table 5. Gene flow N_em among *Pomatoschistus* clades (above diagonal) with pairwise genetic distances (K2P for COI, Tamura–Nei for cyt *b*) from haplotype frequencies (below diagonal)

	LEV	AEG	BLAMAR
COI			
LEV	–	0.013	0.012
AEG	0.139	–	0.041
BLAMAR	0.117	0.027	–
Cyt *b*			
LEV	–	0.002	0.008
AEG	–	–	0.163
BLAMAR	0.193	–	–

LEV, Levantine Sea; AEG, Aegean Sea; BLAMAR, Black Sea & Sea of Marmara.

will keep planktonic larvae away from the protected coastal waters, and reduce the possibility of reaching suitable habitat for settlement (Lutjeharms, 2006).

Coastal surface currents of the northern Levantine Sea are moving westward and change direction in front of Fethiye and Rhodes Island. The main currents in the Aegean Sea are generally south-oriented and have been reported to be directed towards the Ionian Sea before reaching the Levantine Sea (Fernández *et al.*, 2005; Hamad *et al.*, 2006; Patarnello *et al.*, 2007). Taking these into consideration, there are no main coastal surface currents extending from the Levantine Sea towards the Aegean Sea, and this phenomenon creates an effective hydrographic barrier that limits gene flow. In addition, it was observed in our field studies that the Datça peninsula, which is located near the intersection of the southern Aegean and western Levantine coasts, limits gene flow, while deep rocky habitats frequently found along the coastline of the peninsula interrupt the habitat connectivity of *P. marmoratus*. Similar habitat fragmentations such as steep cliff structures and the lack of shallow sandy areas were observed between Fethiye-Antalya and Alanya-Tasucu. However, although suitable habitats have been observed from the Tasucu coasts to Iskenderun Bay, it has been observed that the populations of *P. marmoratus* are either undetectable or are not abundant in these habitats. It was concluded that this might be due to the intense pressure of lessepsian fishes. It is seen that all these factors limit gene flow between the Aegean Sea and Levantine Sea populations and thus negatively affect populations by decreasing the intra and inter-population genetic diversity.

It was understood that conservation of biological diversity alone is not sufficient, but the levels of genetic variation of species and populations, as well as the variation processes of the populations, must also be known. Based on the results presented in this paper, it was concluded that hydrographic factors directly affect gene flow at larval stages and habitat fragmentations limit the gene flow of the poor-swimmer goby species in the adult period. Besides this, the ongoing genetic differentiation process in the Levantine populations caused by palaeo-geographic and palaeo-climatic conditions is still affected by today's hydrographic factors. Moreover, environmental pressures such as lessepsian invasion in the area, pollution and increasing seawater temperature make these populations fragile by decreasing the abundance of the populations.

Supplementary material. The supplementary material for this article can be found at https://doi.org/10.1017/S0025315421000199

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Data availability. The gene sequences are available in GenBank and can be found under the accession numbers MT181761–MT181840 and MT181989–MT182021. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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