

## Research Article

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# Genetic investigation of Cuvier's beaked whale, *Ziphius cavirostris*, along the coast of Türkiye and Northern Cyprus, based on mtDNA sequences

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## Abstract

The Cuvier's beaked whale, *Ziphius cavirostris*, is a cosmopolitan species and the only beaked whale species commonly found in the Mediterranean Sea. Five strandings of Cuvier's beaked whale were reported along the Aegean/Mediterranean Seas coasts of Türkiye and northern coast of Cyprus in 2016–2017. Mitochondrial DNA (mtDNA) control region (430–444 bp) and cytochrome b (cytb) (382–424 bp) sequences each revealed two different haplotypes (four out of five individuals had the same haplotype for each locus) on these stranded animals. The control region haplotypes were identical to two previously identified haplotypes from the Ionian (Greece) and Adriatic (Croatia) Seas. Only one of the cytb haplotypes had previously been described from the Adriatic Sea (Italy) and the other one was detected for the first time. In a comparison of these haplotypes with Cuvier's beaked whale haplotypes previously reported from outside the Mediterranean Sea, the Mediterranean subpopulation shows genetic differentiation based upon the presence of two unique haplotypes. Additional mtDNA sequences from the Mediterranean Sea are needed for a better understanding of the genetic population structure of this species and to elaborate more concrete conservation measures.

## Introduction

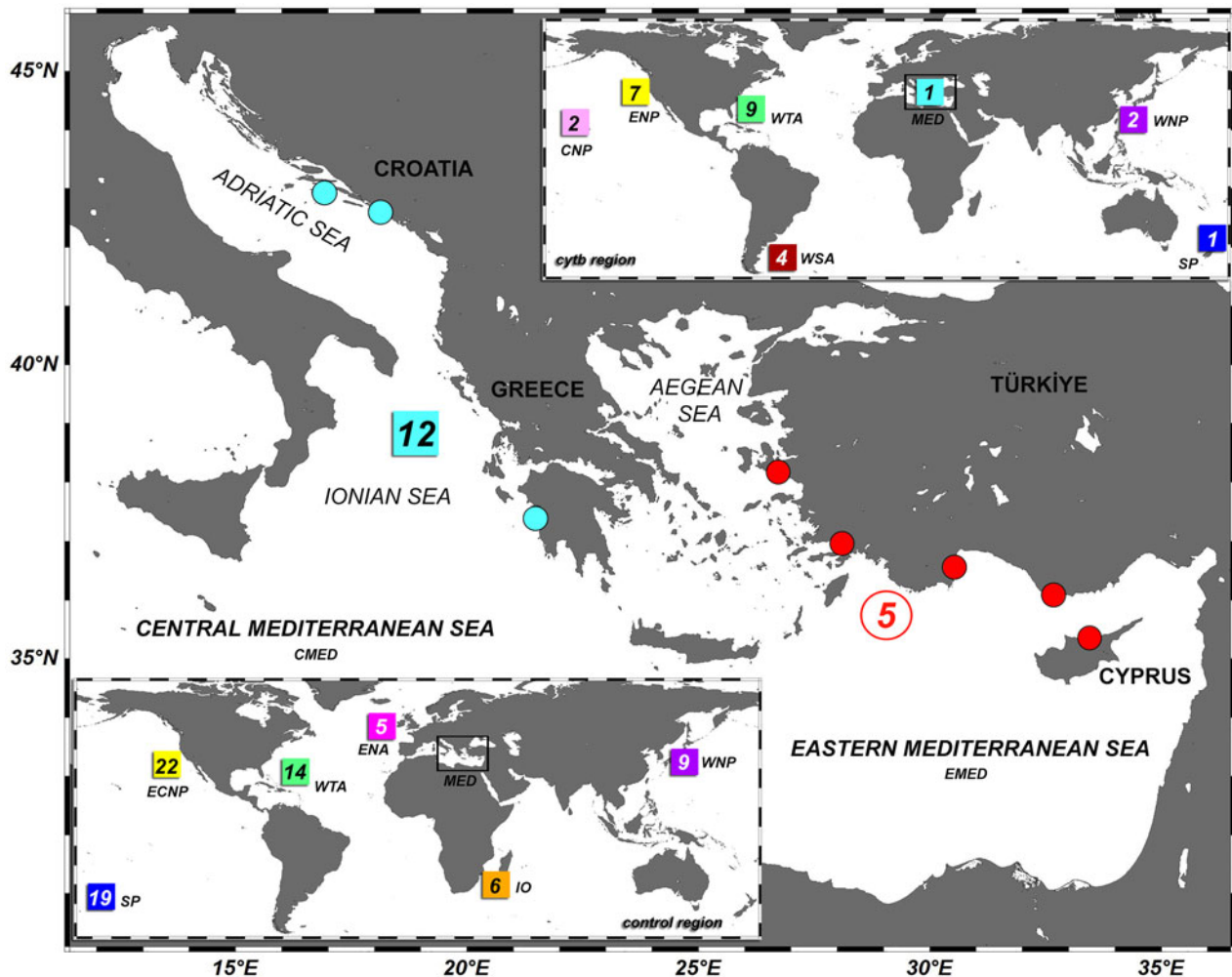
Cuvier's beaked whales, *Ziphius cavirostris*, are widely distributed in the offshore waters of all oceans (Jefferson *et al.*, 2015), including the Mediterranean Sea. Podesta *et al.* (2006) compiled the records of 316 strandings of this species between 1803 and 2003 in the Mediterranean Sea. In Türkiye, 13 stranded Cuvier's beaked whales were reported between 1964 and 2016, according to published records (Öztürk *et al.*, 2011, 2016; Bachara and Norman, 2013). This species is known to be sensitive to noise pollution, indicated by stranding cases due to seismic surveys or naval sonar exercises (Heyning and Mead, 2009; Podesta *et al.*, 2016). Beaked whale strandings in the Mediterranean have been significantly correlated with naval activities (Filadelfo *et al.*, 2009). Sonar-associated Cuvier's beaked whale strandings are well known in the study area, particularly in the Greek islands, some of which are: 14 in 1996 in the Kyparissiakos Gulf, nine in 1997 in the Ionian Sea (Frantzis, 1998, 2004) and seven in 2014 in Crete (Jasny, 2014).

Although the number of samples and studies are limited, Cuvier's beaked whales in the Mediterranean diverged approximately 0.5 mya and are considered to be genetically isolated from those in the Atlantic, due to substantial differentiation in mitochondrial DNA (mtDNA) and nuclear DNA, low genetic diversity and private haplotypes found only in the Mediterranean (Dalebout *et al.*, 2005; Onoufriou *et al.*, 2022). Within the Mediterranean, two genetically distinct populations, western (samples from Ligurian Sea) and eastern (samples from Ionian, Adriatic, Aegean Seas, south of Crete and Israel coast), have been identified (Onoufriou *et al.*, 2022).

The goal of this study is to compare the mtDNA data of new Cuvier's beaked whale specimens from the Aegean Sea and the eastern Mediterranean Sea, a poorly sampled region, with the rest of the Mediterranean and world oceans. This provides a better understanding of the population genetic structure of Cuvier's beaked whales in the Mediterranean Sea in comparison with those in the broader geographic area and contributes to the body of knowledge required to assess the conservation status of the populations in the Mediterranean Sea.

## Materials and methods

Tissue samples were obtained from Cuvier's beaked whales found stranded along the Aegean/Mediterranean Seas coasts of Türkiye ( $N=4$ , June 2016 and November 2017) and northern Cyprus Island ( $N=1$ , March 2017) (Figure 1) (Supplementary Table S1). In Figure 1, the



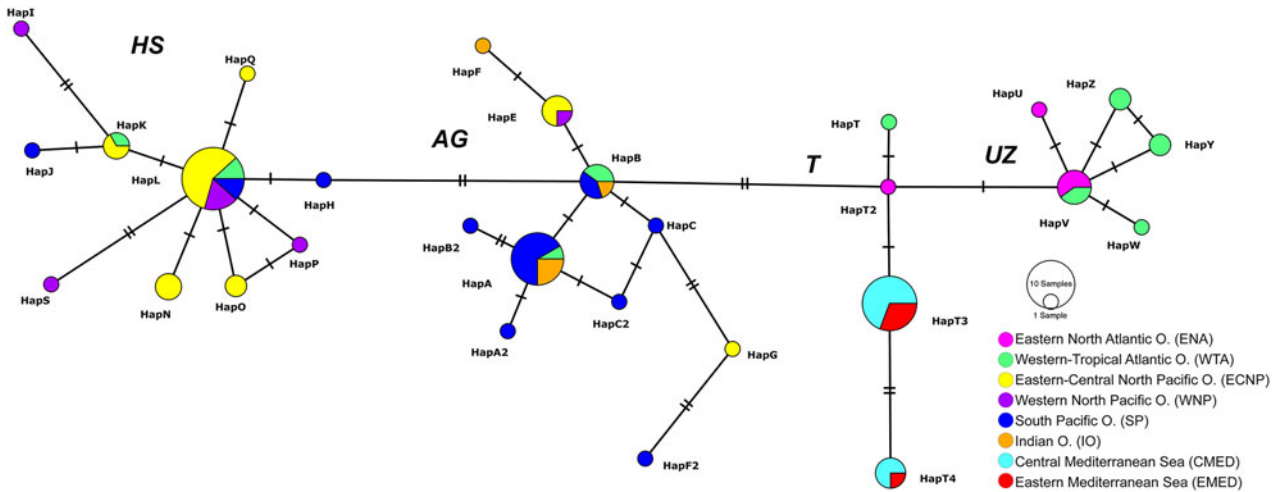
**Figure 1.** Locations of Cuvier's beaked whale samples used in the study. Numbers indicate sample sizes. Circled numbers represent sequences generated in this study; numbers in squares refer to sequences obtained from GenBank. Main and bottom left map/control region: Dalebout *et al.* (2005); top-right/cybt region: Otley *et al.* (2012), Morin *et al.* (2013) and Montelli *et al.* (2016).

locations of the samples used in this study are indicated by red dots. Additionally, Figure 1 displays the oceanic regions from which other sequences were obtained from GenBank. The details of sample collection have been reported in Öztürk *et al.* (2018). The samples were collected from skin or heart tissues and preserved in 20% DMSO saturated with sodium chloride or 70% ethanol at 20°C. DNA was extracted using QIAGEN MagAttract HMW DNA Kit following the manufacturer's protocol. The mtDNA control region was amplified using the PCR primers M13-Dlp1.5-L (5'-TGTA AACGACGGCCAGTTCACCCAAA GCTGRARTTCTA-3') and Dlp5-H (5'-CCATCGWGA TGTCTTATTTAAGRGAA-3') (Dalebout *et al.*, 2005), and for the cytochrome b (cytb) gene, the GLUDG-L (5'-TGA CTTGAARAACCAAYCGTTG-3') and CB2-H (5'-CCCTCA GAATGATATTTGTCCTCA-3') primer set was used (Dalebout *et al.*, 2004). PCR reactions were carried out in 25 µl volume including 0.5 µl of each 10 µM primer, 12.5 µl of 2× Fast PCR Master mix with Taq polymerase (AMBRD Laboratories, Türkiye), 1 µl of 25 mM MgCl<sub>2</sub> and 1 µl of DNA and 9.5 µl of ddH<sub>2</sub>O. The amplification profile consisted of a 4 min preliminary denaturation at 95°C, followed by 30 cycles of denaturation at 95°C for 10 s, annealing at 55°C for 30 s and extension at 72°C for 45 s. An extension step at 72°C for 5 min completed the reaction. Nanodrop was used to measure concentration of the PCR products. The amplified DNA products were sequenced commercially (Macrogen, Korea). Sequences were edited with Sequencher

v.4.8 (Gene Codes Corp., USA). Previously recorded control region (Dalebout *et al.*, 2005) and cytb gene sequences (Otley *et al.*, 2012; Morin *et al.*, 2013; Montelli *et al.*, 2016) of *Z. cavirostris* were retrieved from GenBank (the accession numbers are listed in Supplementary Tables S2, S3 and localities are shown in Figure 1) and aligned with the new sequences using CLUSTALX 2.1 (Larkin *et al.*, 2007). Haplotype diversity (Hd), nucleotide diversity ( $\pi$ ) and number of haplotypes (h) were computed with DnaSP v.6 (Rozas *et al.*, 2017). Minimum spanning haplotype networks were constructed via PopART (Bandelt *et al.*, 1999). Because gaps of cytb gene are treated as missing data by PopART, 16 indel-containing haplotypes were treated as identical to 13 other indel-free haplotypes (Hap\_3 and 4 in Hap\_2, Hap\_15 in Hap\_9) by the program.

## Results

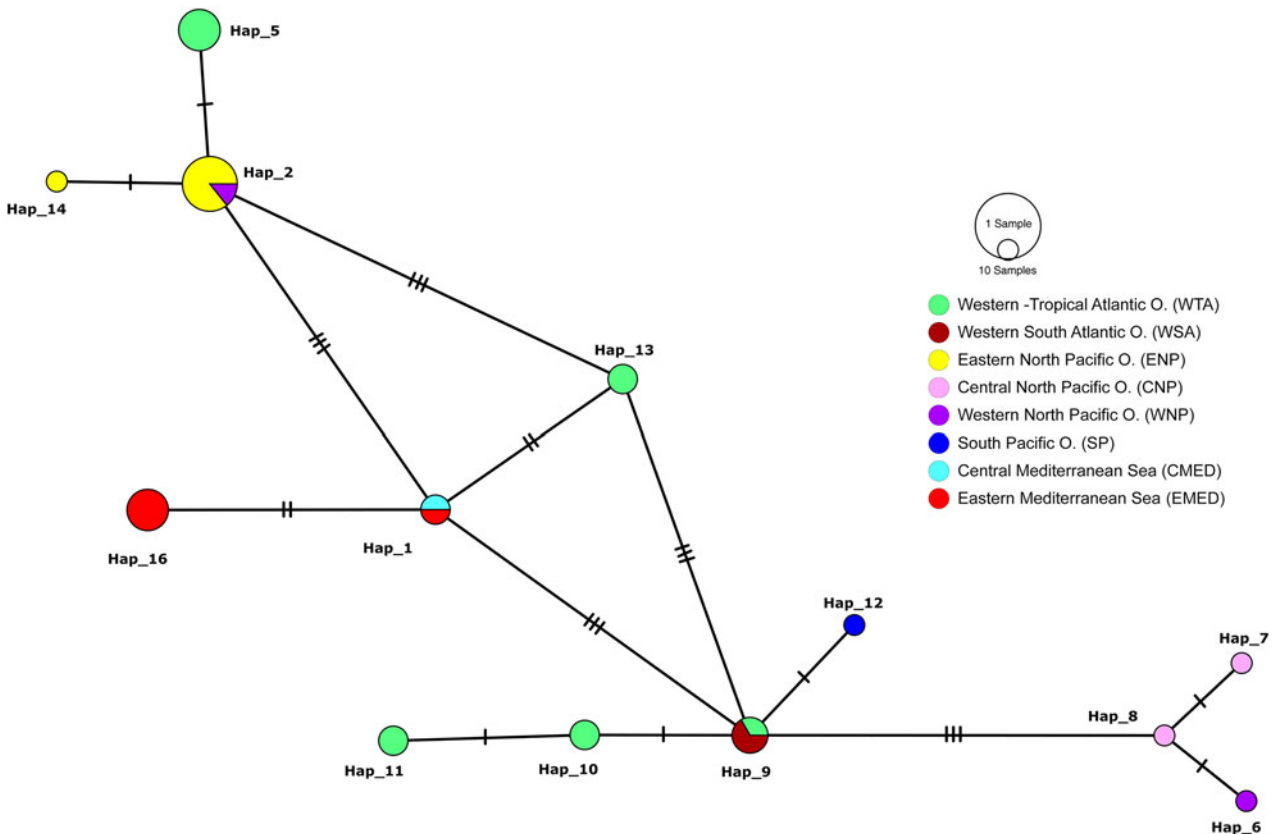
New mtDNA control region and cytb sequences of lengths ranging between 430–444 and 382–424 bp, respectively, were obtained from five stranded Cuvier's beaked whales found along the coasts of Türkiye and Cyprus. Two haplotypes were obtained for the control region, and the first of these haplotypes was found in four individuals while the second haplotype was found in one individual. The same pattern of two haplotypes being found in four and one individuals, respectively, was observed for the cytb region (Supplementary Table S1). The second haplotype detected



**Figure 2.** Minimum spanning haplotype network for mtDNA control region of *Ziphius cavirostris*. Haplotype group definitions (HS, AG, T, UZ) are from Dalebout *et al.* (2005).

in both the control region and *cytb* was found in the same individual, which was sampled from Seferihisar, İzmir, Türkiye. The *cytb* haplotype in this individual was a new one recorded for the first time in this study. These sequences have been submitted to GenBank with accession numbers OK570378-9 and OK584066-7. As shown in the haplotype networks for the two genes (Figures 2, 3), there are two nucleotide differences between the two haplotypes for each gene. One of our eastern Mediterranean haplotypes in both control region and *cytb* networks was shared with central Mediterranean (the Ionian Sea and the Adriatic), suggesting connectivity of the two populations.

Also, the low nucleotide diversity was observed for both genes, with the values of 0.00186 and 0.00203 for control region and for *cytb*, respectively (Table 1) support the notion of high connectivity of the populations or even that they belong to the same population. The control region haplotypes found in this study were identical to two previously identified haplotypes from the Ionian (Greece) and Adriatic (Croatia) Seas (T3 and T4 haplotypes), which are the only additional areas within the Mediterranean Sea (Dalebout *et al.*, 2005) with mtDNA data for *Z. cavirostris*. One *cytb* haplotype (Hap1) was the same as a previously described haplotype from the Adriatic (Italy) Sea



**Figure 3.** Minimum spanning haplotype network for mtDNA *cytb* region of *Ziphius cavirostris*. Circles represent the haplotypes identified by Otley *et al.* (2012), Morin *et al.* (2013) and Montelli *et al.* (2016), except those in red (this study).

**Table 1.** Population diversity parameters for control and cytb regions

	Number of sequences	Number of haplotypes (h)	Haplotype diversity Hd	Nucleotide diversity $\pi$
Control region	5	2	0.4	0.00186
Cytb region	5	2	0.4	0.00203

(Montelli *et al.*, 2016), but the second haplotype (Hap16) has not previously been reported.

## Discussion

The previous studies on *Z. cavirostris* phylogeography have indicated that the Mediterranean basin was significantly differentiated from the other global basins (Dalebout *et al.*, 2005; Onoufriou *et al.*, 2022). These studies support our results by finding that the isolated Mediterranean subpopulation of *Z. cavirostris* was found to be differentiated based upon the presence of unique haplotypes, which were not found outside the Mediterranean Sea. At the same time, the results suggest that the Cuvier's beaked whales in the Aegean Sea and Levantine Basin (eastern Mediterranean Sea) have some degree of genetic connectivity to the Adriatic and Ionian populations (central Mediterranean Sea). As the haplotypes discovered in this study in both Dloop and cytb genes only matched those in the Mediterranean or were novel haplotypes, the results of our study add partial support to the notion of differentiation of the Mediterranean *Z. cavirostris*.

*Ziphius cavirostris* is an oceanic species that prefers steep slope habitats, especially those indented by underwater canyons and other complex topographic features (Cañadas *et al.*, 2017). The conservation status of its Mediterranean population has been changed from Data Deficient (DD) to Vulnerable (VU) in the IUCN Red List of Threatened Species (Cañadas and Notarbartolo di Sciarra, 2018). In the eastern Mediterranean Sea, this species is regularly seen in the southern Adriatic Sea, along the Hellenic Trench to the west of Cyprus, especially around the Finike (Anaximander) Seamount and the eastern Mediterranean Sea off Lebanon and Israel (Notarbartolo di Sciarra and Tonay, 2021). The northern part of Levantine Basin, precisely the area between the Finike Seamounts, Antalya Gulf and Adana Trough, could be an important habitat for this species (Woodside *et al.*, 2006; Akkaya Baş *et al.*, 2016; Awbery *et al.*, 2022; Dede *et al.*, 2022). The Rhodes Basin and Anatolian Canyons, which comprise deep-sea upwelling zones, provide feeding grounds for cetaceans (Öztürk *et al.*, 2012). Four areas have been suggested as High Sea Marine Protected Areas (HSMPAs) in the eastern Mediterranean Sea by Öztürk (2009). One of them is between Rhodes and Finike, where the most distinctive features include submarine canyons and the Finike (Anaximander) Seamounts, which had been declared as a Special Environment Protected Area (SEPA) since 2013 by the Republic of Türkiye. As a result, additional research on this species in this important area is required to support the possibility of expanding the existing SEPA to encompass the canyon region.

During the 5<sup>th</sup> Meeting of the Parties for the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic Area (ACCOBAMS) (Morocco, November 2013), the member states adopted a resolution on the areas where sonar should be avoided to protect the Cuvier's beaked whales in the Mediterranean Sea (ACCOBAMS, 2013). Therefore, a precautionary approach in terms of their conservation has been initiated, although the Eastern Mediterranean Sea was shown as one of the gaps. The present study provided some further (or additional) evidence for the genetic isolation

of the Mediterranean population from the other seas, which should be taken into consideration when elaborating conservation measures for this species. Further studies are needed to understand their distribution in the Mediterranean Sea, so that conservation measures against anthropogenic stress such as seismic surveys and naval exercises can be managed appropriately.

Onoufriou *et al.* (2022) showed that the differentiation observed in marine taxa due to the Sicilian Strait, which divides the Mediterranean Sea into west and east, is also seen in *Z. cavirostris*. We acknowledge that our inferences are based on a limited number of samples; it is important that it fills the sample gap especially eastern Mediterranean population of *Z. cavirostris*. We believe that although not many, the genetic studies on beaked whale provide a good starting point for initiation of conservation efforts and the formulation of effective management strategies. For instance, Onoufriou *et al.* (2022) proposed the two populations in the Mediterranean Sea to be categorized as ESUs (Evolutionarily Significant Units) and DIPs (Demographically Independent Populations). Based on our results, it would be appropriate to extend this categorization for the eastern Mediterranean population to include the waters of Türkiye and Cyprus. To gain more comprehensive insights into the population structure of this species within the Mediterranean Sea, particularly in the western Mediterranean (such as the Alboran Sea), it is essential to analyse additional samples using the same genetic markers. This broader dataset will enable the formulation of more precise conservation strategies for the species in this region.

**Supplementary material.** The supplementary material for this article can be found at <https://doi.org/10.1017/S0025315424000079>

**Data availability.** The mtDNA control region and cytb sequences are available in GenBank and can be found under the accession numbers OK570378-9 and OK584066-7.

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**Author contributions.** A. M. T. and R. B. planned the study. A. M. T., A. D., E. D., I. A. D., İ. T., C. D. and A. A. Ö. collected the samples. K. K., B. U. and R. B. performed the laboratory work and conducted data analysis. A. M. T. and R. B. wrote the initial manuscript draft with input from all the authors. All authors contributed to the article and approved the submitted version.

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**Competing interest.** No potential conflict of interest was reported by the authors.

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