

Genetic structure and demographic history of whiting *Merlangius merlangus* (Linnaeus, 1758) populations distributed in Turkey inferred from variation in mitochondrial DNA sequences

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Abstract: The genetic diversity, structure, and demographic history of the economically important and overfished Gadidae species *Merlangius merlangus* were investigated using the non-coding mitochondrial Control Region (CR) from five different sites in the Sea of Marmara and the Black Sea in Turkey. The populations of *M. merlangus* were found to be genetically diverse, with 14 haplotypes and 15 polymorphic regions. The overall haploid diversity was 0.910 ± 0.024 , and the nucleotide diversity was 0.003 ± 0.0003 . Genetic distances between populations varied between 0.13% and 8.02%, while genetic distances within *M. merlangus* populations varied between 0.09% and 0.42%. Principle Coordinates Analysis showed that Marmara, Black Sea, and Karadeniz Ereğli populations were clearly separated. Pairwise F_{ST} values varied from 0.12 to 0.69, highlighting high genetic variation among populations. The Black Sea and Marmara lineages of *M. merlangus* diverged from the North Sea lineage 1.65 (1.08-2.29) mya, whereas the separation between the Atlantic lineage occurred about 0.84 (0.51-1.2) mya. The recent expansion of the whiting population was identified through neutrality tests and mismatch distribution analyses. This study provides important insight into the genetic structure, conservation, and management of this species.

Keywords: population genetics; Black Sea; D-loop; genetic diversity; population expansion

Sažetak: GENETSKA STRUKTURA I DEMOGRAFSKA PROŠLOST POPULACIJA PIŠMOLJA *MERLANGIUS MERLANGUS* (LINNAEUS, 1758) S PODRUČJA TURSKE ODREĐENE NA TEMELJU VARIJACIJA MITOHONDRIJSKIH DNA SEKVENCI. Genetska raznolikost, struktura i demografska prošlost ekonomski važne i prelovom ugrožene vrste pišmolja *Merlangius merlangus* istraživane su korištenjem nekodirajuće mitohondrijske kontrolne regije (CR) s pet različitih lokaliteta u Mramornom i Crnom moru u Turskoj. Populacije *M. merlangus* pokazale su genetičku raznolikost s 14 haplotipova i 15 polimorfnih regija. Ukupna haplotipna raznolikost iznosila je 0.910 ± 0.024 , a nukleotidna raznolikost 0.003 ± 0.0003 . Genetske udaljenosti između populacija varirale su između 0.13% i 8.02%, dok su genetske udaljenosti unutar populacija *M. merlangus* varirale između 0.09% i 0.42%. Analiza glavnih koordinata (PCoA) pokazala je jasnu razdvojenost populacija iz Mramornog i Crnog mora te Karadeniz Ereğli područja. Uparene F_{st} vrijednosti varirale su od 0.12 do 0.69, ukazujući na visoku genetičku varijabilnost između populacija. Genealoške linije pišmolja iz Mramornog i Crnog mora odvojile su se od linija iz Sjevernog mora prije 1.65 (1.08-2.29) milijuna godina, dok se odvajanje od atlantske linije dogodilo prije oko 0.84 (0.51-1.2) milijuna godina. Nedavno širenje populacije pišmolja utvrđeno je putem testova neutralnosti i analize neusklađenosti distribucije. Ovo istraživanje donosi bitne spoznaje o genetskoj strukturi, očuvanju i upravljanju ovom vrstom.

Ključne riječi: populacijska genetika; Crno more; D-petlja; genetska raznolikost; ekspanzija populacije

INTRODUCTION

The excessive use of fish populations for commercial purposes, overfishing, and other activities such as pollution and habitat degradation are leading to the loss of fish genetic resources and even extinction of fish populations (Çiftci and Okumuş, 2002). The situation for sturgeons is a good example of the interaction of multiple factors threatening the continued survival of species, as all species of sturgeons are now listed by the Convention on the International Trade in Endangered Species of Wild Flora and Fauna (CITES), with most of

them on Appendix II (Powles *et al.*, 2000). According to FAO statistics, the annual world catches of whiting *Merlangius merlangus* (Linnaeus, 1758) increased from 73400 tons in 1950 to 283616 tons in 1976, followed by a gradual decline (FAO, 2023). In 2021, the amount of whiting caught worldwide reached 45248.37 tons. In Turkish seas, the highest recorded catch was in 1988 with 30488 tons, but it significantly decreased to 10379.9 tons in 2021 (FAO, 2023). The International Union for Conservation of Nature (IUCN) has identified commercial passive fishing methods (i.e. netting,

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long lining) and active fishing techniques (i.e. demersal and pelagic trawling) as major threats to commercially important whiting populations in the Mediterranean and Black Seas. Currently, whiting is listed as 'Least Concern' (Nedreaas *et al.*, 2014).

Population genetics studies aim to elucidate the genetic diversity within current populations and the alterations in genotype and allele frequencies over time (Keats and Sherman, 2014). Increased homozygosity, genetic drift, and population subdivision can lead to a loss of genetic diversity, which could further threaten small, isolated populations (Ferguson *et al.*, 1995; Jamieson *et al.*, 2008). Reduced diversity could compromise the adaptability of many species when confronted with emerging challenges such as climate change and new diseases (Minter *et al.*, 2021). To preserve fish populations and secure their long-term benefits, two approaches are recommended: the preservation of the gene pool and the maintenance of genetic diversity over time (Çiftci and Okumuş, 2002). Molecular genetics research utilizing advances in technology, such as DNA sequencing, Single Nucleotide Polymorphism (SNP) analysis, and Next-Generation Sequencing (NGS), is an efficient method for assessing the level of variation within and between populations, among species, and within species. It also aids in determining gene flow and migration between populations (Okumuş and Çiftci, 2003). Genetic studies are being conducted on endemic and endangered species of commercial importance in many countries with objectives to preserve their genetic resources and develop conservation strategies for their future sustainability (Carlsson *et al.*, 2004; Corral-Lou *et al.*, 2021; Rossi *et al.*, 2021). Limited population

genetics studies have been conducted in Turkey. However, such studies represent an important milestone in achieving sustainable, long-term fisheries management.

The whiting *Merlangius merlangus* (Linnaeus, 1758) is distributed along the coasts of the Sea of Marmara and Black Sea and the Mediterranean in Europe (Akşiray, 1987). Although there are studies in the literature showing the distribution of this species in the Adriatic Sea (Svetovidov, 1986), a study by Milić and Kraljević (2011) drew attention to the morphological differences related to the eastern part of the northern Adriatic. Furthermore, Milić and Kraljević (2011) identified differences in meristic and morphometric characteristics among whiting populations in the Atlantic Ocean, Black Sea, and Adriatic and emphasized that the taxonomic status of *M. merlangus* species is controversial and further phylogenetic studies are needed. To date, there has been a limited number of investigations focused on the whiting population within Turkey. Using Random Amplified Polymorphic DNA (RAPD) analysis, Bektaş and Beldüz (2007) detected weak genetic differentiation of whiting (*Merlangius merlangus euxinus*) populations sampled along the Black Sea coast of Turkey. Şalcıoğlu *et al.* (2020) examined the taxonomic and phylogenetic differences between the two proposed subspecies of *Merlangius merlangus* - *M. m. merlangus* and *M. m. euxinus* - by analyzing the mitochondrial and nuclear gene regions. Limitation in genetic resolution and/or the possibility of gene flow between the two subspecies have been proposed, leading to a lack of clear genetic differentiation. In addition, Tayhan (2014) studied the benthic distribution of whiting populations in the Central and Eastern Black Sea, examining their

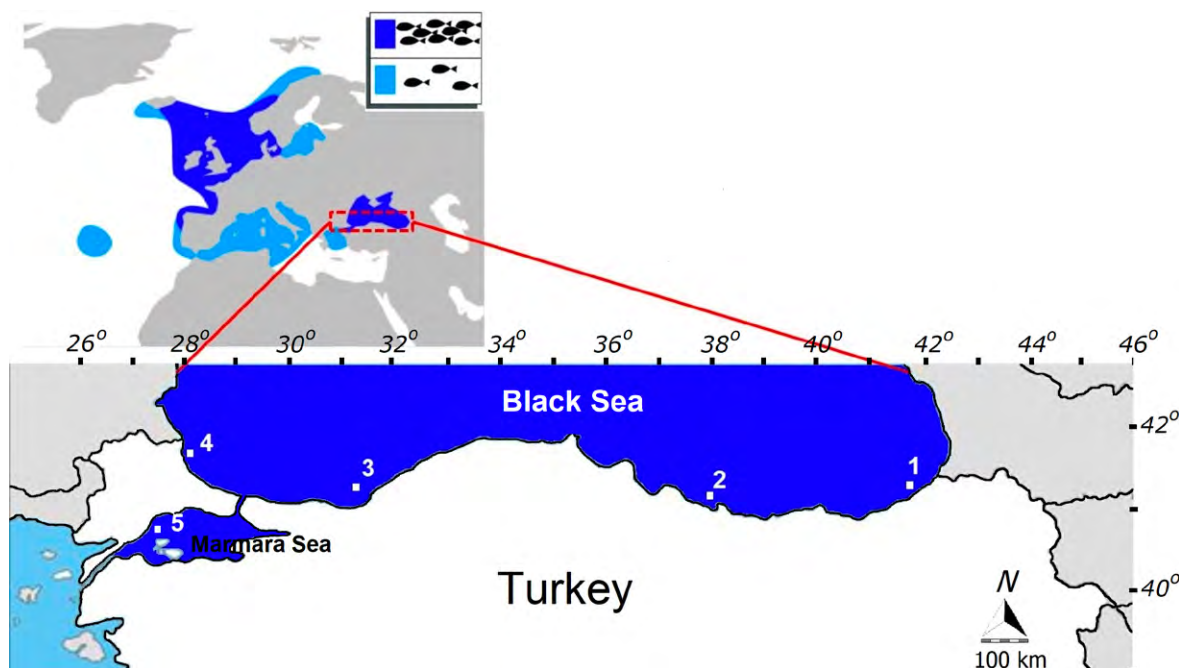


Fig. 1. Sampling localities for *Merlangius merlangus* populations in the Black Sea and the Sea of Marmara (1. Hopa; 2. Ordu; 3. Karadeniz Ereğli; 4. Iğneada; 5. Marmaraereğlisi). Map of the distribution of whiting (*M. merlangus*) modified from Wikimedia commons (2020).

Table 1. Sampling locations and molecular diversity indices for *Merlangius merlangus*.

ID	Sampling locations	Geographic location	Latitude/Longitude	N	h	S	Hd ± s.d.	π ± s.d.
HPA	Hopa	East Black Sea	41°23'59.7"N/ 41°23'58.4"E	7	5	8	0.857±0.137	0.003±0.0010
ORD	Ordu	Central Black Sea	41°04'14.8"N/ 37°47'22.4"E	8	3	2	0.714±0.123	0.001±0.0003
KDE	Karadeniz Ereğli	West Black Sea	41°14'40.4"N/ 31°23'07.9"E	7	3	3	0.667±0.160	0.002±0.0005
IGN	İğneada	West Black Sea	41°50'59.5"N/ 28°00'01.8"E	8	4	4	0.821±0.101	0.002±0.0005
MME	Marmaraereğlisi	Marmara Sea	40°57'07.5"N/ 27°55'01.2"E	8	3	2	0.679±0.122	0.001±0.0002
TOTAL				38	14	15	0.910±0.024	0.003±0.0004

Hd, haplotype diversity; s.d., standard deviation; N, samples size; h, number of haplotypes; S, Number of variable sites; π , nucleotide diversity.

morphologies and genetic characteristics through the analysis of otolith shape, the mitochondrial partial Cyt b gene, and microsatellite loci. Significant genetic differentiation at the nuclear level was recorded for two localities represented by a total of 60 samples. The study concluded that enhancing sample sizes on a broader geographical scale would lead to a better understanding of population dynamics and connectivity, facilitating more reliable stock management of whiting in the Black Sea.

Therefore, in support of the conservation of genetic resources and the investigation of population genetic connectivity, the present study aims to assess the genetic diversity, genetic structure, and demographic history of whiting populations in Turkish waters using mtDNA regions.

MATERIALS AND METHODS

Sample collection

The study covered the Eastern, Central, and Western Black Sea and Marmara Sea, where the distribution of fishing grounds is shown in Fig. 1. Sampling locations were selected mainly based on intensive fishing activity and geographically divided localities (Table 1). The fish material for the study was obtained from commercial fishing boats, especially those targeting whiting, between July 2019 and January 2020. The locations where the samples were taken, their coordinates, and the number of samples taken are given in Table 1. During the sampling activity, a 2-3 cm² piece of the caudal fin tissue of *M. merlangus* was clipped and stored in 95% ethanol for subsequent genetic analysis.

DNA extractions and PCR amplification

Total genomic DNA from fins (n=38) was extracted using the PureLink® Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocol. DNA qualities were checked on an agarose gel. The primers CR2F (5'- TCC CAC CAC TAG CTC CCA AAG C -3') and CR2R (5'- CGG GAC TTT CTA GGG CCC ATC CT -3') were employed to amplify the mtDNA D-loop Control Region (CR) (Gür, 2023). PCR amplification was carried out using

a Thermal Cycler (Techne, TC-Plus, Staffordshire, UK) with a final volume of 25 μ L, comprising 12.5 μ L of 2X GoTaq® Colorless Master Mix (Promega, Madison, USA), 0.4 pmol of each primer, approximately 1 μ g of genomic DNA, and nuclease-free water to attain the ultimate reaction volume. The thermal cycling procedure consisted of an initial denaturation stage at 95°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 53°C for 1 min, and extension at 72°C for 2 min, concluding with a final elongation at 72°C for 10 min, and eventually cooling down to 4°C. The quality and size of PCR products were visualized on a 1.0% agarose gel stained with ethidium bromide (1 mg/100 ml) using a gel documentation system (Vilber Lourmat, Marne La Vallee, France). Product purification and sequencing were performed by Macrogen Inc (Amsterdam, Netherlands) on an ABI 3730 automatic sequencer.

Data analysis

Haplotypes and genetic diversity

Sequence alignment was performed using the Clustal W algorithm (Thompson *et al.*, 1994) implemented in Bioedit 7.2.5 (Hall, 1999). Data set was inspected for genetic diversity indices, including nucleotide composition, nucleotide variable sites, number of polymorphic sites (S), parsimony informative sites, number of haplotypes (h), haplotype distributions, transitions and transversions, nucleotide diversity (π ; Lynch and Crease, 1990) and haplotype diversity (Hd; Nei, 1987) in DnaSP v6.0 (Rozas *et al.*, 2017). All sequences were uploaded to the GenBank database via the Submission Portal (accession numbers: OQ981486 - OQ981523 for D-loop; Sup. Table 1). The Kimura Two-Parameter (K2P) model in MEGA X (Kumar *et al.*, 2018) was used to calculate the pairwise genetic distances among various populations.

Phylogenetic analyses and haplotype network analysis

Phylogenetic analyses were conducted on 781 base pairs (bp) aligned nucleotides containing polymorphic regions for the mitochondrial D-loop control region.

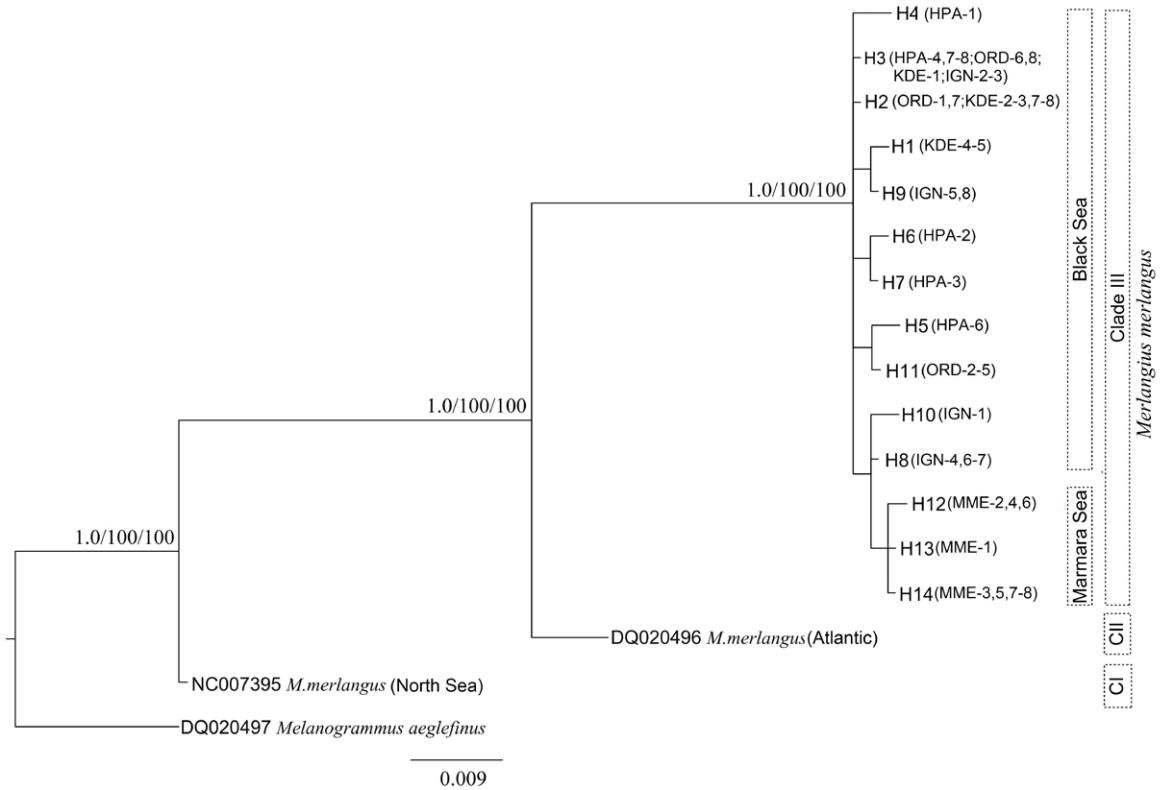


Fig. 2. Phylogenetic tree of *Merlangius merlangus* haplotypes based on mtDNA D-loop control region sequences. BI, ML and MP yielded identical topologies, so only the Bayesian tree is shown. Posterior probability, ML and MP bootstrap values are indicated near the branches.

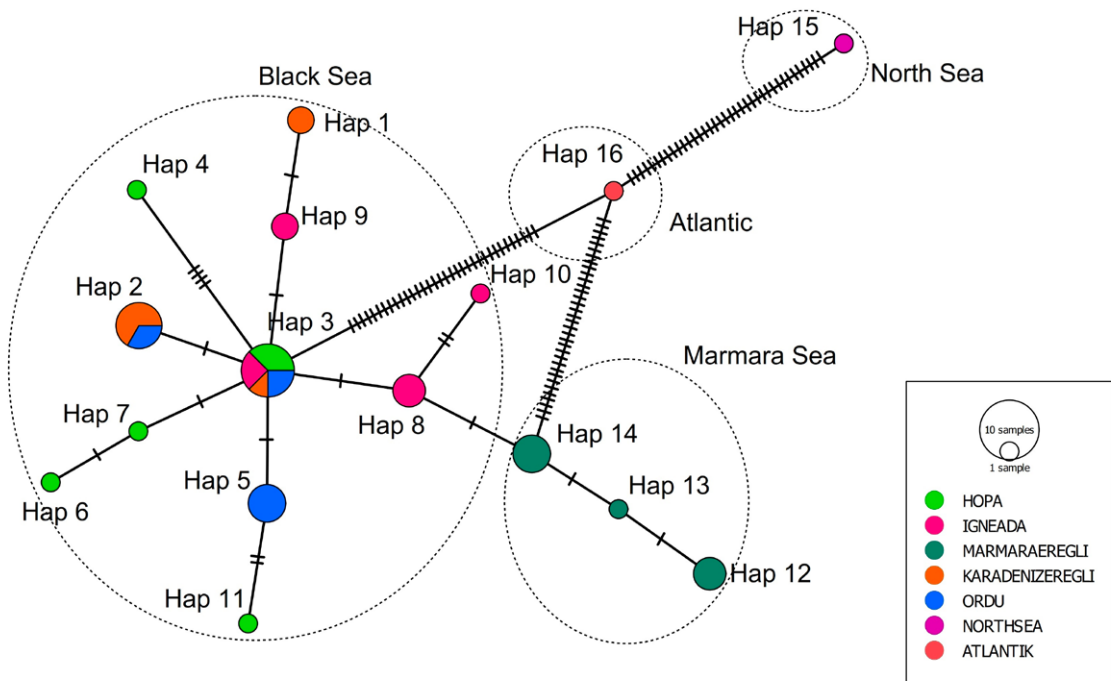


Fig. 3. Minimum spanning haplotype network based on mtDNA D-loop control region haplotypes. The numbers and each circle represent unique haplotypes; the circle size is proportional to their frequency, and colors indicate populations; short lines in branches represent the number of mutation steps.

Phylogenetic trees of D-loop haplotypes were constructed using Bayesian inference (BI), Maximum likelihood (ML), and Maximum parsimony (MP) methods. jModeltest ver. 0.1.1 (Posada, 2008) was used to statistically select the most suitable nucleotide substitution model for the DNA dataset according to the Bayesian Information Criterion (BIC) (Schwarz, 1978) and the Akaike Information Criterion (AIC) (Akaike, 1974), suggesting the TPM3uf+G model as the best-fit model for the dataset. The ML analysis of the D-loop sequence dataset was performed under the most suitable model using PhyML version 3.0 (Guindon and Gascuel, 2003) with 1000 bootstrap replicates. The BI analysis used four independent Markov Chain Monte Carlo (MCMC) with 1,000,000 generations in MrBayes 3.2 (Ronquist *et al.*, 2012), with trees corresponding to the 25% burn-in value discarded. *Melanogrammus aeglefinus* (Accession No. DQ020497) was used as outgroup species. Phylogenetic trees were created in PAUP 4.0b10 (Swofford, 1998) and FigTree v.1.4.4 (Rambaut, 2018). Minimum spanning network was constructed in PopART software (Leigh and Bryant, 2015).

Population genetic structure and geographical patterns

Principal coordinate analysis (PCoA) based on covariance a matrix of genetic distances was performed using GenALEX 6.5 (Peakall and Smouse, 2012). The distribution of genetic variation within and among populations was assessed using AMOVA in Arlequin 3.5 software (Excoffier and Lischer, 2010). Pairwise F_{ST} , the mean gene flow and the absolute number of migrating individuals between populations ($M = Nm$, for haploid data) were calculated using the Arlequin 3.5 with 1000 permutations applied. Correlation between genetic and geographic distances among populations was evaluated using the MANTEL test (Mantel, 1967) in GenALEX 6.5, with 10,000 permutations applied. The matrix of geographic distance (km) between sampling locations was created using Google Earth, version 7.1.7.2606, based on the distance along the coastline between sampled locations.

Divergence time estimates

Divergence times were calculated using Bayesian MCMC analysis in BEAST 2.6.2 (Bouckaert *et al.*, 2019). A strict clock was employed, assuming molecular evolutionary rates that were uncorrelated but lognormally distributed (Drummond *et al.*, 2006). We incorporated two calibration points from published Gadidae phylogenies into our own phylogenetic tree: the estimated divergence time of *Trisopterus luscus* from other gadid species, ranging from 17 million years (my) with a confidence interval of 10-24 my, and 7 my with a confidence interval of 5-9 my, along with the divergence time of *M. merlangus* from *Gadus* species as indicated by Owens (2015). The “Calibrated Yule model” was used as it better represents the species-level phylogenetic processes for evolutionary rates. An

MCMC analysis was conducted, sampling every 2000 generations for 20,000,000 generations. Distributions for different parameters sampled by the Markov chain were examined using Tracer version 1.7.1 (Rambaut *et al.*, 2018) with an effective sample size (ESS) of 200 used for all parameters, and a maximum clade credibility tree was generated with TreeAnnotator version 2.6.0 (Bouckaert *et al.*, 2019) using a 10% burn-in. Finally, the tree was visualized using FigTree v.1.4.4 (Rambaut, 2018).

Demographic history and selective neutrality

Three methods were employed to monitor the demographic history of three sub-groups detected with PCoA. To estimate the demographic model of *M. merlangus*, neutral tests including Fu’s F_s (Fu, 1997) and Tajima’s D (Tajima, 1989) tests were used, as well as a mismatch distribution analysis with 10,000 permutations in Arlequin 3.5. Additionally, Arlequin 3.5 was used to calculate the sum of squared deviations (SSD) between the expected and observed mismatch distributions, as well as Harpending’s raggedness index (Harpending, 1994). The demographic expansion factor τ with a 95% confidence interval (CI) was also calculated. The expansion time (t) was estimated based on the equation $t = \tau/2u$ (Rogers and Harpending, 1992), where u is the mutation rate per generation for the DNA sequence studied and is calculated as $u = \mu k$. In this context, μ represents the mutation rate per nucleotide, while k refers to the number of nucleotides present in the sequence. A mutation rate of 3-10% per million years previously applied to the D-loop control region of marine fish was used (Dowling *et al.*, 2002). Graphs for the mismatch distribution were generated using DnaSP v6.0 (Rozas *et al.*, 2017). Finally, changes in the relative population size over time were estimated using Bayesian skyline plot (BSP) analysis (Drummond *et al.*, 2005) implemented in BEAST 2.6.2 (Bouckaert *et al.*, 2019).

RESULTS

Genetic diversity

Sequences of 781 bp of the mitochondrial D-loop gene were obtained for 38 individuals of *M. merlangus* species from five localities in the Sea of Marmara and Black Sea. With 766 fixed characters (98.1%) and 15 polymorphic nucleotide positions, nine were parsimony informative. A total of 14 haplotypes were determined. The most common haplotype Hap3 was found in all populations except Marmaraeğlisi (MME), while haplotype Hap2 was shared by the Karadeniz Ereğli (KDE) and Ordu (ORD) populations. Other haplotypes were shared by only one population. Haplotype (Hd) and nucleotide (π) diversity ranged from 0.667-0.857 and 0.0013-0.0034, respectively, for all populations (Table 1). Hopa (HPA) population showed the highest haplotype (Hd: 0.857) and nucleotide diversity (π : 0.003).

Phylogenetic analyses and haplotype network analysis

Phylogenetic trees (BI, ML, and MP) were constructed using haplotype sequences and exhibited consistent topologies. The topology of the MP tree with 50% majority rule consensus (CI= 0.926, HI= 0.074, RI= 0.916, RC= 0.8480, and tree length 108) matched the ML and BI topologies and included all clades that had bootstrap support >50% on the BI tree. The trees included three clades (Clades I, II, and III) as depicted in Fig. 2, all of which had high bootstrap values (100/1.0/100) supporting their reliability. Clade I was composed of the North Sea sample, while Clade II comprised the Atlantic sample. Clade III consisted of 14 haplotypes from 5 sampling localities in the Sea of Marmara and Black Sea. The average sequence divergence between Clades I and II was 4.89%, between Clades I and III was 7.95% and between Clades II and III was 4.77%. In addition, divergence between the Black Sea and the Sea of Marmara samples was 0.46%.

The minimum spanning haplotype network (Fig. 3) showed a star-shaped phylogeny with many haplotypes emanating from a central haplotype Hap3 and separated by several mutation steps. Furthermore, a clear segregation of the haplotypes with respect to the geographical origin was noted.

Population genetic structure and geographical patterns

According to the PCoA and the first principal component, which explained 75.53% of the variance, the Marmara Sea (MME), Black Sea (HPA, IGN, ORD) and KDE populations were clearly separated (Fig. 4). AMOVA analysis showed that 41.83% of the variance was distributed among populations and 58.17% within populations ($P < 0.001$). When populations were grouped by geographic origin, it was found that 34.95% of the variation was distributed among the groups, 13.08% among populations within groups, and 51.97% within populations ($P < 0.001$).

The pairwise F_{ST} values ranged from 0.109 to 0.688 (Table 2). The highest F_{ST} value was observed between the ORD and MME populations while the lowest F_{ST} value was observed between the ORD and HPA populations. All pairwise F_{ST} values were moderate and statistically significant ($P < 0.05$), except for ORD-KDE and ORD-HPA populations. The highest migration

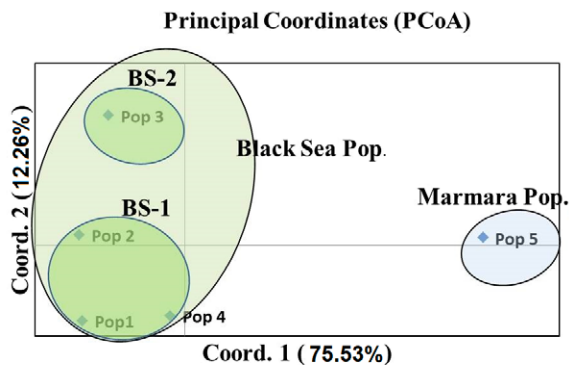


Fig. 4. The clustering of the populations of whiting based on the principal coordinate analysis. Pop 1: Hopa, Pop 2: Ordu, Pop 3: Karadeniz Ereğli, Pop 4: İğneada, Pop 5: Marmaraereğlisi. Pop = population.

value (N_m) of 4.05 was recorded between ORD-HPA populations, while the lowest value of 0.23 between ORD-MME populations (Table 2).

The isolation by distance (IBD) hypothesis was rejected as relationship between genetic distance and geographic distance was not significant ($R^2 = 0.064$, $P = 0.21$).

Divergence time estimates

The estimated evolutionary time scale and 95% HPD (Highest Posterior Density) intervals for the *M. merlangus* dataset are presented in Fig. 5. The divergence of *Trisopterus luscus*, which was used as an outgroup, from species belonging to the genera *Gadus* and *Merlangius* was estimated to be about 33.32 (22.75-45.09) million years ago (mya). The separation of the genus *Gadus* from *Merlangius* occurred 5.05 (3.63-6.64) mya, while *M. merlangus* separated from *Melanogrammus aeglefinus* 2.36 (1.6-3.25) mya. It was estimated that there was a partial separation within the *M. merlangus* samples, and the lineages from the Black Sea and Marmara Sea separated from the North Sea about 1.65 (1.08-2.29) mya, while the separation between the Atlantic lineages occurred approximately 0.84 (0.51-1.2) mya. The separation process within the *M. merlangus* samples from Black Sea and the Sea of Marmara occurred about 0.2 (0.11-0.3) mya.

Table 2. The pairwise F_{ST} values (below the diagonal) and migration ($M = Nm = 1 - F_{ST} / 2F_{ST}$ for haploid data) values (above the diagonal) among five populations of *Merlangius merlangus* based on D-loop sequence dataset.

Pop.	Hopa	Ordu	Karadeiz Ereğli	İğneada	Marmaraereğlisi
Hopa		4.05	2.20	2.86	0.39
Ordu	0.11		1.77	1.25	0.23
Karadeiz Ereğli	0.19*	0.22		1.55	0.30
İğneada	0.15*	0.29*	0.24*		0.41
Marmaraereğlisi	0.56*	0.69*	0.63*	0.55*	

* $p < 0.05$ (Significant F_{ST} values)

Table 3. Results of neutrality tests, mismatch analysis, and the time of expansion for three groups of *Merlangius merlangus* based on D-loop control region sequences.

Populations	Neutrality tests				Goodness of fit test				Mismatch distribution		
	Tajima's D	Tajima's D p-value	Fu's FS	FS p-value	SSD ^a Model	SSD p-value	HRI ^b	HRI p-value	Tau ^c	t ^d = $\tau/2u$ (kya ^e)	
										Mutasyon oranı	
										(%3)	(%10)
Hopa	-0.963	0.214	-0.943	0.170	0.007	0.970	0.020	1.000	4.078	87.03	26.11
Ordu	1.104	0.849	0.204	0.454	0.012	0.270	0.122	0.600	1.000	21.34	6.40
Karadeiz Eregli	1.107	0.854	0.789	0.625	0.071	0.190	0.256	0.370	3.375	72.02	21.61
İğneada	-0.121	0.452	-0.422	0.285	0.011	0.000	0.101	1.000	1.500	32.01	9.60
Marmaraeğlisi	1.621	0.977	0.390	0.498	0.045	0.180	0.221	0.350	1.781	38.01	11.40
Black Sea-1	-1.585	0.035	-4.212	0.011	0.010	0.220	0.082	0.110	1.750	37.35	11.20
Black Sea-2	1.107	0.858	0.789	0.646	0.058	0.270	0.256	0.670	3.375	72.02	21.61
Marmara pop.	1.621	0.976	0.390	0.519	0.040	0.200	0.221	0.590	1.781	38.01	11.40
All populations	-1.029	0.145	-5.655	0.007	0.001	0.710	0.034	0.1900	2.409	51.40	15.40

^aSSD (Sum of squared differences in mismatch analysis); ^bHRI (Harpending's (1994) raggedness index); ^ctau (expansion parameter); ^dt (beginning a time of expansion); ^ekya (thousand years ago).

Demographic history and selective neutrality

Tajima's D and Fu's Fs values for the HPA and IGN populations were negative but not significant, but for the Black Sea-1 (BS-1) group and all populations the values were negative and significant, indicating significant deviations from neutrality and suggesting past population expansion events (Table 3). In addition, the small and not significant values of the SSD and Harpending's raggedness index for all groups and the total population, along with the negative and significant values of Tajima's D and Fu's Fs tests, indicate significant deviations from neutrality. Such finding was consistent with a sudden demographic expansion scenario for the entire dataset (Table 3 and Fig. 6). The single-modal mismatch distribution obtained for all groups and the total population of *M. merlangus* (as presented in Fig. 6) also indicates a recent demographic expansion. Under the assumption of a sudden demographic expansion, the τ value for all samples was 2.4 (1.2–3.1 95% CI). When the mutation rate was assumed to be 3%, the expansion time was estimated to be 51.4 (24.3–67.2) kya (thousand years ago), and when it was assumed to be 10%, it was 15.4 (7.3–20.2) kya (Table 3).

Bayesian Skyline Plot (BSP) analysis showed a significant increase in effective population size over time for the BS-1 and the total population (Fig. 7), indicating that populations have expanded and grown over the last 5000 years (Fig. 7A and D). The estimated timing of the demographic expansion agreed well with the estimate obtained using the mismatch distribution statistics. A long period of constant population size from 3500 years ago to the present was found for the BS-2 and from 100 years ago to the present for the Marmara populations (Fig. 7).

DISCUSSION

Merlangius merlangus is a widespread and economic species in the Sea of Marmara and Black Sea in Turkey and is an important food source for humans. This study was carried out with the use of D-loop control region gene sequences to understand the genetic diversity, demographic history and genetic structure of *M. merlangus*. D-loop control region is a fast-evolving locus in most species and has been used for both phylogenetic analysis and intraspecific phylogeographic studies of fishes (Avice, 2000). Thus, basic aspects of the genetic structure of whiting species distributed in the Sea of Marmara and the Black Sea in Turkey are discussed, as sufficient genetic information is lacking. This knowledge gap poses a substantial challenge, particularly in light of the overfishing pressure on commercially valuable fish species.

The genetic diversity, population structure, and demographic history

Relatively high levels of genetic variation were estimated in *M. merlangus* populations sampled from the Sea of Marmara and Black Sea. The high degree of haplotype diversity and low nucleotide diversity observed in the study can be attributed to a sudden demographic expansion from a small effective population size, assuming that there is enough time to increase haplotype numbers (H) through mutation, but not accumulate large sequence differences (Rogers and Harpending, 1992). Also, it is directly linked with the evolution rate of mtDNA which differ among gene regions (Allio *et al.*, 2017). Most of the haplotypes were found to be specific to a particular sampling region (4 haplotypes for HPA, 3 for IGN, 3 for MME, 1 for KDE, and 1 for ORD) indicating limited gene flow among the studied regions.

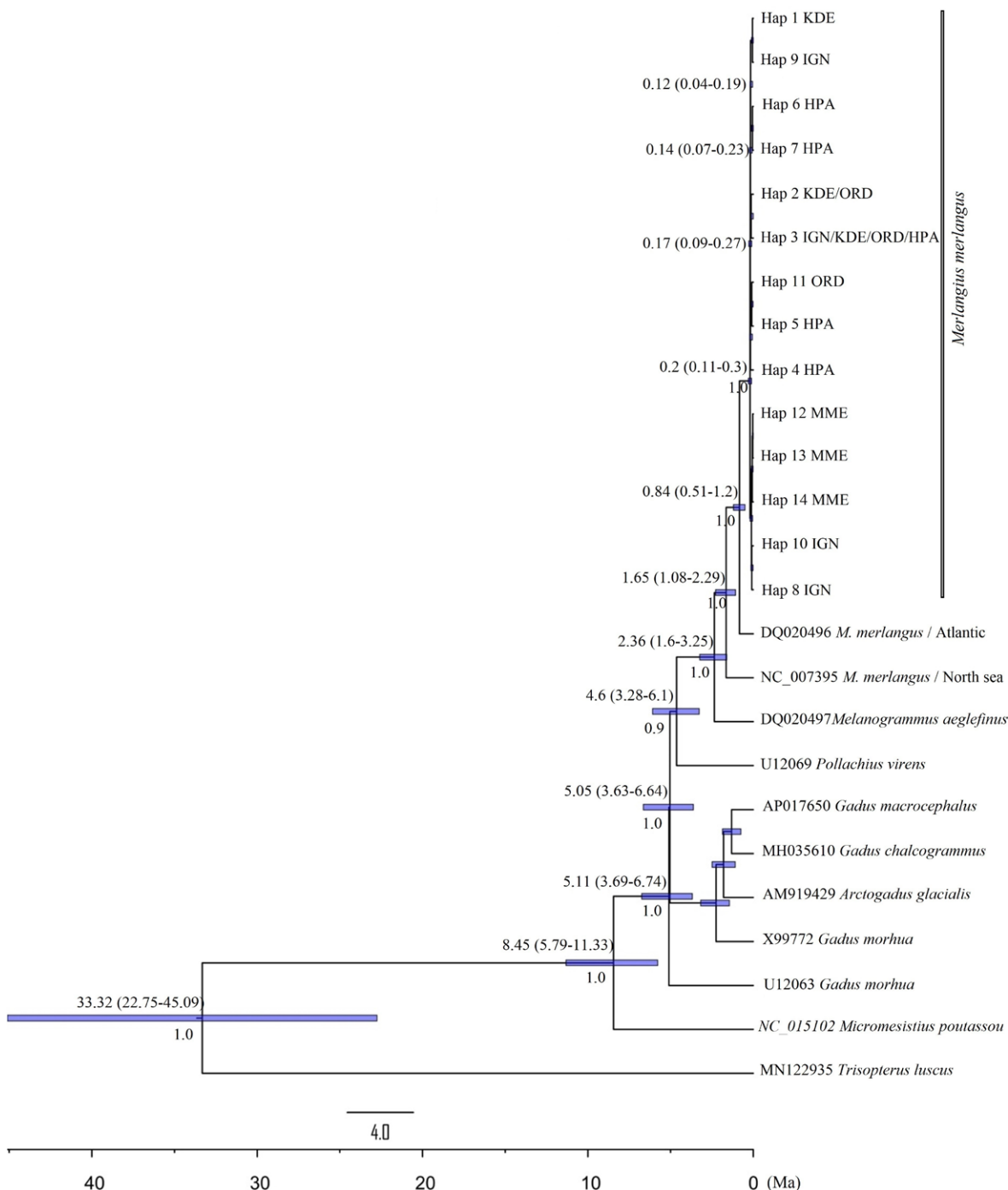


Fig. 5. Bayesian divergence time (Ma) estimates based on lognormal relaxed molecular clock analyses of the D-loop data set. Numbers at the nodes represent the mean estimated divergence times in million years (Ma), along with their 95% HPD confidence intervals in brackets. Numbers below nodes indicate the mean posterior probability values for Bayesian Inference. The bars denote the 95% HPD credibility intervals.

Based on phylogenetic analysis and haplotype networks, distribution of *M. merlangus* includes at least two genetically distinct clades with no shared haplotypes, even though such pattern was not confirmed by Şalcıoğlu *et al.* (2020) which combined COI, Cyt b, and RAG1 gene regions. The first group included individuals from Marmaraeğlisi within the Tekirdağ province of the Marmara Sea (Fig. 2), while the second

group included fish from four different localities in the Turkish Black Sea (IGN, KDE, ORD, and HPA). The phylogenetic trees revealed that Clade II, encompassing the majority of haplotypes, exhibited poor resolution attributed to the limited levels of intraspecific sequence divergence. Haplotype network confirmed population clustering. Members of four populations (IGN, KDE, ORD and HPA) shared many haplotypes within the

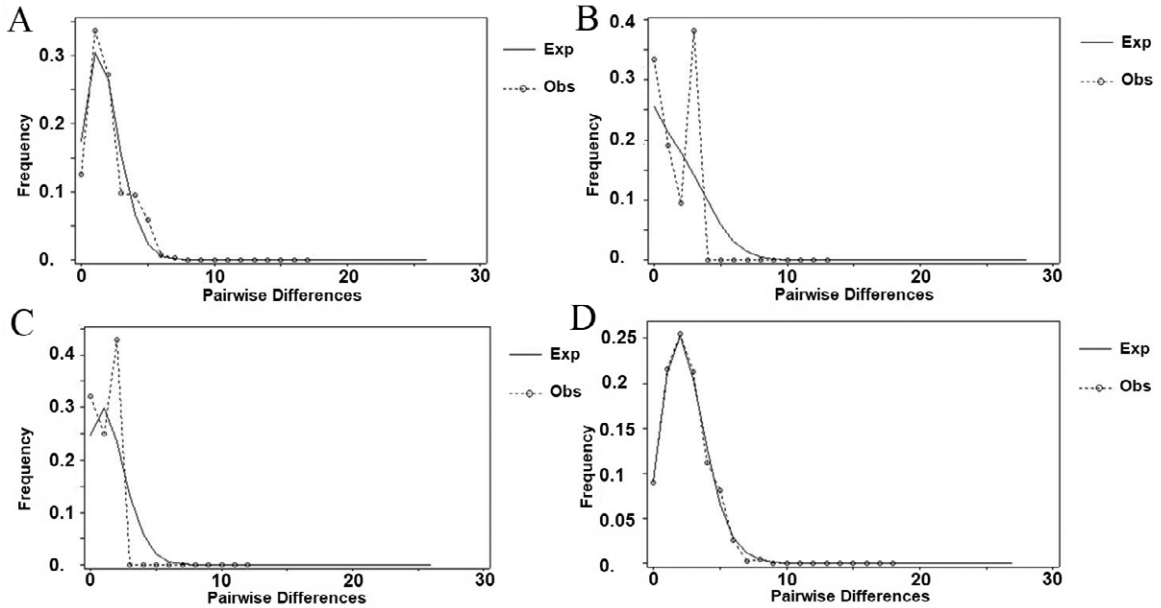


Fig. 6. Observed and expected mismatch distributions for three haplogroups and all populations. BS-1 group(A); BS-2 population (B); Marmara population (C); and (D) all populations (D). Dashed line represents the observed distribution; solid line represents the theoretically expected distribution under an expanding population model.

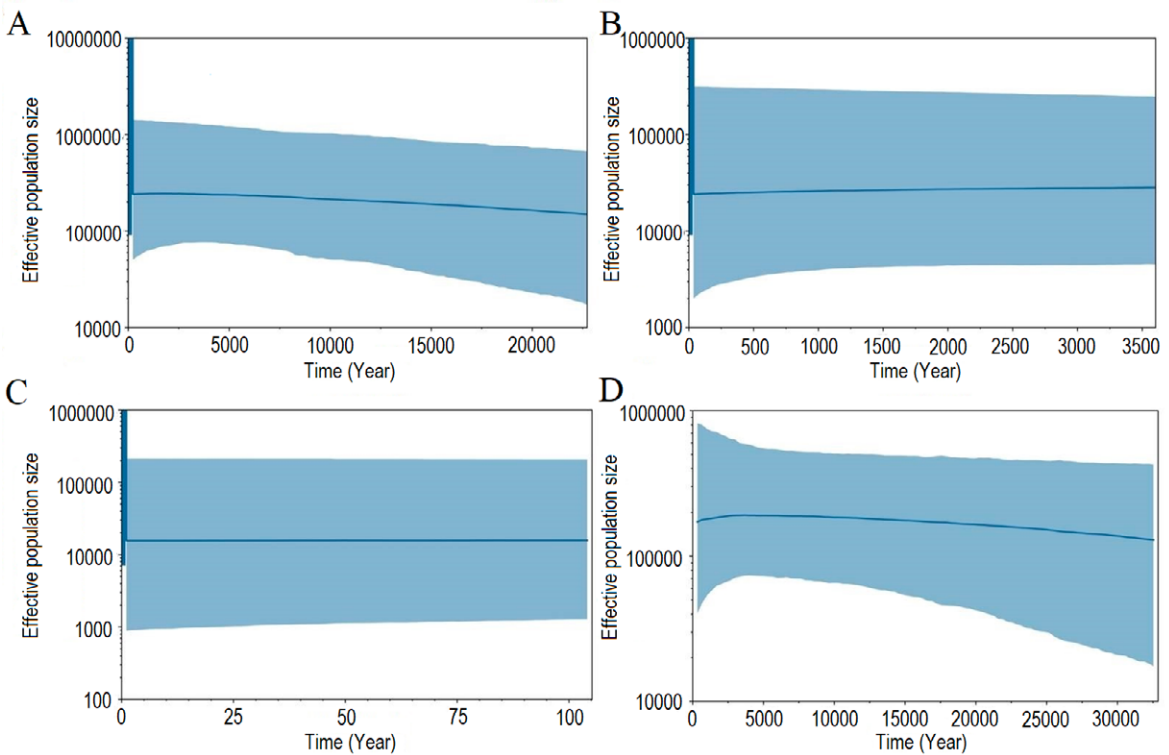


Fig. 7. BSP analysis of D-loop dataset for three haplogroups and the total population. BS-1 group (A); BS-2 population (B); Marmara population (C); and all populations (D). The X-axis represents time before present (year). The Y-axis represents effective population size. The solid line represents the median population size, and the 95% highest posterior density (HPD) interval is shown in blue.

first group, while individuals from the Marmaraeğlisi population formed unique haplogroups. The majority of unique haplotypes showed a close relationship to a common central haplotype (Hap3) with one or two mutational steps. The presence of an ancestral haplotype sug-

gests that both groups originated from a single distinct source in the Marmara Sea and Black Sea. In addition, the star-like shape of the haplotype network indicates that *M. merlangus* populations in different regions have recently diverged or/and have recently experienced a

significant increase in population size, which will be discussed in the following section.

Both, the molecular variance and F_{ST} analyses showed genetic differentiation among populations. AMOVA revealed the structure of three different groups based on Principal Coordinate Analysis (PCoA) and supported by phylogenetic analysis results. Even though the isolation by distance pattern was not confirmed, physical barriers and geographical distance can contribute to observed decrease in genetic connectivity among populations or groups.

Estimation of divergence time revealed deep divergences similar to phylogenetic analysis. Divergence of the *Merlangius* genus began diverging in the early Pliocene, with a significant separation from *Gadus* around 5.05 million years ago (mya). This period coincided with the Messinian Salinity Crisis in the Mediterranean (Krijgsman *et al.*, 1999). The reopening of the Strait of Gibraltar marked the end of this crisis around 5.33 mya (Govers, 2009). During the Quaternary period, rising sea levels led to the formation of the Aegean Sea, and the Black Sea underwent transitions between freshwater and marine environments (O'Regan *et al.*, 2011). Additionally, *M. merlangus* samples separated into different lineages during the Pleistocene, reflecting the dynamic environmental changes of that era.

Demographic history reconstruction can provide valuable insights into various evolutionary and population genetic processes, including correlations between demographic patterns and paleoclimatic events (Drummond *et al.*, 2005). Namely, results from the present study confirmed that BSP-1 group and all populations of *M. merlangus* underwent demographic changes in the past, such as expansion in the Black Sea. Additionally, a large number of rare haplotypes observed in the study can be attributed to populations that experienced bottlenecks and subsequent demographic expansions, as sudden population expansion after periods of low effective population size results in the retention of new mutations and thus an excess of rare haplotypes (Grant and Bowen, 1998). The estimated expansion time (15.4 - 51.4 kya) of *M. merlangus* coincides with the events of marine species migrating to the Black Sea following the invasion of the Black Sea basin by saltwater from the Mediterranean after the formation of the Çanakkale and Istanbul straits at the end of the Pleistocene and during the Holocene. Şalcıoğlu *et al.* (2020) similarly suggested that the whiting populations from Eastern Mediterranean may have spread and expanded to the Black Sea through the straits system, as seen in other fish species (*Mugil cephalus*, Durand *et al.*, 2013; *Trachurus trachurus*, *T. mediterraneus*, and *T. picturatus*, Bektaş and Beldüz, 2008). Additionally, Şalcıoğlu *et al.* (2020) linked the approximately 2000-year time difference between the opening of the Çanakkale Strait (~8 Kya) and the expansion of the Black Sea (~18 Kya) and the TSS (~5-6 Kya) following the last glacial maximum to the time required to create suitable habitat conditions for settlement.

The coalescent approach and Bayesian Skyline Plot (BSP) analysis showed that the BS-1 population and the

total population have expanded and grown over the last 5,000 years, indicating a demographic recovery scenario following a bottleneck in the past (Hoffman *et al.* 2011). The timing of the population growth estimated from the demographic expansion model was consistent with the BSP analysis estimate. This implies that the population growth began about 5,000 years ago, during the Holocene, after the formation of the Çanakkale and Istanbul straits, rather than after the Last Glacial Maximum (LGM), which was the earlier assumption for the North-east Atlantic (Eiriksson and Árnason, 2014).

Implications for conservation and management

Effective fish population management requires using genetically homogenous local groups as the fundamental unit. Genetic changes can create new units, and considering geographical variations in these homogenous groups is vital. In 1994, Moritz introduced two conservation units: Management Units and Evolutionarily Significant Units (ESUs) for separate population management and long-term species diversity preservation. Mitochondrial DNA analysis aids in identifying and managing genetic diversity and ESUs, but to investigate effective population sizes for sustainable fisheries management, future studies should also use nuclear markers. Integrating both markers into management strategies empowers informed decisions, preventing overexploitation, conserving biodiversity, and ensuring aquatic resource viability.

In present study, mitochondrial D-loop sequences revealed important genetic variation and interaction insights within populations. High genetic diversity suggests ancient divergence. Three ESUs (Marmara, BS-1, and BS-2 populations) emerged, indicating isolated lineages, supported by genetic variation and PCoA analyses. These groups may have higher adaptability in their own environments. Therefore, conservation efforts should aim to preserve the genetic integrity of each cluster to maintain the evolutionary capacity of species. Debates exist about the appropriate level of fisheries management, with some proposing local strategies to ensure sustainable resource use and prevent overfishing (Bruckmeier and Neuman, 2005). Conservation genetics aims to sustainably use resources by preserving local genetic structures, which is often overlooked, and managing diverse populations as a single unit can lead to genetic variation depletion (Laikre *et al.*, 2005).

Finally, the results of the study can serve as a valuable basis for the management of whiting, *i.e.* species found in the regions bordering the Mediterranean Sea, the Aegean Sea and the Black Sea, including Turkey. The development of conservation and management plans for whiting, which accounts for a significant proportion of fish catches in Turkey, will not only contribute to economic sustainability, but also represents a remarkable shift in the application of genetic studies aimed at playing a direct role in shaping fisheries management decisions for commercially important species.

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