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Different patterns of population structure and genetic diversity of three mesopelagic fishes in the Greek Seas

Xenia SARROPOULOU[1,2], Dimitris TSAPARIS[1], Konstantinos TSAGARAKIS[3], Nicholas BADOUVAS[3] and Costas S. TSIGENOPOULOS[1]

[1] Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research (HCMR), Thalassocosmos, Heraklion 71500, Crete, Greece
[2] Department of Biology, School of Sciences and Engineering, University of Crete, Heraklion 70013, Greece
[3] Institute of Marine Biological Resources and Inland Waters, Hellenic Centre for Marine Research (HCMR), Anavyssos 19013, Attica, Greece

Corresponding author: Xenia Sarropoulou; x.sarrop@gmail.com

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Abstract

Mesopelagic fishes are among the most abundant groups of vertebrates on Earth. Despite their unique biological and ecological traits, research in this group has been particularly scarce. The present study investigates the intraspecific genetic diversity of three mesopelagic fishes (*Hygophum benoiti, Maurolicus muelleri*, and *Benthosema glaciale*) in the Greek Seas. Analyses of three mitochondrial DNA genes (COI, 12S, and 16S) from a total of 168 samples revealed a lack of genetic structure for *M. muelleri* and *B. glaciale* across the studied area. However, *H. benoiti* specimens from the Corinthian Gulf were differentiated from the rest of the populations, suggesting that the limited connection between the Corinthian and neighboring seas may act as a barrier to gene flow. Furthermore, the COI data of this study were co-analyzed with publicly available sequences, demonstrating lack of phylogeographic structure for all three species through their distribution range. Therefore, even though indications of genetic differentiation were observed, the three mesopelagic fishes are generally characterized by genetic homogeneity, which may be the result of their recent evolutionary history.

Keywords: Phylogeography; mitochondrial DNA; Aegean Sea; Eastern Mediterranean; twilight zone; *Hygophum benoiti; Maurolicus muelleri*; *Benthosema glaciale.*

Introduction

The marine environment is traditionally considered as highly connected due to few barriers to gene flow (Palumbi, 1994). Compared to terrestrial organisms, marine species, and marine fishes in particular, are generally characterized by high genetic diversity and weak population structure (Selkoe *et al*., 2008; Plough, 2016). This is mainly attributed to large population sizes and the existence of a planktonic larval stage, during which larvae can be passively transported over long distances by ocean currents (Calò *et al*., 2013). In general, species with no pelagic larval dispersal tend to exhibit strong population structure suggesting low gene flow (Palumbi, 1994), whereas species with longer, and planktonic, larval duration are expected to present low population differentiation due to high gene flow between remote populations (Bohonak, 1999). However, structural patterns are not always correlated with geographic factors and can be influenced by traits related to life-history and behaviour as well as ocean currents and environmental factors (Hedgecock *et al*., 2007; Galarza *et al*., 2009; Salmenkova, 2011).

During the glacial periods of the Pleistocene era, sea level fluctuations affected the connectivity between the Mediterranean Sea basins. For many taxa, changes in sea level and climate resulted in periods of extinction and recolonization that, combined with present day physical barriers, are considered to be the main factors that shaped the population structure of Atlantic and Mediterranean species (Patarnello *et al*., 2007). The Greek Seas, from the Ionian Sea in the West, to the Aegean Sea in the East, comprise a complex ecosystem combining a highly irregular coastline and semi-isolated deep basins (Olson *et al*., 2007), which has also undergone several sea level changes over the years (Sakellariou & Galanidou, 2016). Genetic differentiation has been reported from different parts across the Greek Seas, and is due to historic demographic processes as well as hydrological and ecological traits (e.g., Gkafas *et al*.,

2013; Kousteni *et al*., 2015; Imsiridou *et al*., 2019).

Mesopelagic fishes constitute the most abundant group of vertebrates in the marine environment (Irigoien *et al*., 2014). They are mainly small-sized species, which inhabit the mesopelagic zone, usually at depths of 200-1,000 m. Recent research suggests that they may represent a total biomass of 10 billion tons (Irigoien *et al*., 2014), an order of magnitude higher than previous estimates (Gjoesaeter & Kawaguchi, 1980). Mesopelagic fishes form an important link between primary consumers -mainly zooplankton- (Contreras *et al*., 2015) and apex predators, such as marine mammals (Giménez *et al*., 2018), sea birds (Barrett *et al*., 2002) and several larger fish species, e.g., the highly commercial tuna and hake (Battaglia *et al*., 2013; Modica *et al*., 2015). Most species of this group display a daily pattern of diurnal vertical migration following their prey into the epipelagic zone to feed at night (Olivar *et al*., 2012). During daytime they stay in the mesopelagic zone to avoid their predators, where they digest and excrete, which is likely responsible for a large fraction of the carbon fluxes in the ocean (Irigoien *et al*., 2014). Mesopelagic fishes have also attracted economic interest due to their high abundance and potential source of food mainly for aquaculture species (John *et al*., 2016; Alvheim *et al*., 2020; Grimaldo *et al*., 2020). Despite their high abundance and integral role in marine food webs and biogeochemical cycles, this group remains significantly understudied (Caiger *et al*., 2021).

Several studies have focused on the intraspecific genetic diversity of mesopelagic fishes, based mainly on allozymes (Suneetha & Salvanes, 2001; Kristoffersen & Salvanes, 2009), mitochondrial markers (Habib *et al*., 2012; Gordeeva, 2014; Pappalardo *et al*., 2015; Christiansen *et al*., 2018; Terada *et al*., 2018; Torri *et al*., 2021), a few microsatellite markers (Gordeeva, 2011; Van de Putte *et al*., 2012) and only one study on genomic-wide SNP data (Rodriguez-Ezpeleta *et al*., 2017). The majority of them concern a limited number of species with samples from scattered parts of their distribution range, using very few genetic markers. Available genetic data about mesopelagic fishes are particularly scarce in the Eastern Mediterranean and inexistent in the Greek Seas, where mesopelagic fishes are found in great abundance (Somarakis *et al*., 2011a, b).

In this study, a comparative genetic analysis of three mesopelagic fish species (the silvery lightfish *Maurolicus muelleri*, the glacier lantern fish *Benthosema glaciale,* and the Benoit's lanternfish *Hygophum benoiti*) is performed to explore patterns of diversity and genetic differentiation in the Greek Seas. To achieve this, samples from the Gulf of Corinth, the Aegean Sea and the Cretan Sea were used, and three mitochondrial DNA gene regions (COI, 12S and 16S) were sequenced. Furthermore, in order to assess the phylogeography of these species and understand the potential processes that shaped their genetic variation, we investigated their historical demography using published COI sequences throughout their distribution range.

Materials and Methods

Sample collection

Mesopelagic fishes were collected from four locations (Gulf of Corinth, Saronic Gulf, North Aegean Sea, Cretan Sea) (Fig. 1) during various scientific expeditions within the framework of the MESOBED (Mesopelagic fish: biology, ecological role and distribution of a disregarded

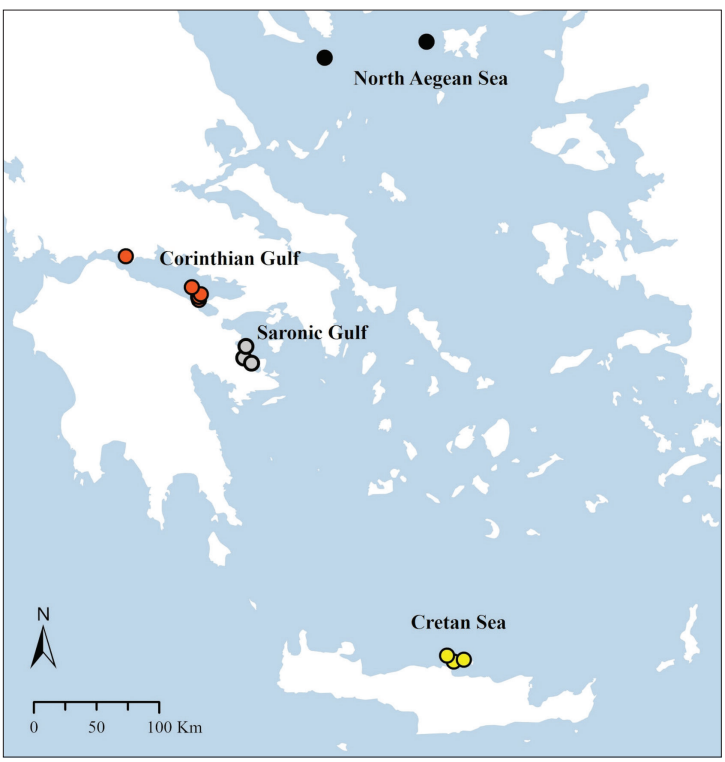

Fig. 1: Map of sampling locations. The colour of sampling locations in each region (Corinthian Gulf, Saronic Gulf, North Aegean Sea, and Cretan Sea) corresponds to the colour that was used for the haplotype networks.

trophic link) and MEDIAS (Mediterranean International Acoustic Surveys) projects. The Gulf of Corinth is a deep inlet of the Ionian Sea and has limited connection with the Aegean Sea; it is connected with the Saronic Gulf through an 8 m deep, 24 m wide and 3.5 nmi long channel. On the contrary, all the other regions are located in the open sea, where no major physical barriers are found. The samplings were carried out between November 2018 and December 2019, and a pelagic trawl was used as the main sampling gear. Each specimen was morphologically identified and then muscle tissue or pelvic fin clips were preserved in absolute ethanol (>99.8%) for genetic analysis.

DNA extraction, amplification and sequencing

Total genomic DNA of 168 specimens (56 for each of the three species) was extracted from the stored samples using the salt extraction protocol of Miller *et al*. (1988) with some modifications (available upon request).

Three mitochondrial DNA genes, namely, cytochrome c oxidase subunit 1 (COI), 16S rDNA and 12S rDNA, were partially amplified by Polymerase Chain Reaction (PCR) using the universal primers FishCoxI_F1 and FishCoxI_R1 (Ward *et al*., 2005), 16SarL and 16SbrH (Palumbi, 1996), L1091 and H1478 (Kocher *et al*., 1989), respectively. The PCR was performed in a total volume of 15 μl containing 1.5 μl of PCR reaction buffer (1 X), 0.3 μl of DNTPs mix (0.2 mM), 0.45 μl of each primer (0.3 μM), 0.6 μl for COI and 0.3 μl for 12S and 16S of $MgCl_2$ (1 mM), 0.3 U of KAPA Taq polymerase (KAPA BIOSYSTEMS) and 20-50 ng of template DNA. The cycling conditions consisted of an initial 3 min step at 95°C, followed by 35 cycles (30 cycles for 12S) of 30s at 95°C, 30s at the annealing temperature (51°C for COI; 50°C for 16S; 55°C for 12S), 45s at 72°C and a final extension step at 72°C for 1.30 min. The PCR products were purified with ethanol/sodium acetate precipitation and sequenced using the forward primer. In cases where the obtained sequences were short or of poor quality, sequencing was repeated with the reverse primer. Sequencing reactions were carried out using the BigDye Terminator version 3.1 Cycle Sequencing Kit (Applied Biosystems) and the products were run on an ABI 3730 capillary sequencer (Applied Biosystems), following the manufacturer's instructions. Raw fluorogram data were imported to MEGA v.7 (Kumar *et al*., 2016) and inspected for quality and accuracy in nucleotide base calling. Sequences for each gene region were aligned using ClustalW as implemented in MEGA v.7. Amino acid translations of the COI datasets were examined to ensure the absence of stop codons in the alignment. The final dataset for all specimens and each species was created by concatenating the sequences of the three gene regions.

Genetic diversity and population differentiation

Molecular diversity indices for the mtDNA fragments including the number of haplotypes (H), haplotype diversity (Hd), nucleotide diversity (π) and the number of private haplotypes (N_{hp}) were estimated for each sampled population using the DNAsp v5 (Librado & Rosas, 2009) software.

The relationships between the concatenated haplotypes were depicted with median-joining networks (Bandelt *et al*., 1999), which were constructed using the PopART (Leigh & Bryant, 2015) software package. To estimate population differentiation, population pairwise Φ_{ST} values were calculated, based on the number of pairwise differences among sequences using the ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010) software package. Statistical significance was assessed using 10,000 permutations. The overall Φ_{ST} value for each species was also calculated using the same parameters.

Phylogeographic analysis

A second dataset was created for each species by combining the COI sequences of this study with published sequences from the Genbank and BOLD databases for further phylogeographic analysis. The 16S and 12S sequences were not used, due to the insufficient number of available published sequences. The retrieved sequences were selected only if they had available information about their origin and their length was long enough in order not to omit genetic information from our dataset. Median-joining networks were also constructed for each dataset using PopART.

Demographic history

To test departures from neutrality, Fu's F test (Fu, 1997) and Tajima's D test (Tajima, 1989) were implemented in ARLEQUIN v.3.5.2.2 (Excoffier & Lischer, 2010). Significantly negative values of Fu's Fs and Tajima's D reflect an excess of rare polymorphisms in a population, which indicates either positive selection or an increase in population size. Both tests were used for both the concatenated mtDNA datasets and the published COI sequences. Significance was assessed by 10,000 simulated samples.

Bayesian skyline plots (BSP) were also applied in order to study the past population dynamics of the three mesopelagic fish species using BEAST v.2.5 (Bouckaert *et al*., 2019). Considering the fact that sample size greatly affects the power of BSPs (Grant, 2015), analyses were conducted only on the datasets that contained both the COI sequences of this study and those retrieved from public databases. The HKY substitution model was used for *M. muelleri* and *B. glaciale* and the JC model for *H. benoiti*, as determined by jMODELTEST v.2.1.10 (Darriba *et al*., 2012). Due to the lack of a species-specific mutation rate for the studied gene, the substitution rate of 1.77×10^{-8} substitutions per site per year that has been reported for reef fish (Eytan & Hellberg, 2010) was applied with a strict molecular clock. Two independent Markov Chain Monte Carlo (MCMC) runs were carried out with

40,000,000 iterations and 10% burn-in (4,000,000 iterations), sampled every 4,000 steps. The convergence of the MCMC runs was evaluated based on whether the effective sample size (ESS) of all parameters was greater than 200 when analyzed in TRACER v.1.7 (Rambaut *et al*., 2018). Then, the same software was used to plot the skylines.

Results

Genetic diversity

Final sequence length for COI ranged from 583 to 612 bp, 16S ranged from 540 to 556 bp and 12S ranged from 355 to 372 bp. All sequences were deposited in Genbank and the accession numbers are presented in Tables S1-S3. Concatenation of the three gene regions resulted in a 1,505 bp dataset for *M. muelleri* and *H. benoiti* and 1,514 bp dataset for *B. glaciale* (Table 1).

Across the studied area, overall haplotype diversity ranged from 0.73 (*H. benoiti*) to 0.85 (*B. glaciale*), while nucleotide diversity ranged from 0.071% (*H. benoiti*) to 0.137% (*B. glaciale*). Haplotype and nucleotide diversity values overall and for each sampling site are presented in Table 1. Private haplotypes were reported in all population samples. Population samples with the highest number of haplotypes and haplotype diversity were found in the Saronic Gulf for *H. benoiti* (Hd=0.84, N_h=9, N_{hp}=6), the N. Aegean for *B. glaciale* (Hd=0.97, N_h=12, N_{hp}=10) and the Gulf of Corinth for *M. muelleri* (Hd=0.92, N_h=10, N_{hp}=8). On the contrary, population samples with the lowest values were found in the Cretan Sea for *H. benoiti* (Hd=0.4, N_h=4, N_{hp}=3) and in the Saronic Gulf for *B. glaciale* and *M. muelleri* (Hd=0.77/0.71, N_h=8/5, N_{hp}=6/2).

Population differentiation

Haplotype networks of *M. muelleri* and *B. glaciale* exhibited star-like patterns, consisting of a dominant haplotype shared by all populations with high frequency (41.1% and 39.3%, respectively). The rest of the haplotypes were mainly singletons or haplotypes shared by two or three regions, separated by one (rarely two or three) mutational steps (Fig. 2B, 2C). *H. benoiti* sequences also exhibited a star-like network, but apart from the main haplotype (50% frequency) there was also another abundant haplotype (17.9%), represented mainly by specimens from the Gulf of Corinth (Fig. 2A). The rest of the haplotypes, apart from one, were singletons.

Pairwise Φ_{ST} values ranged from 0 to 0.016 between populations of *M. muelleri* and from 0 to 0.04 for *B. glaciale*; overall, Φ_{ST} values were 0.0003 and 0.013 respectively (Table 2). However, none of these values were statistically significant. For *H. benoiti*, all Φ_{ST} values including those of the Gulf of Corinth were significant and ranged from 0.332 to 0.430, whereas the Φ_{ST} values of all the other pairwise comparisons were not significant (Table 2); this species showed significant genetic heterogeneity between the different sampling sites (Φ_{ST}=0.217, p<0.01).

Table 1. Molecular diversity indices and results of the Tajima's D and Fu's Fs neutrality tests for the *H. benoiti*, *B. glaciale* and *M. muelleri* samples inferred from the concatenated mtDNA datasets (N: number of samples, N_h: number of haplotypes, N_{hp}: number of private haplotypes, Hd: haplotype diversity, π: nucleotide diversity).

Species	Location	N	N_h (N_{hp})	Hd	π%	Fu's Fs	Tajima's D
H. benoiti	Corinthian G.	14	4 (2)	0.58	0.051		
	Saronic G.	14	9 (6)	0.84	0.084		
	N. Aegean	14	6 (5)	0.60	0.048		
	Cretan Sea	14	4 (3)	0.40	0.048		
	(All)	56	19 (16)	0.73	0.071	**-20.61775**	**-2.45707**
M. muelleri	Corinthian G.	14	10 (8)	0.92	0.118		
	Saronic G.	14	5 (2)	0.51	0.047		
	N. Aegean	14	9 (4)	0.88	0.099		
	Cretan Sea	14	10 (7)	0.92	0.12		
	(All)	56	26 (21)	0.83	0.096	**-27.89441**	**-2.40482**
B. glaciale	Corinthian G.	14	9 (8)	0.84	0.131		
	Saronic G.	14	8 (6)	0.77	0.102		
	N. Aegean	14	12 (10)	0.97	0.197		
	Cretan Sea	14	8 (7)	0.82	0.113		
	(All)	56	33 (31)	0.85	0.137	**-27.15325**	**-2.66376**

Statistically significant values are indicated in bold characters (p<0.01).

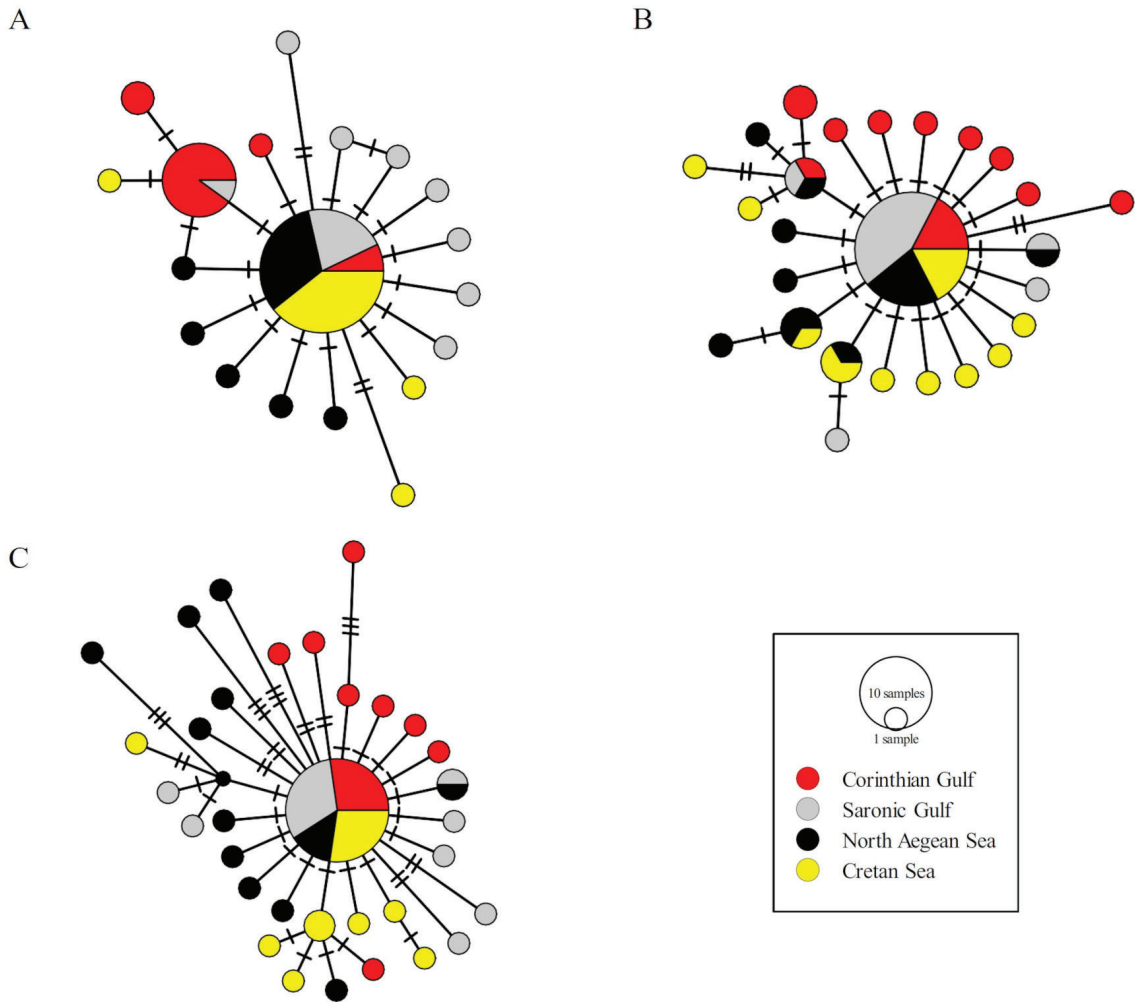

Fig. 2: Median-joining haplotype networks of the concatenated mtDNA datasets of (A) *H. benoiti*, (B) *M. muelleri* and (C) *B. glaciale.* Each circle represents a haplotype and its size is proportional to its relative frequency. Each colour corresponds to a different sampling site. Bars indicate the number of nucleotide differences between haplotypes.

Table 2. Pairwise and overall Φ_{ST} values for the concatenated mtDNA datasets among *H. benoiti*, *B. glaciale* and *M. muelleri* samples.

		Corinthian G.	Saronic G.	N. Aegean	Cretan S.
H. benoiti Φ_{ST} = **0.217**	Corinthian G.				
	Saronic G.	**0.332**			
	N. Aegean	**0.430**	0.000		
	Cretan S.	**0.404**	-0.005	-0.007	
		Corinthian G.	Saronic G.	N. Aegean	Cretan S.
M. muelleri Φ_{ST} = 0.0003	Corinthian G.				
	Saronic G.	0.011			
	N. Aegean	0.016	0.003		
	Cretan S.	0.003	-0.015	-0.016	
		Corinthian G.	Saronic G.	N. Aegean	Cretan S.
B. glaciale Φ_{ST} = 0.013	Corinthian G.				
	Saronic G.	0.007			
	N. Aegean	0.005	-0.008		
	Cretan S.	0.025	0.040	0.016	

Statistically significant values are indicated in bold characters ($p<0.01$).

Phylogeographic analyses

Apart from the sequences of this study, published COI sequences were retrieved from BOLD and Genbank for each species. A total of 80 sequences were downloaded for *M. muelleri*, 23 for *B. glaciale* and 24 for *H. benoiti* (Table S4) and added to the respective final datasets. Haplotype networks (Fig. 3) exhibited star-like patterns with one main haplotype and several minor haplotypes that were separated by a small number of mutational steps from the main one. The main haplotype in the *H. benoiti* (Fig. 3A) and *M. muelleri* (Fig. 3B) networks was found in all the studied regions, whereas in the *B. glaciale* network it was represented mainly by samples from Greece and a few from Greenland (Fig. 3C).

Demographic history

Tajima's D and Fu's F neutrality tests presented negative and statistically significant values both in the concatenated (Table 1) and the COI datasets (Fig. 3). Thus, the D and Fs values indicate demographic expansion for all three species both in the Aegean Sea and the wider range of distribution of each species.

Skyline plot analyses of the COI datasets, on the contrary, suggest that the median estimate of population size remained almost constant over time for *H. benoiti* and *M. muelleri*. Specifically, the patterns of both species were those of a stable population with a slight upward trend for the last 15 ky (Fig. 3A, 3B). However, the BSP of *B. glaciale* revealed population growth, which was estimated to have started around 80 ky BP (Fig. 3C).

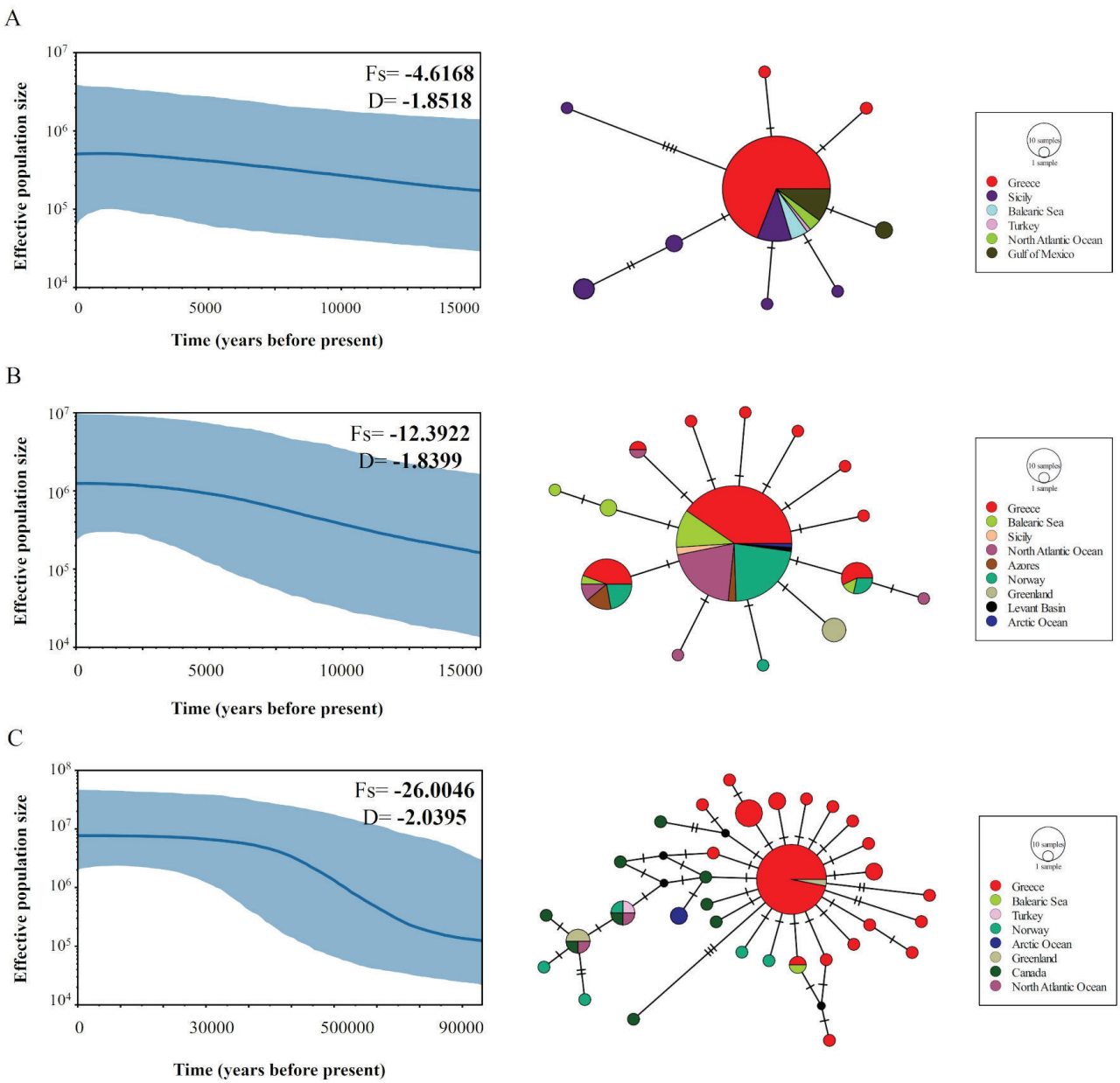

Fig. 3: Bayesian Skyline Plot (BSP) demographic analyses, median-joining networks and results of Fu's F and Tajima's D neutrality tests for the mtDNA COI dataset with the published sequences of (A) *H. benoiti*, (B) *M. muelleri* and (C) *B. glaciale*. Statistically significant values of D and Fs are indicated in bold characters. For BSPs, the thick solid line depicts the median estimate, and the margins of the blue area represent the highest 95% posterior density intervals.

Discussion

Patterns of genetic diversity of H. benoiti, M. muelleri *and* B. glaciale *in the Greek Seas*

Mitochondrial data analyses of the three mesopelagic fish species showed different patterns of genetic diversity and differentiation. Overall, high haplotype diversity and low nucleotide diversity were observed in all three species, which indicates rapid population expansion (Grant & Bowen, 1998). The star-like phylogenies of the haplotypes also suggest recent population expansion from a population with a small effective size (Avise, 2000), which was statistically confirmed by the significantly negative values of the two neutrality tests. Furthermore, all sampling regions presented several private haplotypes that, in conjunction with the fact that the main haplotype was found in different frequencies in each region, is an indication of limited gene flow and genetic differentiation (Slatkin, 1985). While no phylogeographic structure was observed in *M. muelleri* and *B. glaciale*, *H. benoiti* specimens from the Gulf of Corinth presented significant genetic differentiation from the rest of the samples. This was evident in the haplotype network by the dominance of a haplotype in the Gulf of Corinth samples, which differed from the main haplotype shared by the individuals from the Saronic Gulf and the North Aegean Sea. The statistically significant and high pairwise Φ_{ST} values between the Gulf of Corinth and the other two regions further support this finding.

The Gulf of Corinth is semi-closed with limited communication to the open sea via shallow and narrow channels, and is characterized by unique hydrological and topographical traits (Drakopoulos & Lascaratos, 1998; Ramfos *et al*., 2005). Specifically, there is practically no water exchange between the Saronic Gulf and the Gulf of Corinth, while the relatively shallow (<130 m) Gulf of Patras in the west is where water exchange takes place between the Gulf of Corinth and the open Ionian Sea (Friligos *et al*., 1985). The isolation of the Gulf of Corinth is supported in this study by the high number of private haplotypes in the case of *M. muelleri* and *B. glaciale* and the genetic differentiation of *H. benoiti* samples from the rest of the population samples. Therefore, certain characteristics of the Gulf of Corinth may act as a barrier to gene flow between the Gulf of Corinth and the Aegean Sea. Nevertheless, it should be mentioned that owing to the limited number of samples examined in this study, these insights have to be considered as tentative and be confirmed analyses of a more extensive dataset. Moderate but statistically significant genetic differentiation between the Gulf of Corinth and the Aegean Sea has been reported in the small-spotted catshark *Scyliorhinus canicula* (Kousteni *et al*., 2015). Moreover, partial genetic isolation between semi-closed ecosystems (fjords) and the open sea has been observed in *B. glaciale* in the Norwegian Sea (Suneetha & Salvanes, 2001; Kristoffersen & Salvanes, 2009).

All three species considered in this study present similarities as regards the depth they inhabit, both during daytime and at night, and their life cycles are highly associated with the epipelagic zone, where larvae occur and juveniles and adults feed (Salvanes & Kristoffersen, 2001; Olivar *et al*., 2012; Bernal *et al*., 2015; Christiansen *et al*., 2019). The observed discordance between the patterns of genetic structure is not surprising as similar cases have been reported for other mesopelagic fishes (Gordeeva, 2011; Gordeeva, 2014; Pappalardo *et al*., 2015). Several comparative phylogeographical analyses have shown that even phylogenetically closely related species with similar life-histories and distribution ranges can exhibit different patterns of genetic diversity and population structure (Patarnello *et al*., 2007; Bowen *et al*., 2014). Specific characteristics related to life-history and behavioural traits as well as the influence of ocean currents on dispersal and connectivity have been suggested as possible causes of genetic differentiation (Hedgecock *et al*., 2007). Currently, there is limited available information on the ecology and biology of mesopelagic fishes and, therefore, extensive research is necessary to make comparisons and explain the observed patterns.

Phylogeography and demographic history of H. benoiti, M. muelleri *and* B. glaciale

In order to draw a more comprehensive picture of the phylogeography of the studied species, published data from BOLD and Genbank were added to the mtDNA COI datasets of this study. The median-joining networks of *H. benoiti* and *M. muelleri* (Fig. 3A and 3B, respectively) show that sequences from different regions are randomly dispatched in the networks, with no evidence of structure. Although there are several private or geographically restricted haplotypes, the most frequent ones are found in all or most of the regions, thus indicating homogeneity. Lack of phylogeographic structure has been reported both in *M. muelleri* and other species of the genus *Maurolicus* (Rees *et al*., 2017; Terada *et al*., 2018; Rees *et al*., 2020) as well as *H. benoiti* (Pappalardo *et al*., 2015). No clear phylogeographic pattern was found in *B. glaciale* either (Fig. 3C), but the dominant haplotype was shared by the sequences from Greece and Greenland. Moreover, the Mediterranean and Atlantic sequences were generally represented by different haplotypes, which suggests genetic differentiation. This may be due to the small number of available sequences from the Atlantic (Table S4), which is not representative of the genetic diversity of this region. Therefore, more samples are necessary for genetic analysis and validation of this finding.

The demographic analyses indicate the different historical population dynamics of each species. The neutrality tests suggest recent population expansion for the three species, which agrees with the star-like patterns of the haplotype networks. However, BSP analyses revealed moderate demographic expansion over the last 15 ky for the *H. benoiti* and *M. muelleri* samples, but considerable population expansion for *B. glaciale* that started approximately 80 ky BP. These dates are consistent with several sea level changes that occurred during the last glacial period and affected the genetic structure of many marine

organisms (Avise, 2000; Hewitt, 2000). It is important to point out that the power of BSP analyses to reconstruct demographic histories is greatly affected both by sample size and the levels of polymorphism of the molecular markers used (Grant, 2015). Therefore, the general stability observed in *H. benoiti* and *M. muelleri* samples compared to *B. glaciale* samples could be caused by lack of statistical power due to lower levels of polymorphism. On the contrary, Tajima's D and Fu's F neutrality tests can be particularly sensitive in detecting population growth even with a limited number of sequences (Ramos-Onsins & Rozas, 2002). These results are considered preliminary as they are based on the sequences of a single gene (COI) and need further validation by additional highly polymorphic markers.

Multiple studies have revealed generally low levels of genetic structure in marine fishes (e.g., Cano *et al.*, 2008; Selkoe *et al*., 2016 and references therein) and the results of this study are consistent with this broad picture. This genetic homogeneity is commonly related to large effective population sizes that limit the effects of genetic drift and life-history characteristics, which favour dispersal by ocean currents (Waples, 1998). This high connectivity may be due to the active migration of adults and/or larval dispersal (Cowen & Sponaugle, 2009). Therefore, the observed general absence of genetic structure in such a wide range, from the Mediterranean to the Atlantic, is not uncommon in marine organisms. Similar cases of panmixia have been reported in several bony fishes, such as *Dentex dentex* (Viret *et al*., 2018), *Sardinella aurita* (Stern *et al*., 2018), *Conger conger* (Miralles *et al*., 2016), *Epinephelus marginatus* (Schunter *et al*., 2011) and *Scomber japonicus* (Zardoya *et al*., 2004), thus underlining the important influence of ocean currents on larval dispersal.

The exploration of genetic diversity across the surveyed distribution, both at small-scale (Greek Seas) and large-scale (whole distribution range), was based on the use of three and one mitochondrial markers, respectively. The potential future use of multiple and highly polymorphic markers, such as microsatellite loci with different evolution rates and coalescent histories, may provide a more accurate view of the patterns and origin of the genetic diversity of the studied species. Furthermore, well-representative sampling, including a sufficient number of sequences from different parts of the distribution range of each species, would also contribute to achieving this goal. This study constitutes an important contribution to the phylogeography of mesopelagic fishes in the Eastern Mediterranean Sea and provides important data for future phylogeographic studies.

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Supplementary Data. The following supplementary information is available online for the article:
Table S1. General information on the H. benoiti specimens used in the present study. **Table S2.** General information on the M. muelleri specimens used in the present study. **Table S3.** General information on the M. muelleri specimens used in the present study. **Table S4.** Information on the COI sequences from Genbank and BOLD databases used for phylogeagraphic analyses.