



GENETIC DIVERSITY PATTERNS OF MESOPELAGIC FISH IN THE GREEK SEAS

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Abstract

Mesopelagic fish comprise the most abundant group of vertebrates in the marine environment as well as in the total biosphere. Despite their unique biological and ecological traits, research on this group has not been implemented extensively. The present study investigates the intraspecific genetic diversity of three mesopelagic fishes (*Maurollicus muelleri*, *Benthoosema glaciale*, *Hygophum benoiti*) in the Greek Seas. Analyses of three mitochondrial genes (COI, 12S, 16S) from 223 samples revealed a lack of phylogeographic structure for *M. muelleri* and *B. glaciale* across the studied area. However, *H. benoiti* specimens from the Corinthian Gulf differed from the rest of the populations, suggesting that the limited connection with the open sea may act as a barrier to gene flow. The shallow haplotype genealogy (high haplotype diversity and low nucleotide diversity) in association with recent demographic expansion events can be considered as the result of the recent evolutionary history of these species in the Greek seas.

Keywords: Phylogeography, genetic structure, genetic differentiation, mitochondrial DNA

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1. Introduction

Mesopelagic fishes are usually small sized species, which inhabit the twilight zone, usually defined as the depths between 200-1,000 m. Most species of this group undertake diurnal vertical migrations following their prey into the epipelagic zone to feed at night (Olivar *et al.* 2012). Mesopelagic fish play an integral role in marine food webs and global biochemical cycles (Irigoien *et al.* 2014). Furthermore, their large abundance makes them particularly attractive for exploitation (John *et al.* 2016). Yet, mesopelagic fauna still remains largely unknown and genetic studies for these species have been particularly scarce.

The aim of the present study is to explore the genetic diversity and phylogeographical patterns of three mesopelagic fishes (*M. muelleri*, *B. glaciale*, *H. benoiti*) in the Greek Seas.

2. Materials and Methods

Samples were collected from six locations in Greek Seas (Corinthian Gulf, Saronic Gulf, North Aegean Sea, Euboean Gulf, Cretan Sea, Ionian Sea) and from two locations of the Spanish Mediterranean, during various scientific expeditions carried out between November 2018 and October 2020. Mitochondrial genes cytochrome c oxidase subunit 1 (COI), 16S rDNA and 12S rDNA were partially sequenced and analysed. Molecular diversity indices for the concatenated dataset including the number of haplotypes (H), haplotype diversity (h), nucleotide diversity (π) and the number of private haplotypes (Hpr) were estimated for each sampled population using the software DNAsp v.5. The relationships between concatenated haplotypes were depicted with median-joining networks which were constructed in the software PopART. To test departures from neutrality Fu's F test and Tajima's D test were employed, both implemented in ARLEQUIN v.3.5.2.2.

3. Results and Discussion

Haplotype networks exhibited star-like shapes, which in combination with the observed high haplotype diversity and low nucleotide diversity values (Tables 1-3) indicate a signature of recent population expansion. Negative and statistically significant values of Tajima's D and Fu's F (Figure 1) further suggest recent demographic expansion for the three mesopelagic species.

According to the median-joining networks, no phylogeographic pattern was found among *M. muelleri* (Figure 1A) or *B. glaciale* (Figure 1B) populations. On the contrary, *H. benoiti* individuals from the Corinthian Gulf were differentiated from the rest of the studied populations. This was evident by the dominance of a haplotype in the samples from the Corinthian Gulf, which was different from the main haplotype shared by the individuals from the Saronic Gulf and the North Aegean Sea (Figure 1C).



The Corinthian Gulf is semi-closed with limited connection to the open sea and has unique hydrological and topographical characteristics. Therefore, gene flow between the Corinthian Gulf and the open sea could also be limited. One possible explanation of the fact that genetic differentiation was evident only in one of the three species may be that *H. benoiti* potentially has unique traits compared to the other species, limiting the distance of passive transfer by the currents. Strong currents and wind-driven circulation has been reported in the Corinthian Gulf (Drakopoulos & Lascaratos, 1998), which may affect the distribution of *H. benoiti* in the area.

Table 1: Molecular diversity indices for *M. muelleri* samples.

	N	Nh (Nph)	Hd	π (%)
Corinthian G.	14	10 (7)	0.923	0.118
Saronic G.	14	5 (2)	0.505	0.047
N. Aegean	14	9 (3)	0.879	0.099
Euboean G.	14	3 (1)	0.385	0.027
Cretan Sea	14	10 (6)	0.923	0.120
Ionian Sea	14	4 (2)	0.495	0.043
Spain (GSA01)	12	6 (2)	0.682	0.066
Spain (GSA06)	15	9 (5)	0.8	0.096
Total	111	36	0.722	0.078

N: number of samples, Nh: number of haplotypes, Nph: number of private haplotypes, Hd: haplotype diversity, π : nucleotide diversity

Table 2: Molecular diversity indices for *B. glaciale* samples.

	N	Nh (Nph)	Hd	π (%)
Corinthian G.	14	9 (8)	0.835	0.131
Saronic G.	14	8 (6)	0.769	0.102
N. Aegean	14	12 (9)	0.967	0.231
Euboean G.	14	9 (7)	0.879	0.122
Cretan Sea	14	8 (6)	0.824	0.113
Total	6	39	0.848	0.133

Table 3: Molecular diversity indices for *H. benoiti* samples.

	N	Nh (Nph)	Hd	π (%)
Corinthian G.	10	3 (2)	0.511	0.036
Saronic G.	10	5 (3)	0.533	0.052
N. Aegean	14	6 (5)	0.505	0.047
Total	34	12	0.761	0.042

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