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Range Extension of European Hake from The Eastern Black Sea Coasts of Turkey

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Abstract: Eight specimens of European hake, *Merluccius merluccius* (Linnaeus, 1758) were caught in 2021 by commercial gill net from the Eastern Black Sea coasts of Turkey. The total length of the specimens ranged from 12.3 to 22.3 cm. Mitochondrial gene regions of 16S rRNA and COI were analyzed to genetically characterize *M. merluccius* specimens. This record is the first confirmed report suggesting that *M. merluccius* has expanded its distribution range eastward in the Black Sea. The occurrence of the species at different times and in a wide depth range (20-92 m) strengthens our opinion that this species is adapted to the region. We highlight that the current status of environmental factors for a productive habitat may increase the biomass level of European hake in the long run in the Eastern Black Sea.

Keywords: Eastern Black Sea, First record, Expansion, Merlucciidae, Morphology, mtDNA

Berlam Balığının Türkiye'nin Doğu Karadeniz Kıyılarında Dağılımının Genişlemesi

Öz: Sekiz adet Berlam, *Merluccius merluccius* (Linnaeus, 1758), 2021 yılında Doğu Karadeniz'in Türkiye kıyılarında Ordu'da ticari galsama ağları ile yakalanmıştır. Bireylerin toplam boyları 12,3 cm ile 22,3 cm arasında değişmektedir. Mitokondriyal DNA'nın 16S rRNA ve COI gen bölgeleri analiz edilerek *M. merluccius* genetik olarak karakterize edilmiştir. Bu kayıt, *M. merluccius*'un Karadeniz'deki dağılım alanını doğuya doğru genişlettiğini gösteren ilk doğrulanmış rapordur. Türün farklı zamanlarda ve geniş derinlik aralığında (20-92 m) ortaya çıkması, bu türün bölgeye uyum sağladığı kanaatimizi güçlendirmektedir. Verimli bir habitat için mevcut çevresel faktörlerin Doğu Karadeniz'de uzun vadede Avrupa Berlamı'nın biyokütle seviyelerini artırabileceğini vurguluyoruz.

Anahtar Kelimeler: Doğu Karadeniz, İlk kayıt, Genişleme, Merlucciidae, Morfoloji, MtDNA

1. Introduction

The European hake, *Merluccius merluccius* (Linnaeus, 1758), is a demersal and benthopelagic species that mainly inhabits muddy bottoms of shallow (30 m) and deep (1000 m) waters. Adults feed mainly on fish (small hakes, sardines, anchovies, pilchard), while juveniles feed on crustaceans (Preciado et al., 2008). The European hake is among the main target species of demersal fisheries in the Western and Eastern Mediterranean Sea (Gücü & Bingel, 2011). It is one of the most heavily exploited fish species on the west coast of Europe (Casey & Pereiro, 1995).

The genus *Merluccius* comprises 12 species widely distributed along the coasts of Europe, America, and Africa. Phylogenetic analysis based on mitochondrial and nuclear sequences indicates the presence of two distinct clades: American clade and Euro-African clade (Campo et al., 2007; Perez et al., 2021; Quinteiro et al., 2000). The Euro-African clade includes the European hake. *M. merluccius* is widely distributed over the northeast Atlantic shelf (Arancibia, 2015), being more abundant from the British Isles to the south of Spain (Alvarez et al., 2004). Its range extends from Mauritania to off the western coast of the waters south of Iceland and Norway (Casey & Pereiro, 1995; International Council for the Exploration of the Sea [ICES], 2008). There is a limited number of available DNA sequences of *M. merluccius* throughout its distribution range.

The first occurrence of *M. merluccius* in the Black Sea was mentioned in the marine fish checklist for the Black Sea by Bilecenoğlu et al. (2014), referring to Ninni (1923). Various studies (Geldiay, 1969; Svetovidov, 1986) claim that European hake was found in the Black Sea. Different researchers have reported that European hake is sparsely distributed in the Eastern Black Sea, referring to Casey and Pereiro (1995). Still, there is no evidence of its existence in the Eastern Black Sea. Fishermen and fishmongers in the region were also unable to recognize the species and stated seeing it for the first time. This paper aimed to highlight, for the first time, the presence of European hake in the Eastern Black Sea.

2. Materials and Methods

Eight specimens of European hake were caught from the Eastern Black Sea coast, Fatsa, Ordu (41°02′21.61″N − 37°30′06.13″E) (Figure 1) between May and July in 2021. Specimens were obtained between 20 − 92 m depth by a commercial whiting gill net (18 mm mesh size). Specimens were identified morphologically according to Fischer et al. (1987) and genetically based on mtDNA sequences. Total length (TL) and total weight (TW) were measured to nearest 0.1 cm and 0.01 g, respectively. Sex was determined macroscopically, according to Gunderson (1993).

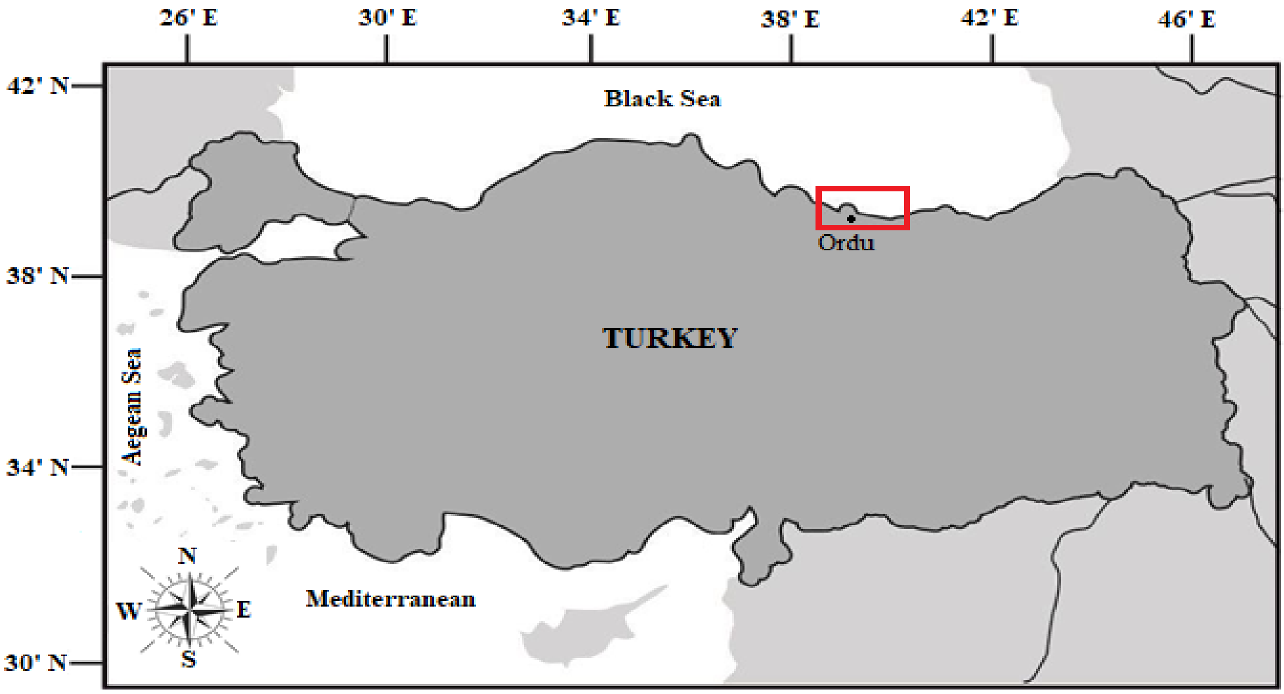

Figure 1. Study area. The red frame indicates the capture site of European hake in the eastern Black Sea coasts of Turkey.
Şekil.1. *Çalışma sahası. Kırmızı alan Türkiye'nin Doğu Karadeniz kıyılarında Berlam Balığının yakalandığı sahaı gösterir.*

Sequence analysis of two mitochondrial gene regions, 16S rRNA and cytochrome c oxidase subunit I (COI), were performed to identify and characterize specimens genetically. Genomic DNA was isolated from the fin clips of eight specimens using the Wizard SV Genomic DNA Purification Kit (Promega) following the manufacturer's protocol. Quantity and purity of DNA were assessed using nanodrop (NanoDrop 8000, Thermo Fisher). The 16S rRNA gene region was amplified with 16Sbr-H and 16Sar-L (Palumbi, 1996). The COI gene region was amplified with primers of Fish-F1 and Fish-F2 (Ward et al., 2005). PCR assay was performed in a total volume of 25 µl containing 12.5 µl 2X Master mix (Hibrigen), 1µM of each primer (forward and reverse), 100 ng DNA, and ultrapure water. The thermal cycling condition was as follows: 95 ºC for 3 min, followed by 35 cycles of 95 ºC for 50 s, 54 ºC for 45 s, and 72 ºC for 45 s with a final extension step of 5 min at 72 ºC. Amplicons were visualized on agarose gel and sequenced on ABI 3500 Genetic Analyzer (Thermo Fisher) with a Big Dye v.3.1 Terminator Cycle Sequencing Kit.

Raw sequences were trimmed and aligned in BioEdit (Hall, 1999) using the ClustalW algorithm (Thompson et al., 1994). Quality checked sequences were compared with reference sequences in the GenBank database (https://www.ncbi.nlm.nih.gov) using BLAST (Basic Local Alignment Search Tool), and species identification was performed by comparing sequence similarity. Phylogenetic relationships were inferred with a maximum likelihood tree using available COI and 16S rRNA sequences of *M. merluccius* with known geographic information. The reference COI sequences of *M. merluccius* (Accession numbers: KX782994, KJ709559, KJ205034, KC500932, JQ775075), *M. senegalensis* (GQ988403), *M. capensis* (JF286820), *M. albidus* (KF930125), *M. bilinearis* (MT455831) and 16S rRNA sequences of *M. merluccius* (FN688058, FN688061, FB688064, FN688067, FN688070, KC980962, DQ304654, KJ128827), *M. senegalensis*

(DQ274040), *M. capensis* (HQ592194), *M. albidus* (DQ274018), *M. bilinearis* (DQ274021) were retrieved from NCBI GenBank database. *Merlangius merlangus* was used as an outgroup. The appropriate model of sequence evolution for 16S rRNA and COI genes was determined based on the Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC) as Tamura-Nei (COI) and Kimura 2-parameter (16S rRNA). The robustness of the trees was tested with 1000 bootstrap replicates in Mega X (Kumar et al., 2018).

3. Results and Discussion

The generated partial COI and 16S rRNA sequences were 589 bp and 568 bp, respectively. A total of two haplotypes for COI and three haplotypes for 16S rRNA were identified from eight specimens. Comparison of COI and 16S rRNA sequences against the GenBank database using BLAST gave a successful match with available *M. merluccius* sequences with pairwise sequence identity similarity of 100% for COI and 99.65% for 16S rRNA genes. Generated COI and 16S rRNA sequences were deposited in GenBank (Accession numbers: COI, MZ540345-MZ540346; 16S rRNA, MZ540342-MZ540344). The maximum likelihood tree generated with COI and 16S rRNA revealed a clear separation of *M. merluccius* from other *Merluccius* species, in harmony with previous reports (Campo et al., 2007; Perez et al., 2021; Quinteiro et al., 2000). The Black Sea specimens nested with reference *M. merluccius* sequences, yet there was no clear separation based on geographic origin (Figure 2).

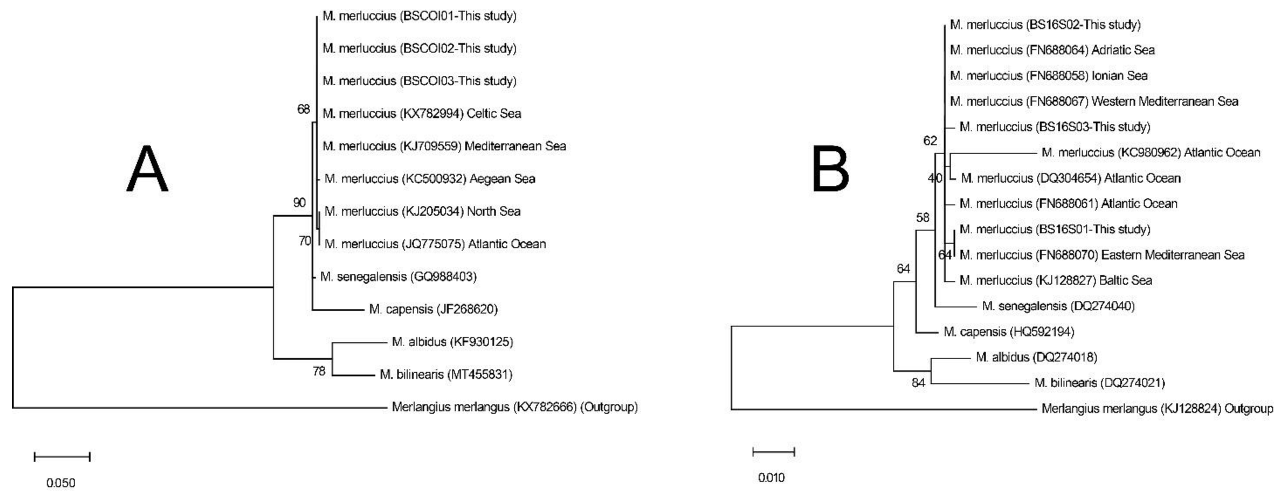

Figure 2. Maximum likelihood tree constructed with the COI (A) and 16S rRNA sequences (B) of *Merluccius merluccius* along with reference sequences obtained from the NCBI GenBank database.
Şekil 2. *Merluccius merluccius'un COI (A) ve 16S rRNA dizileri (B) ile birlikte NCBI GenBank veri tabanından elde edilen referans dizileri ile oluşturulan maksimum olasılık ağacı.*

The TL and TW of the sampled specimens ranged between 12.3 – 22.3 cm and 12.63 – 81.86 g, respectively (Figure 3). Sampling and morphological details of the European hake specimens are given in Table 1. The presence of the European hake was previously reported from the Mediterranean Sea (Çiçek & Avşar, 2010; Özvarol, 2014; Sangun et al., 2007), the Aegean Sea (Gurbet et al., 2013; Soykan et al., 2015), and the Marmara Sea coasts (Daban et al., 2020; Gül et al., 2019) of Turkey. The only evidence suggesting the presence of *M. merluccius* existence in the Black Sea was claimed by Türker and Bal (2018). The researchers caught the European hake during the bottom trawl surveys conducted in the Western Black Sea (Zonguldak-Amasra). To obtain more reliable results from the studies on fish stocks, if possible, different studies must be carried out separately for each fish species along with its length-weight relationships and updated within specific periods.

The Black Sea is a semi-enclosed basin connected to the Mediterranean Sea via the Bosporus Strait and Dardanelles. In recent years, the physical, chemical, and biological properties of the Black Sea have changed significantly with the impact of global climate change. In this process, fish species that have settled in the Black Sea ecosystem have been in constant change/development (van der Voo, 1990). New species are settling in the Black Sea, and the first sightings and new geographical records are increasing day by day (Aydın, 2015; Aydın, 2017; Aydın, 2020; Aydın & Bodur, 2018; Aydın & Gül, 2021; Engin et al., 2015; Göktürk et al., 2012; Öztürk & Özbulut, 2016).

Figure 3. The sampled specimen of *Merluccius merluccius* with 22.3 cm TL, captured from the eastern Black Sea coast on July 18, 2021

Şekil.3. *Merluccius merluccius'un Doğu Karadeniz kıyısından yakalanan 22,3 cm TL boya sahip örneği, 18 Temmuz 2021*

Table 1. Capturing details, total length (cm) and total weight (g) of sampled *Merluccius merluccius* individuals by sex

Çizelge 1. *Örneklenen Merluccius merluccius bireylerinin cinsiyete göre yakalama detayları, toplam uzunluk (cm) ve toplam ağırlıkları (g)*

Capture date	Capturing depth (m)	Total length (cm)	Total weight (g)	Sex
03.05.2021	90	12.4	12.63	Male
28.05.2021	84	12.3	13.85	Male
13.06.2021	92	15.3	27.09	Female
22.06.2021	20	15.0	22.29	Female
09.07.2021	45	16.8	35.60	Female
09.07.2021	58	16.9	32.41	Female
14.07.2021	64	15.5	26.05	Male
18.07.2021	50	22.3	81.86	Female

Environmental factors are usually admitted as the main factors controlling the spatio-temporal distribution of fish populations (Planque et al., 2011). Fish distribution is affected by several biotic and abiotic factors, such as food availability and temperature and may be influenced at the same time by various variables and conditions (Zheng et al., 2002). The environmental conditions such as temperature (11.8 – 15.0 °C), chlorophyll-*a* (0.1 – 0.9 mg·m^3) and food availability (crustaceans and small pelagic) might play a key role in the spatial distribution of the biomass of European hake as previously reported (de Pontual et al., 2015; Druon et al., 2015; Sion et al., 2019; Vasilakopoulos et al., 2014). Yalçın and Gurbet (2016) described that the higher *M. merluccius* abundances were found in deeper from 50 m with salinity >38.55 ‰ and water temperature ranging from 14.5 to 19 °C. Sakallı and Başusta (2018) reported that the mean annual surface water temperature has varied between 12 and 17 °C in the last 34 years in the Black Sea, and the relative increase in average surface water temperature is predicted to be 5.1 °C at the end of this century. In the coastal waters of Turkey in the Black Sea, the Chl-*a* concentrations vary from 0.20 to 1.23 mg·L^{-1} in the coastal waters and decrease in the open waters to 0.22 − 0.90 mg·L^{-1} (Polat Beken et al., 2017). The potential of crustaceans and small pelagic fish (anchovy and sardine) in the Black Sea (Gücü et al., 2017) might support *M. merluccius* existence in the long term. The European hake stocks in the Mediterranean Sea suffer from the fishing pressure, with a fishing mortality rate that is on average five times higher than the target fishing mortality level (Food and Agriculture Organization [FAO], 2016). Despite its regional importance, exploitation due to fishing mortality is also present in the Aegean Sea (Gurbet et al., 2013) and the Sea of Marmara (Gül et al., 2019). All types of trawling (bottom and beam) are prohibited in the Eastern Black Sea (from Ordu to Turkish Georgian border) due to having a very narrow continental shelf. Gillnets, hand lines and deep-water cast nets that are less harmful to the benthic ecosystem are used for demersal fisheries (especially whiting) in the Eastern Black Sea (Karadurmuş et al., 2021). If the European hake forms a population in this area, it is thought that it will be less exposed to overfishing, unlike other areas (the Mediterranean Sea, the Aegean Sea, the Sea of Marmara, and even the Western Black Sea).

4. Conclusion

The present individuals were obtained through fishing. This finding is the first confirmed report suggesting that *M. merluccius* has an eastward distribution range in the Black Sea. The occurrence of the species at different times and in a wide depth range strengthens our opinion that this species has adapted to the region. We highlight that the current status of environmental factors for a productive habitat may increase the biomass level of European hake in the long run in the Eastern Black Sea. We estimate that if European hake adopts this region, it may create a population in the long term and become a sustainable fishery resource thanks to comparatively lower fishing pressure in the area. It should be noted that further studies are needed to understand the existence of female adults and juveniles.

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