Mammal Review

PRACTICE INSIGHTS OPEN ACCESS

Seeing Through the "Clouds" With Molecular "Eyes". First eDNA-Based Detections of Pygmy Sperm Whale (*Kogia breviceps*) in the Mediterranean Sea

Elena Valsecchi[1,2] | Alessia Rota[1,3] | Graziella Pupillo[1] | Natalia Fraija-Fernández[4] | Juan-Antonio Raga[4] | Fulvio Maffucci[5] | Paolo Galli[1,2] | Antonella Arcangeli[6]

[1]Department of Environmental and Earth Sciences, University of Milano-Bicocca, Milan, Italy | [2]MaRHE Center, Magoodhoo, Republic of Maldives | [3]National Biodiversity Future Center (NBFC), Palermo, Italy | [4]Marine Zoology Unit, Cavanilles Institute of Biodiversity and Evolutionary Biology, University of Valencia, Valencia, Spain | [5]Marine Animal Conservation and Public Engagement Department, Stazione Zoologica Anton Dohrn, Naples, Italy | [6]ISPRA BIO Dep, Rome, Italy

Correspondence: Elena Valsecchi (elena.valsecchi@unimib.it)

Received: 8 September 2025 | **Revised:** 18 February 2026 | **Accepted:** 24 February 2026

Keywords: cetaceans | ferry | Kogidae | marine mammals | MarVer | metabarcoding

ABSTRACT

Introduction: The pygmy sperm whale (*Kogia breviceps*) is globally distributed but considered absent in the Mediterranean Sea, with no confirmed sightings to date. Environmental DNA (eDNA) analysis offers a non-invasive, cost-effective, and highly sensitive tool for detecting marine species where direct observation is challenging.

Methods: An extensive eDNA metabarcoding analysis was conducted on 393 samples collected during the LIFE-CONCEPTU MARIS multidisciplinary monitoring programme (October 2022–October 2024). Mitochondrial 12S-rDNA and 16S-rDNA amplicons were compared with reference databases and with sequences from stranded *Kogia* individuals from Atlantic regions adjacent to the Mediterranean and from the Mediterranean itself to ensure robust species identification.

Results: At least five independent detections of *K. breviceps* were recorded from Gibraltar to the Tyrrhenian Sea. Repeated signals and high read abundance support detection reliability. Spatio-temporal patterns and haplotype diversity suggest the presence of multiple individuals, possible seasonal occurrence, with detections significantly associated with nocturnal sampling.

Discussion: The study provides the first molecular detection of *K. breviceps* in the Mediterranean and shows that eDNA is effective in detecting the species, possibly facilitated by the release of DNA-rich defensive fluids characteristic of the genus. The lack of visual records likely reflects limited surface activity and historical exclusion from Mediterranean cetacean check-lists, suggesting that some unidentified small-cetacean sightings may correspond to this species.

Synthesis and Recommendations: This study highlights the value of integrated molecular, acoustic and visual monitoring and recommends enhancing multidisciplinary monitoring frameworks to better understand the distribution and ecology of this poorly known, rare and elusive species.

1 | Introduction

The genus *Kogia* (Family Kogiidae) comprises two extant small cetacean species: the pygmy sperm whale (*Kogia breviceps*, de Blainville 1838) and the dwarf sperm whale (*Kogia sima*, Owen 1866). These elusive Odontocetes are considered inhabitants of warm-temperate to tropical oceans globally, including continental slope regions, although they are rarely observed alive and most knowledge derives from strandings and necropsy data (Baird 2026). Safeguarding lesser-known cetacean species, such

as members of the Kogiidae family, is crucial to ensuring a comprehensive understanding of marine biodiversity and the ecological balance of oceanic ecosystems.

Kogia breviceps typically grows to about 3.3 m, feeding by suction on squid at mesopelagic depths, whereas *K. sima* is slightly smaller (hardly reaches 3 m), differentiated in part by dorsal-fin placement and cranial morphology (Bloodworth and Odell 2008). Phylogenetic studies employing mitochondrial markers have confirmed their distinct species status and suggest that *K. sima* may actually comprise multiple cryptic lineages (Chivers et al. 2005; Maggioni et al. Submitted).

The Mediterranean Sea is a unique marine region, shaped by its geological history, physical dynamics, and exceptional biodiversity (Coll et al. 2010). Being a semi-enclosed basin acts like a crossroads for species from the Atlantic, Red Sea, and Indo-Pacific region. Among the 27 cetacean species listed as either regular ($n = 14$), visitor ($n = 3$), or vagrant ($n = 10$) in the Mediterranean Sea, Black Sea, and adjacent areas, the pygmy sperm whale (*Kogia breviceps*) is not listed (diNotarbartolo Sciara and Tonay 2021).

Records of the genus *Kogia* in the Mediterranean Sea are extremely rare. Fossil evidence indicates that some kogiid taxa (e.g., *K. pusilla*) occurred in the Mediterranean during the Pliocene, based on teeth, periotic and skull remains found in Tuscany and Malta (Collareta et al. 2019). In contrast, modern occurrences are limited to a few strandings, all referring to the dwarf sperm whale, *K. sima*. Three cases along the Italian coasts: one in 1988 in the North Eastern Tyrrhenian Sea (Baccetti et al. 1991), one in 2002 in Southern Sicily (Bortolotto et al. 2003), and a third one in 2017 in Central-Eastern Tyrrhenian close to Salerno (Maio et al. 2017). The two latter stranded specimens were genetically confirmed as *K. sima* in Maio et al. (2024). An additional stranding event along the Moroccan Mediterranean coast (Mnar-Tanger, Strait of Gibraltar, April 2015) also refers to a dwarf sperm whale specimen (Benchoucha et al. 2018). *K. breviceps* has lacked confirmed strandings in the Mediterranean Sea, although it is present in adjacent NE Atlantic waters (e.g., Martín et al. 2021). Thus, only the dwarf sperm whale, *K. sima*, is listed under ACCOBAMS (Agreement on the Conservation of Cetaceans in the Black Sea, Mediterranean Sea and Contiguous Atlantic Area) Annex I, affording it protective status even when accidental or occasional records occur within the Agreement area (diNotarbartolo Sciara and Tonay 2021).

Environmental DNA (eDNA) refers to genetic material obtained directly from environmental samples (such as water, soil, or air) without the need to capture or observe the target organism/s (Taberlet et al. 2012; Thomsen and Willerslev 2015). It encompasses both cellular and extracellular DNA fragments that organisms release into their surroundings through processes including excretion, secretion, skin sloughing, reproduction, or decomposition (Turner et al. 2014). By analysing this genetic material, eDNA methods enable the detection and monitoring of species presence, distribution, and community composition, providing a powerful and non-invasive tool for biodiversity assessment (Bohmann et al. 2014; Barnes and Turner 2016). Environmental DNA (eDNA) has recently emerged as a novel and transformative tool for monitoring marine mammals (Suarez-Bregua et al. 2022).

In the present communication we produce molecular evidence of the presence of *K. breviceps* in Mediterranean waters, probably not as a vagrant species. Consequently, we urgently advocate for its formal inclusion in the ACCOBAMS protection list. Recognising this cryptic population is a critical first step toward targeted monitoring, habitat protection, and ultimately, safeguarding this poorly understood yet persistent component of Mediterranean biodiversity.

2 | Methods

This first-occurrence report emerges as a by-product of an extensive eDNA monitoring program within the LIFE-CONCEPTU MARIS project (https://webgate.ec.europa.eu/life/publicWebsite/project/details/5707), which uses commercial ferries as an opportunistic platform for collecting data on cetofauna and sea turtles in the Central-Western Mediterranean, using a multidisciplinary approach that combines visual observation with new monitoring methodologies including extensive environmental DNA sampling (Arcangeli et al. 2022).

A total of 393 eDNA samples were collected from operating ferries according to the protocol described in Valsecchi et al. (2021) and now visible also in the video-publication by Rota and Valsecchi (2025). Samples were collected either seasonally in correspondence to 81 Fix Sampling Stations (FSSs) scattered along the monitored routes spread out from Gibraltar to the Adriatic-Ionian ($n = 364$) or on occasion of cetacean sightings regarding rare species ($n = 29$) (Figure 1). The vast majority of samples ($n = 373$, 94.9%) were collected between the 4th of October 2022 and the 23rd of October 2024, while the remaining samples ($n = 20$, 5.1%) were collected in summer 2019 (from the 20th of June to the 17th of September). The samples were collected during 39 "Sampling Cruises", hereafter SC, defined as a discrete period in which the research teams get on board in a port and return to the same port, remaining on board from 1 to 7 days for sampling and collecting visual data. Each SC touches from two up to five different ports and, depending on the length, surveys 4 to up to 15 FSSs.

Each sample consisted of 12 L of seawater, collected in the ferries' engine rooms via a dedicated seawater intake and temporarily stored in a Bag-in-Box container. Seawater was continuously running through the collection pipe to ensure that each sample represented local seawater, rather than residual water from previous collections. Filtration was performed on board immediately after collection, with each water bag (i.e., sample) yielding three replicates of 4 L each. To prevent cross-contamination and exposure to ambient air, the filtering station was sterilised between samples with a 10% bleach solution, and the entire water flow path (from the Bag-in-Box tap to the filter membrane) was housed within a sealed chamber. This procedure is illustrated in the video associated to Rota and Valsecchi (2025). Each replicate was filtered through a mixed cellulose ester (MCE) membrane with 0.45 μm–porosity, 4.7 cm–diameter (Rota and Valsecchi 2025). After filtration, filters were stored in a freezer on board, and transported frozen to

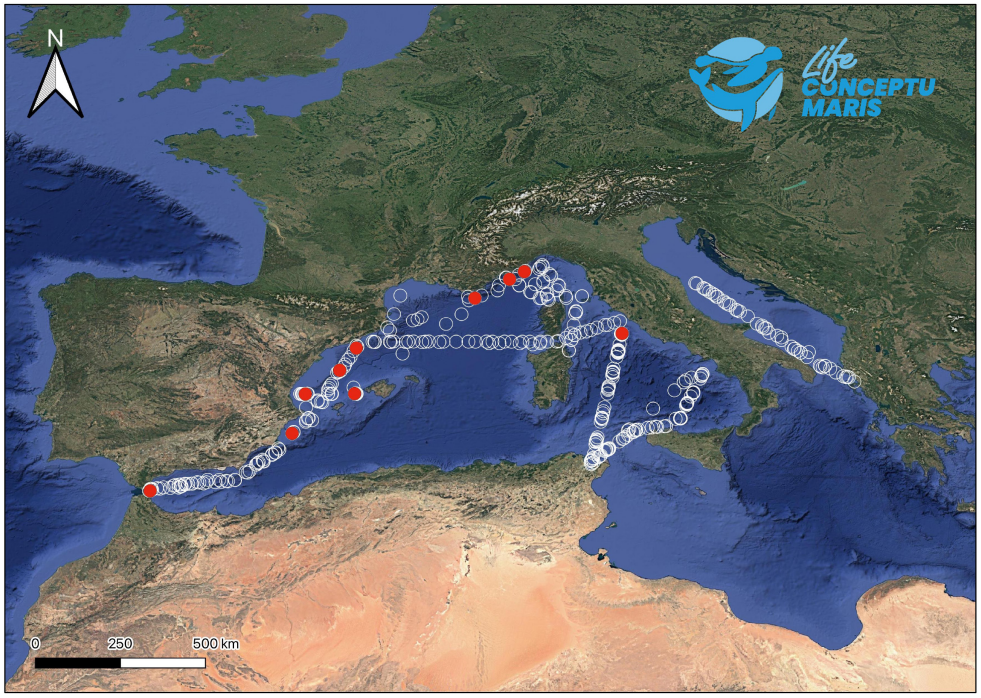


FIGURE 1 | Map showing the 10 positive detections of *Kogia breviceps* (red dots). White-lined circles indicate the eDNA sampling sites of the LIFE-CONCEPTU MARIS project, representing the locations where 393 samples were collected between October 2022 and October 2024 and summer 2019.

the University of Milano-Bicocca laboratory for further processing. Environmental DNA (eDNA) was extracted from the filters using the DNeasy PowerSoil Pro DNA Extraction Kit (Qiagen), following the manufacturer's protocol. Each of the three sample's replicates were extracted and amplified separately and eventually pooled together before subsequent steps (library preparation and sequencing).

Samples were subjected to metabarcoding analysis using two vertebrate-specific markers, targeting portions of the mitochondrial 12S-rDNA (ca 202 bp) and 16S-rDNA (ca 245 bp) genes and amplified with the primers MarVer1 and MarVer3, respectively (Valsecchi et al. 2020). The use of two distinct sets of vertebrate primers was intended to achieve broader taxonomic coverage for both mammals and other vertebrates (including fish and turtles) by spanning two mitochondrial DNA regions (e.g., De Barba et al. 2014). Each region may perform better for certain vertebrate classes, and previous studies have demonstrated that these markers provide overlapping yet complementary coverage (Valsecchi et al. 2020; Boyse et al. 2024). A positive control, which contained a mock freshwater pond community spiked with two non-Mediterranean cetacean species, was included in all runs to assess cross-contamination. DNA metabarcoding library preparation and sequencing were carried out by AllGenetics & Biology SL (www.allgenetics.com) on a NovaSeq PE250 Illumina flow cell. The same company performed quality control (FastQC, Andrews 2010) and reads demultiplexing.

Raw paired-end reads were analysed using the automated eDNAFlow pipeline, which performs in subsequent steps: adapters and primers removal, quality filtering, merging and denoising (Mousavi-Derazmahalleh et al. 2021). During primer removal, Phred quality score was set to 20, while mismatches allowed were set to 2. In this step, untrimmed reads (i.e., reads for which neither the forward nor the reverse primer sequence has been found) were discarded. When merging, minimum overlap between forward and reverse reads was set to 12 bp, whereas minimum length to be merged was 120 and 150 bp respectively for 12S-rDNA, 16S-rDNA fragment reads. The obtained ZOTUs were blasted against the NCBI core nucleotide database (core_nt, updated to March 2025) and assigned taxonomically setting the percentage of identity to 97% (Mousavi-Derazmahalleh et al. 2021). As the reads assigned to cetacean species were too little to run a post-taxonomic assignment decontamination tool efficiently, reads filtering was carried out manually. Based on the results shown by the extraction ($n = 50$) and amplification ($n = 10$) blanks, all reads below 15 were considered as background noise.

Conversely, and only for cetaceans, any detection resolved to the species level with ≥ 15 reads was considered a true positive. This threshold was implemented to mitigate PCR template competition (Stat et al. 2017), a phenomenon where high-biomass species, like fish, outcompete rarer templates. Cetaceans are particularly susceptible to this effect due to their lower abundance and lower mass-specific eDNA shedding rates compared to dense fish schools (Sassoubre et al. 2016).

For the purposes of this new-occurrence record, only partial results are presented, that is, solely those related to the detection of one (out of nine found in the entire sample set) cetacean species, the pygmy sperm whale (*K. breviceps*), as its presence has never been previously reported in the Mediterranean Sea.

Publicly available *Kogia* spp. references and newly generated sequences were employed to contextualise the environmental

TABLE 1 | Metadata for the 10 LIFE-CONCEPTU MARIS eDNA samples tested positive for *Kogia breviceps*. For each sample, the collection date and time, diel period (N: Nocturnal; D: Diurnal), geographic coordies (latitude, longitude), and the number of sequencing reads assigned to *K. breviceps* are provided.

Sample	Date	Time	Diel	Latitude	Longitude	Read number
9VaPa1	15-11-23	22:07	N	39.414198	0.242561	159
9VaPa3	16-11-23	11:03	D	39.42834	2.002331	18
6MTL14	28-02-24	22:58	N	41.576667	11.565	98
2GeBaTa29	17-03-24	14:46	D	41.0533	2.065	2302
2GeBaTa30	17-03-24	17:40	D	40.2603	1.4706	1493
2GeBaTa32	18-03-24	00:38	N	38.0091	−0.2318	312
4GeBaTa59	28-09-24	20:42	N	43.793111	8.072417	153
4GeBaTa60	29-09-24	00:44	N	42.846791	6.304291	8050
4GeBaTa64	03-10-24	04:00	N	43.522225	7.5441583	21
5BaTa36	14-10-24	22:23	N	35.959167	−5.306389	31

sequences, with a dual objective: (1) to confirm species identification (*K. breviceps* versus *K. sima*), and (2) to evaluate their genetic affinity with conspecifics from adjacent regions. These analyses were based on the same two 12S-rDNA and 16S-rDNA mitochondrial markers, incorporating both existing *Kogia* spp. sequences from GenBank (derived from specimens of unspecified geographic origin) and a novel dataset. The new sequences were generated from tissue samples of specimens (4 *K. breviceps* and 3 *K. sima*) stranded in the Eastern Atlantic (Portugal, France, Spain, Scotland, Canary Islands) and the Mediterranean Sea (Italy), as sourced from a population genetic study of Eastern Atlantic *Kogia* (Maggioni et al. Submitted).

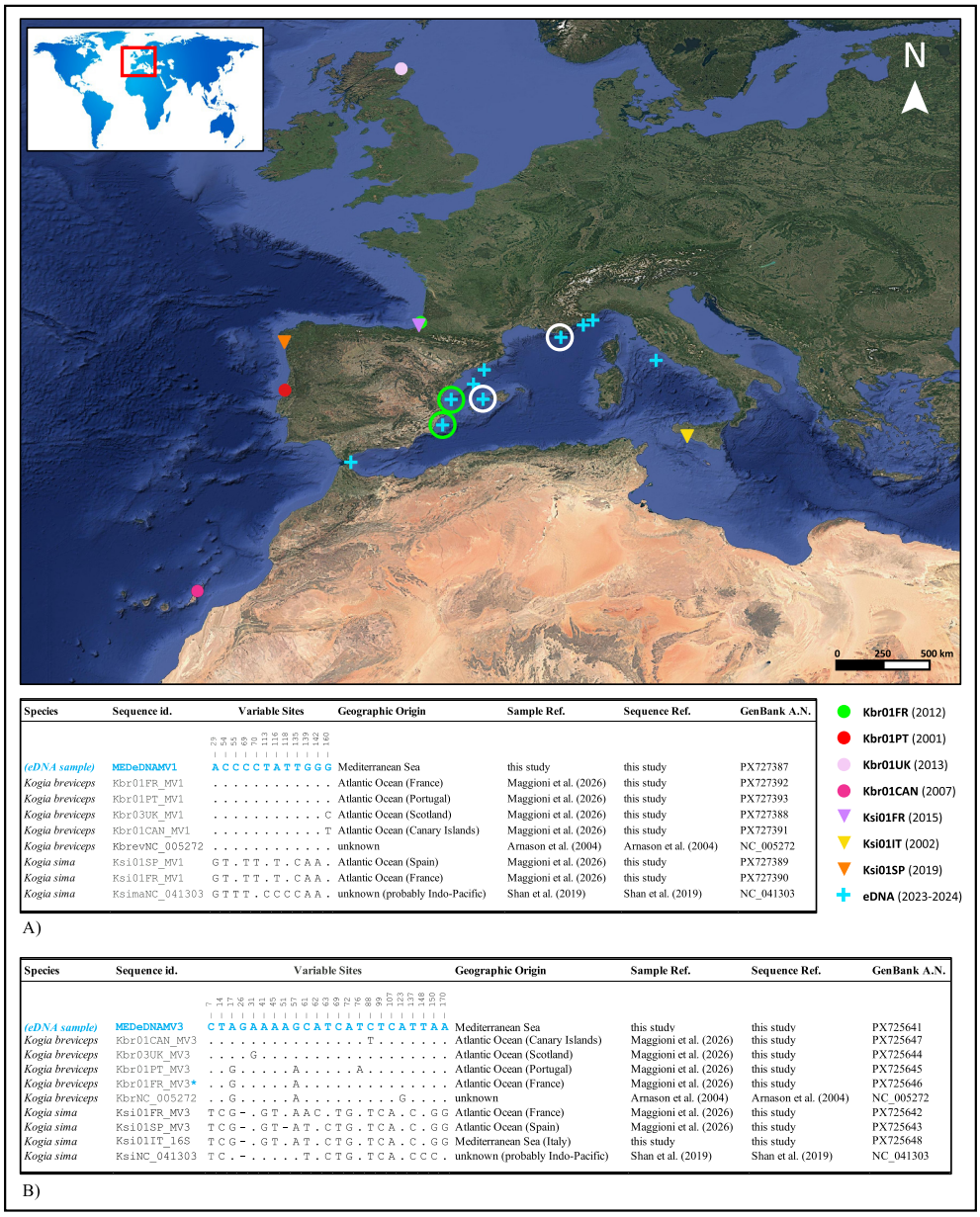
For both loci, polymerase chain reactions (PCRs) were performed in 25 μL reaction volumes containing 2.5 μL of template DNA, 1 μL of each primer, 5 μL of Wonder Taq reaction buffer, 0.3 μL of Wonder Taq DNA polymerase (Euroclone, Milan, Italy), and 15.2 μL of Milli-Q water. A ramping PCR protocol was employed, consisting of an initial denaturation at 95°C for 2 min, followed by 38 cycles of denaturation at 93°C for 25 s, annealing for 25 s, extension at 72°C for 15 s, and a final extension at 72°C for 5 min. The annealing temperature was progressively increased in a stepwise manner over the course of the reaction: 54°C for the first 8 cycles, 55°C for the next 10 cycles, 56°C for the subsequent 10 cycles, and 57°C for the final 10 cycles. PCR products were visualised on a 1% agarose gel, purified, and Sanger sequenced in both directions using the original amplification primers on an ABI 3730xl DNA Analyser (Applied Biosystems, Waltham, MA, USA). Chromatograms were manually inspected and aligned using reciprocal sequence alignment.

Basic statistical analyses were conducted on the *K. breviceps* positive samples. For example, to assess whether molecular detections were more prevalent in diurnal or nocturnal samples, a two-tailed Fisher's exact test was applied, as appropriate for small sample sizes. Similarly, positive detections were plotted along a temporal scale to explore potential seasonal patterns and temporal correlations, with statistical testing conducted while recognizing the inherent limitations imposed by the small sample size.

3 | Results

In 10 (2.5%) of the 393 samples, identified reads (18–8050 reads per sample) referable to the pygmy sperm whale (*K. breviceps*) (Figure 1; Table 1). The traces were detected with either or both the markers targeting vertebrates, namely MarVer1 (12S-rDNA) and MarVer3 (16S-rDNA), with the former performing more efficiently (more samples −8− tested positive and higher read numbers) than the latter (4 samples tested positive). The 10 eDNA samples in which pygmy sperm whale traces were identified generated an identical sequence for the 12S-rDNA and two 16S-rDNA amplicons: a complete one (197 bp, found in samples 4GeBaTa60 and 9VaPa3) and a partial one (153 bp, found in samples 2GeBaTa32 and 9VaPa1). The two full haplotypes have been designated MEDeDNAMV1 (163 bp) and MEDeDNAMV3 (197 bp), for the 12S-rDNA and the 16S-rDNA amplicons respectively, and their corresponding sequences have been deposited in GenBank under accession numbers PX727387 and PX725641, respectively (Figure 2A,B). The second (partial) 16S-rDNA amplicon was found to be identical to the first 153 bp of the sequence obtained in one of the control tissue samples (the *K. breviceps* stranded in Atlantic French waters, see Figure 2B).

The 12S-rDNA fragment was found to be relatively conserved across Kogiidae. For *K. breviceps*, the two Portuguese and French specimens stranded along the Atlantic coast shared an identical haplotype. This haplotype matched also both the GenBank reference sequence (accession NC_005272) and the environmental DNA sequence obtained in this study. Specimens from the Canary Islands and Scotland possessed a slightly different haplotype, differing by only one base pair from the remaining haplotype. For *K. sima*, specimens stranded in Spain and France (Atlantic coast) shared the same haplotype, which differed by five base pairs from the GenBank reference sequence (accession



Species	Sequence id.	Variable Sites	Geographic Origin	Sample Ref.	Sequence Ref.	GenBank A.N.
		29 54 65 69 70 113 116 118 135 139 142 160				
(eDNA sample)	MEDeDNAMV1	A C C C C T A T T G G G	Mediterranean Sea	this study	this study	PX727387
Kogia breviceps	Kbr01FR_MV1	. . . . .	Atlantic Ocean (France)	Maggioni et al. (2026)	this study	PX727392
Kogia breviceps	Kbr01PT_MV1	. . . . .	Atlantic Ocean (Portugal)	Maggioni et al. (2026)	this study	PX727393
Kogia breviceps	Kbr03UK_MV1	 C	Atlantic Ocean (Scotland)	Maggioni et al. (2026)	this study	PX727388
Kogia breviceps	Kbr01CAN_MV1	. . . . . T	Atlantic Ocean (Canary Islands)	Maggioni et al. (2026)	this study	PX727391
Kogia breviceps	KbrevNC_005272	. . . . .	unknown	Arnason et al. (2004)	Arnason et al. (2004)	NC_005272
Kogia sima	Ksi01SP_MV1	G T . T T . T . C A A .	Atlantic Ocean (Spain)	Maggioni et al. (2026)	this study	PX727389
Kogia sima	Ksi01FR_MV1	G T . T T . T . C A A .	Atlantic Ocean (France)	Maggioni et al. (2026)	this study	PX727390
Kogia sima	KsimaNC_041303	G T T T . C C C C A A .	unknown (probably Indo-Pacific)	Shan et al. (2019)	Shan et al. (2019)	NC_041303

A)

Species	Sequence id.	Variable Sites	Geographic Origin	Sample Ref.	Sequence Ref.	GenBank A.N.
		7 14 17 26 31 41 46 51 57 61 62 63 69 72 78 88 99 107 123 137 148 150 170				
(eDNA sample)	MEDeDNAMV3	C T A G A A A A G C A T C A T C T C A T T A A	Mediterranean Sea	this study	this study	PX725641
Kogia breviceps	Kbr01CAN_MV3	. . . . . T	Atlantic Ocean (Canary Islands)	Maggioni et al. (2026)	this study	PX725647
Kogia breviceps	Kbr03UK_MV3	. . . . G	Atlantic Ocean (Scotland)	Maggioni et al. (2026)	this study	PX725644
Kogia breviceps	Kbr01PT_MV3	. . G . . . . A . . . . . A	Atlantic Ocean (Portugal)	Maggioni et al. (2026)	this study	PX725645
Kogia breviceps	Kbr01FR_MV3*	. . G . . . . A	Atlantic Ocean (France)	Maggioni et al. (2026)	this study	PX725646
Kogia breviceps	KbrNC_005272	. . G . . . . A . . . . . G	unknown	Arnason et al. (2004)	Arnason et al. (2004)	NC_005272
Kogia sima	Ksi01FR_MV3	T C G - . G T . A A C . T G . T C A . C . G G	Atlantic Ocean (France)	Maggioni et al. (2026)	this study	PX725642
Kogia sima	Ksi01SP_MV3	T C G - . G T - A T . C T G . T C A . C . G G	Atlantic Ocean (Spain)	Maggioni et al. (2026)	this study	PX725643
Kogia sima	Ksi01IT_16S	T C G - . G T . A T . C T G . T C A . C . G G	Mediterranean Sea (Italy)	this study	this study	PX725648
Kogia sima	KsiNC_041303	T C . - . . . . . T . C T G . T C A . C C C .	unknown (probably Indo-Pacific)	Shan et al. (2019)	Shan et al. (2019)	NC_041303

B)

FIGURE 2 | Geographic and genetic comparison of eDNA sequences with reference specimens. (Main panel) Map showing stranding locations of the seven *Kogia spp* reference specimens (4 *K. breviceps*, 3 *K. sima*) and positive eDNA sampling sites (light-blue crosses). White circles surround eDNA samples carrying the 16S-rDNA *Kogia breviceps* variant sequence MEDeDNAMV3 while green circles enclose eDNA samples carrying the Kbr01FR_MV3 (partial sequence) variant. (Panels A and B) Sequence alignment tables for the (A) 12S-rDNA and (B) 16S-rDNA amplicons, comparing the eDNA haplotype (light-blue font) with homologous sequences from the reference individuals. The second, partial (153 bp), eDNA 16S-rDNA haplotype was identical to the first 153 bp of the Kbr01FR_MV3 haplotype (light-blue asterisk). Vertical numbers indicate the nucleotide positions of variable sites. Dots (.) indicate identity with the eDNA sequence. GenBank accession numbers for the 15 newly sequenced fragments are listed in the rightmost column.

NC_041303). The latter likely represents an Indo-Pacific *K. sima* individual.

In contrast, in our comparison, the 16S-rDNA fragment exhibited greater variability across Kogiidae. For *K. breviceps*, each of the five analysed sequences (four newly generated and one from GenBank, NC_005272) presented a distinct haplotype. One of the two haplotypes detected in environmental samples also differed uniquely (Figure 2B). Among *K. sima* specimens, those from Spain, France (Atlantic), and Italy each carried different haplotypes, varying by one to three base pairs from one another.

For both loci, the *Kogia* spp. sequences retrieved from the 10 eDNA samples consistently clustered with homologous *K. breviceps* sequences (Figure 2A,B). Genetic divergence between eDNA-derived sequences and reference *K. breviceps* sequences ranged from 0% to 0.6% for 12SrDNA and 0.5% to 1.5% for 16SrDNA. In contrast, sequence divergence between eDNA sequences and known *K. sima* references was substantially higher, reaching up to 6.7% (12SrDNA) and 8.6% (16SrDNA).

The 10 samples tested positive for *K. breviceps* were not evenly distributed. In three instances, positive samples formed

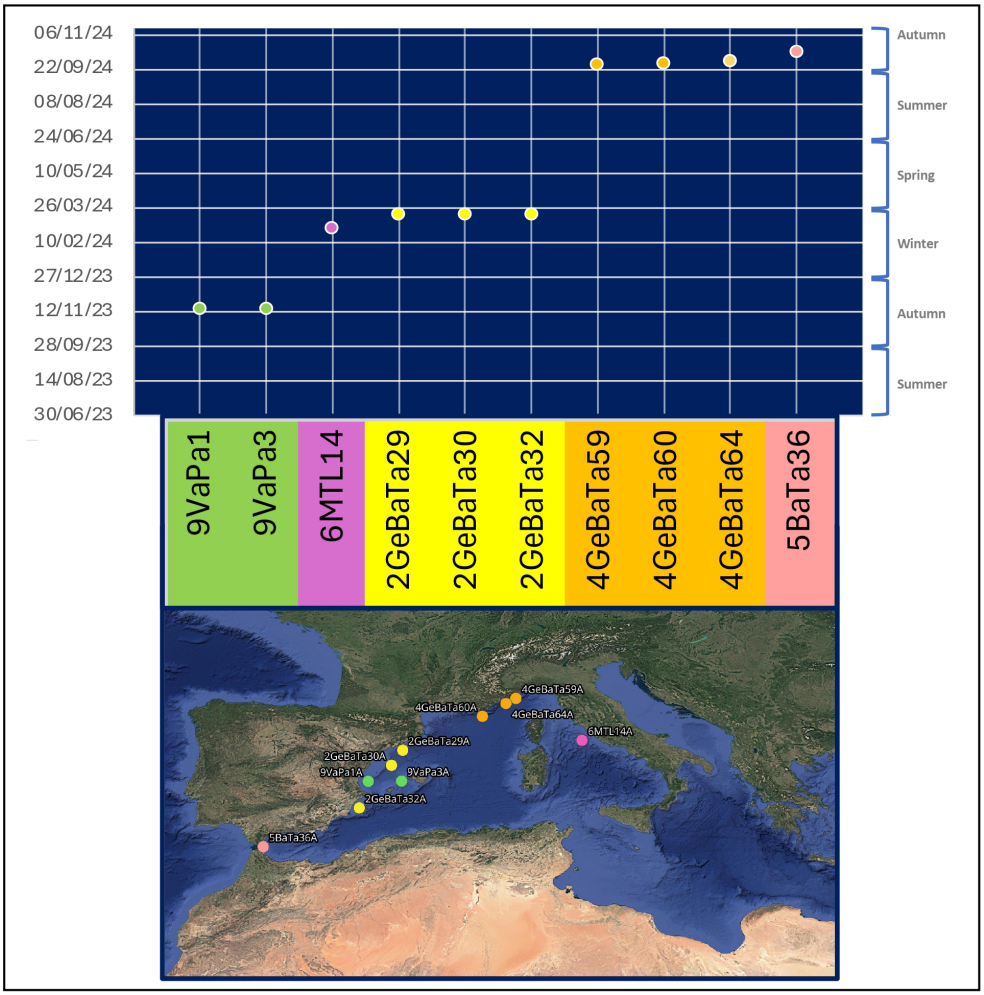


FIGURE 3 | Temporal distribution of the *Kogia breviceps* detections in the study area. The temporal range displayed has been stretched to include the time interval (02/07/23–23/10/24) in which sampling effort was active in the area where detections were found.

spatiotemporal clusters, being collected on the same or consecutive days during the same survey cruise. As such clusters likely represent repeated detections of the same individual or group, the 10 positive samples correspond to a minimum of five independent molecular detection events. In order to distinguish the three samples' clusters and the two individual detections, a colour-code has been used in both Figures 3 and 4.

The 10 *K. breviceps* DNA detections were spread-out across 1 year, spanning from November 2023 to October 2024 (Figure 3). Of the 393 samples collected, 171 were taken in Autumn, 50 in Winter, 83 in Spring, and 89 in Summer, while the 10 recorded detections occurred in Autumn (6) and Winter (4), with none in Spring or Summer. A chi-squared goodness-of-fit test based on seasonal sampling effort indicated a statistically significant deviation from a random distribution ($\chi^2 = 10.87 \backslash \chi^2 = 10.87 \chi^2 = 10.87$, df = 3, $p = 0.013$), with encounters more frequent than expected in Autumn and Winter. However, considering only the five truly independent events (counting each cluster of detection as a single event), these occurred 3 in Autumn and 2 in the Winter. In this case the sample size is too small to establish statistical significance. Even so, rerunning the chi-squared test using only the 5 independent detections it is found no statistically significant deviation from a random distribution ($\chi^2 = 5.39 \backslash \chi^2 = 5.39 \chi^2 = 5.39$, df = 3, $p = 0.145$), though a trend toward higher encounter rates in Autumn and Winter was noted.

Of the 10 samples that tested positive for pygmy sperm whale eDNA, seven were collected at night (Figure 4), despite nocturnal samples representing only 39.4% ($n = 155$) of the total 393 samples. A two-tailed Fisher's Exact Test indicated a statistically significant association between nocturnal sampling and positive detection rates ($p = 0.041$). Also, the positive sample (4GeBaTa60) yielding the highest read number (8050, specifically 5586 and 2464 reads obtained with primer sets MarVer1 and MarVer3 respectively) was collected at 00:44 (on the September 29, 2024) off the coast of Port-Cros National Park (France), approximately 10 miles southeast from the Iles d'Hyères (Figure S1).

4 | Discussion

Though widespread globally, *Kogia* species are extremely uncommon in the Mediterranean Sea. Occasional *K. sima* strandings have been confirmed, but no Mediterranean *K. breviceps* records are available at present. Here we present the first (molecular) evidence of the presence of the pygmy sperm whale in Mediterranean waters, with at least five distinct detections

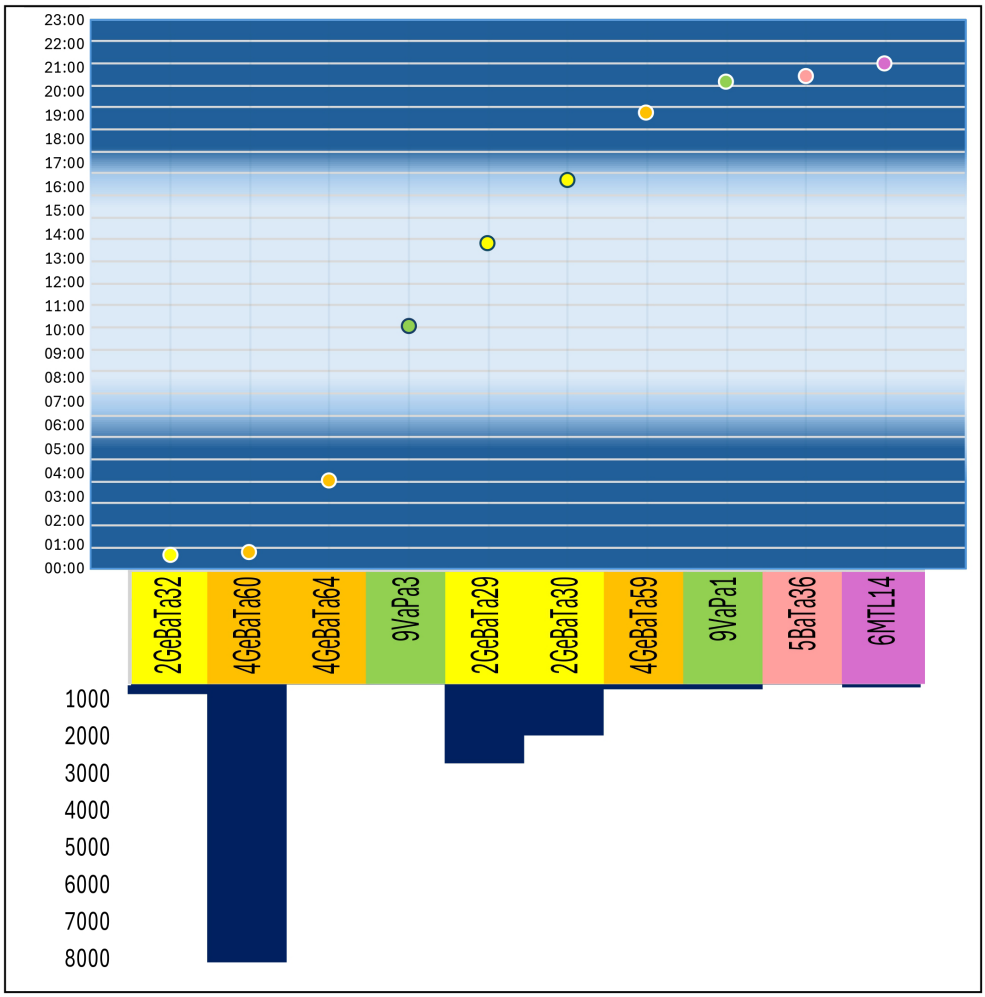


FIGURE 4 | Diel distribution of positive samples based on the time of collection (hour of day). Samples are ranked according to collection time. The lower panel displays the combined number of *Kogia breviceps* metabarcoding reads detected in each sample, aggregated across both vertebrate genetic markers.

occurring across a 12-month period, from November 2023 to October 2024, in the central-north western Mediterranean. Some points and considerations can be extrapolated from these results.

4.1 | First Mediterranean Record: Assessing the Potential for Species Misidentification

This study provides the first molecular evidence supporting the non-occasional presence of the pygmy sperm whale (*K. breviceps*) in the Mediterranean Sea. Given that this species has not previously been observed in the region, the validity of this detection may be questioned. To address this, we examined the molecular distinctiveness of the homologous mitochondrial regions between the two species by comparing the sequences retrieved from the whole mitogenome of both species: GenBank Accession Numbers: NC_041303 for *K. sima* and NC_005272 for *K. breviceps*. Since the geographic origin of specimens producing these GenBank entries was unknown, the comparison was implemented using the sequences obtained from seven Kogidae individuals known to be stranded in waters internal or adjacent to the Mediterranean Sea.

Species attribution of the environmental sequences to *K. breviceps* was unambiguous. The number of variable sites (i.e., specific nucleotide positions that differ between the two otherwise similar DNA sequences), defining the haplotypes was far lower between the eDNA sequence and known *K. breviceps* sequences (1 and 6 variable sites for the 12S-rDNA and 16S-rDNA markers, respectively) than between the eDNA sequence and known *K. sima* sequences (11 and 20 variable sites) (Figure 2). Notably, the comparison set includes the 16S-rDNA sequence from a *K. sima* individual stranded in Eraclea Minoa (Italy, Mediterranean Sea) in 2002, which differs from the environmental sequence by 17 variable sites across 197 bp. These differences provide unambiguous support for the taxonomic assignment of the sequences retrieved in the eDNA metabarcoding analysis, confirming the presence of *K. breviceps* in Mediterranean waters.

Further corroborating this identification is (a) the intensity of the signal, particularly in sample 4GeBaTa60, with more than twice the number of reads that allowed the detection of dwarf sperm whale (*K. sima*) in the Colombian waters of Malpelo Island using a similar approach (Juhel et al. 2021); (b) the repeated detection of the species' eDNA signal across multiple samples and cluster of

positives detected on the same or consecutive days (indicating a persistent signal in the area during the sampling period). For instance, during the Sampling Cruise 4GeBaTa, in the same area where the first two samples (4GeBaTa59 and 4GeBaTa60) were collected during the outward journey, a positive signal (4GeBaTa64) was again detected on the return journey, 4 days later, within the stretch of sea between 4GeBaTa59 and 4GeBaTa60 (Figure 3). This observation could indicate that the signal persisted for several days. However, given the (although limited) data available from tagged animals, it is more plausible that the animals remained in that area throughout the entire period (Scott et al. 2001). All this evidence not only reinforces the reliability of the finding, but also suggests a sustained presence of pygmy sperm whale in the region.

4.2 | Inference of Potential Distribution Range. Vagrant or Migrant?

Inferring population data for species that have not been directly observed remains inherently challenging. However, the spatiotemporal distribution and genetic sequence data from molecular traces allow for cautious ecological inference. In this study, the chronology and sites of the five independent detections, combined with the distinct 16S-rDNA haplotype relative to adjacent Atlantic conspecifics, suggest the presence of not only multiple pods, but also a long-established population in the Mediterranean basin. The evidence supporting these inferences is examined in the following paragraphs.

Firstly, the 10 positive detections were dispersed across a broad geographic area of the central and western Mediterranean, ranging from the Strait of Gibraltar to the central Tyrrhenian Sea. Secondly, the temporal sequencing of these detections indicates that, were they attributable to a single individual or group, the animal(s) would have had to move repeatedly across large distances, including east–west trajectories exceeding 1000 km (> 550 nautical miles) within narrow time windows. For example, samples 6MTL14 and 2GeBaTa29 were collected only 17 days apart, yet are separated by the extensive insular barriers of Sardinia and Corsica (Figure 3). Such rapid and repetitive translocations appear inconsistent with what is known, albeit limited, about the ecology of the genus *Kogia*, which is thought to favour site fidelity in areas with sufficient trophic resources (Scott et al. 2001). These data are supported by long-term photo-identification records of resighted dwarf sperm whales in Hawaiian waters, including one individual resighted after 14.9 years (Baird et al. 2021), and in Mexican waters (Mansilla 2007).

While sample 5BaTa36, collected near the Strait of Gibraltar, might reflect transient incursions by Atlantic individuals and thus does not confirm the species' established presence in the Mediterranean Sea, the remaining nine detections suggest a more stable presence. These data point to at least three Mediterranean sub-regions where the species may occur: the Spanish corridor, the Pelagos Sanctuary, and the Tyrrhenian Sea. Moreover, the hypothesis of multiple individuals/pods within the Mediterranean is further supported by the detection of at least two distinct mitochondrial 16S-rDNA haplotypes (Figure 2).

With regard to seasonality, the monitored area, subjected to systematic molecular surveillance from July 2023 through October 2024, yielded positive detections predominantly during the Autumn (for both years) and Winter months. Although the limited number of truly independent events (reduced from 10 to 5 due to potential spatial and temporal clustering) precludes robust statistical inference, the observed pattern may suggest the possibility of seasonal movements. Future research should aim to clarify the drivers and patterns of such movements, ideally integrating molecular, acoustic, and ecological data.

Interpretation of these data, due to the small sample size and the fact that one key haplotype derives solely from eDNA records, requires extreme caution. Although detailed demographic reconstruction was not the primary aim, the observed spatial distribution of haplotypes invites preliminary population genetic considerations. This analysis revealed unexpected polymorphism in the 16S-rDNA marker. Four *K. breviceps* specimens from the Canary Islands, Portugal, France, and Scotland each possessed a unique haplotype. A fifth distinct haplotype was recovered from two Mediterranean eDNA samples (Figure 2B). This level of polymorphism (five haplotypes from five samples) is exceptionally high for this marker, which typically shows minimal variation even in larger studies of other cetaceans (Valsecchi et al. 2020). The distinct Mediterranean haplotype denotes a long-term separation from Atlantic conspecifics. Its genetic profile, more similar to haplotypes from the Canary Islands and Scotland than to geographically closer ones from Portugal and France, is consistent with a small, isolated Mediterranean subpopulation of older origin.

Speculatively, the high diversity in the central eastern Atlantic may indicate a long-term stable population, possibly reinforced by kogiid site fidelity, which structures genetic variation in semi-isolated units. This pattern could be a legacy of isolation in multiple micro-refugia during past glacial periods. Subsequent dispersal may have intermingled these haplotypes in the open ocean, consistent with the lack of broad-scale structure shown by other markers (Maggioni et al. Submitted).

In this framework, the Mediterranean population could represent a relic refuge that has maintained isolation via strong site fidelity and the biogeographic filter of the Strait of Gibraltar. Nevertheless, occasional ingress from Atlantic conspecifics is suggested by the detection of a second (partial) 16S-rDNA haplotype identical to one found on the French Atlantic coast (Figure 2B). Arguably, gene flow between the two basins may be predominantly unidirectional, with entry into the Mediterranean being more likely. This asymmetry could be driven by the narrow, predator-dense (e.g., Iberian killer whales, Esteban et al. 2016), and heavily trafficked Strait of Gibraltar, which may constrain dispersal out of the enclosed basin. However, this interpretation remains tentative, as the short eDNA sequences (and their mitochondrial, thus solely matrilineal, signal) provide limited resolution for reconstructing the complex demographic history of these cryptic odontocetes.

4.3 | The Detectability Discrepancy: eDNA Evidence in the Absence of Visual Sightings

This section of the Discussion explores the potential reasons for the absence of visual confirmations despite molecular detection of the pygmy sperm whale, focusing on two key questions.

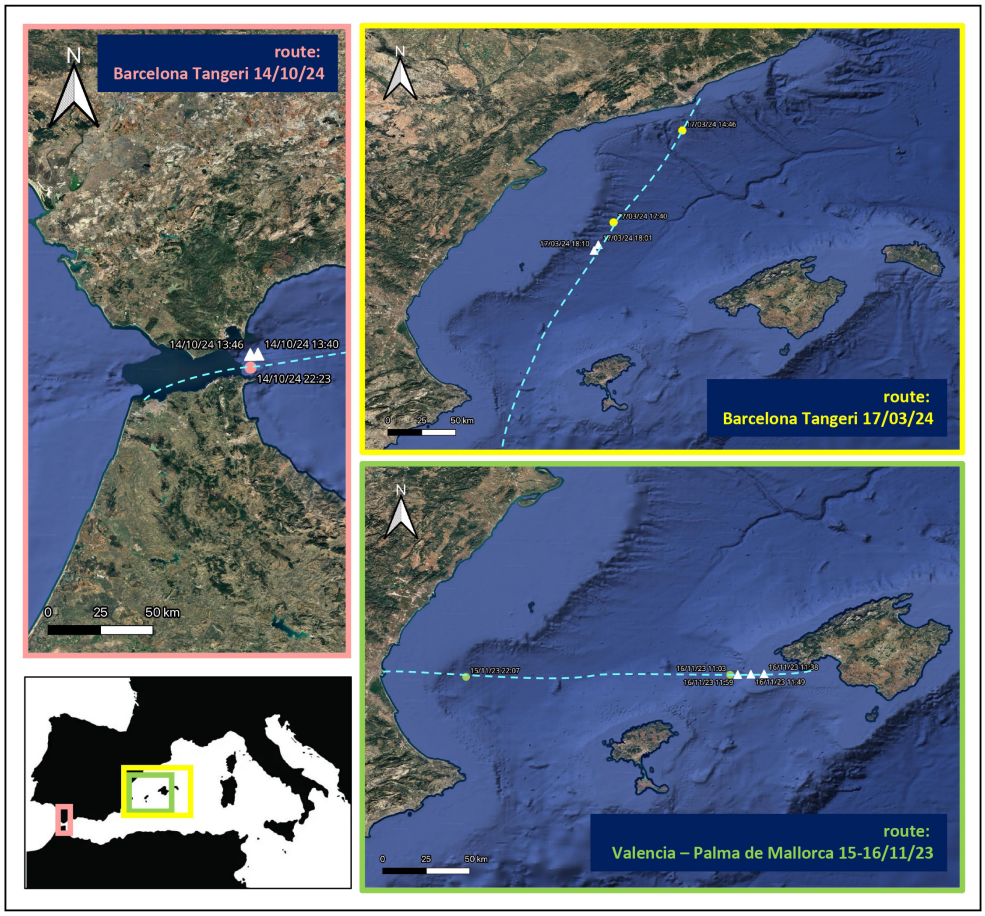


FIGURE 5 | For three out of the five independent clusters or single detections of eDNA, the visual team reported sightings of a small-sized, unidentified cetacean species (US) near the corresponding sampling locations (indicated by white triangles). Coloured dots represent sampling sites where eDNA analysis yielded a positive result for pygmy sperm whale. The light blue dashed line indicates the ferry route. It should be noted that such visual/molecular associations are not possible for nocturnal samples, as no visual survey effort can be conducted during nighttime.

The first is why a species, that has never been recorded at sea, is relatively easy to be detected via molecular analyses. In fact, in our data set, the number of positive samples for pygmy sperm whale exceeds the number of positive detections of other cetacean species with similar habits (deep divers feeding on cephalopods) that, although rare, nevertheless are relatively frequently sighted, such as the Cuvier beaked whale (*Ziphius cavirostris*) and the sperm whale (*Physeter macrocephalus*) (Arcangeli et al. 2022). The pygmy sperm whale was only detected through eDNA, which may be due to the peculiar characteristic of both Kogiid species (the pygmy and dwarf sperm whales) to release a reddish-brown fluid from a specialised "ink sac", located in a modified section of the colon near the anus, typically when they are stressed or threatened (Yamada 1954). This fluid forms a diffuse cloud in the water, sometimes covering up to ~100 m^2 (Scott and Cordaro 1987), functioning probably as a visual deterrent or distraction for predators like sharks or killer whales (Dávila et al. 2026). The excreta, probably rich in DNA like other internal biological fluids (e.g., urine, saliva), are expelled in large quantities, up to approximately 11 L (~3 gal) in a single event, as documented in a video recorded off the coast of Cape Town (South Africa) in 2020. More recently, in Madeira, direct photographic evidence of this instictive reaction during a killer whale attack (Dávila et al. 2026) captures both the moment of fluid release (Figure 5b in Dávila et al. 2026) and the resultant ~24 m-long brownish "cloud" released in the wake of a chased pygmy sperm whale (Figure 4 in Dávila et al. 2026). Although the potential for such excretions to serve as a source of environmental DNA in Kogiidae remains unexamined, the documented behaviour (a substantial, sudden and localised body-fluid excretions) provides a direct pathway for the episodic input of whale DNA into the water column. Thus, the same adaptive mechanism, which allows the animal to evade visual detection by predators, paradoxically makes it more "visible" to molecular detection methods compared to other cetacean species.

The second question that arises is whether the pygmy sperm whale is truly invisible to observers, or rather simply unrecognisable. This issue emerges directly from the findings reported here. A potential clue lies in the nature of the CONCEPTU MARIS project: a multidisciplinary research initiative in which eDNA analysis represents the most recent addition to a long-standing survey protocol. This protocol, in operation for nearly two decades, is based on visual observations conducted from the command deck of commercial ferries during daylight hours by four trained observers (two per side). The visual survey aims to monitor the presence of cetaceans and sea turtles, recording the species, number of individuals, location, behaviour, and

movement direction. During each multidisciplinary SC, the eDNA team worked alongside the visual observation team, and the data collected by both teams were subsequently combined and compared. Across the entire project, in the 39 SC that included eDNA sampling, the visual team recorded 75 sightings of unidentified cetaceans, classified by size as small ($n = 66$), medium ($n = 8$), and large ($n = 1$). Notably, in the subset of five SC (namely 9VaPa, 6MTL, 2GeBaTa, 4GeBaTa, and 5BaTa) in which molecular analyses confirmed the presence of the pygmy sperm whale, the number of unidentified cetaceans was disproportionately high: 27 sightings, comprising 26 small-sized and 1 medium-sized cetacean, representing more than one-third of all unidentified sightings recorded during the 39 SC. Furthermore, when focusing on sighting events occurring in close proximity to the locations of positive eDNA detections, we find that in at least three of these five SC, seven sightings of unidentified small cetaceans occurred near sampling sites where pygmy sperm whale DNA was detected (Figure 5). This co-occurrence suggests that some of these visual sightings of unidentified cetaceans are likely attributable to pygmy sperm whales.

Both points highlight the value of eDNA as a novel and powerful tool for detecting elusive marine species, overcoming a key limitation of structured visual surveys: the reliance on a predefined list of species for identification. In our case, observers were trained to identify cetacean species known to be resident or common in the Mediterranean Sea (a list that does not include the pygmy sperm whale) (Arcangeli et al. 2022). In such protocols, detection is guided by established morpho-behavioural identikits, meaning that species outside this expected list are often recorded as "unknown" rather than correctly identified. For certain elusive species, including the one investigated here, such profiles remain poorly defined, which may explain their underrepresentation in visual records (Baird et al. 2021; Baird 2026). In contrast, eDNA analysis is not constrained by these perceptual limitations. Moreover, when eDNA is collected from operating ferries, it has proven to be a promising approach for investigating marine biodiversity in offshore waters (Boyse et al. 2023).

4.4 | Temporal Signal in eDNA Detection

Although the data presented here do not provide sufficient statistical power to determine whether detections are significantly more prevalent in Autumn and Winter, they unequivocally reveal a statistically significant association between the molecular detection of *K. breviceps* traces and nocturnal sampling. The reasons for this association remain unclear, and several hypotheses can be proposed. For example, pygmy sperm whales may exhibit increased surface activity during nighttime hours, or they may be more frequently exposed to predator interactions at night, thereby triggering the behavioural response described above.

5 | Conclusions

The pygmy sperm whale is regarded as a cosmopolitan species, yet until now it had never been recorded in the Mediterranean Sea. We present data from a spatially and temporally extensive eDNA survey revealing its presence in these waters for the first time. Multiple detections, distributed over a 12-month period and spanning from the Strait of Gibraltar to the Tyrrhenian Sea, were recorded, with a prevalence in nocturnal samples.

This newly recognised distribution pattern raises fundamental questions regarding the origin and status of the population. The observed spatio-temporal signal distribution supports the hypothesis of a refuge subpopulation, although expanded sampling, particularly along the North African coast, will be essential to refine this assessment. Also, haplotypes' divergence suggests the possibility of the Mediterranean specimens belonging to a genetically diverse, ancestral lineage that has persisted in a stable refugium.

Ideally, should additional (environmental/tissue) samples become available, future studies should analyse more genetic loci (or whole genomes) to test the generality of the observed patterns, incorporate nuclear DNA markers to assess potential sex-biased dispersal, and apply coalescent modelling to estimate divergence times and demographic history. Integrating these genetic results with paleo-environmental reconstructions would further help identify potential refugia and colonisation routes.

This study demonstrates the considerable potential of eDNA surveys for detecting elusive marine species when conducted: (a) in offshore waters, (b) across multiple stations along a route, (c) using sufficiently large water volumes (≥ 12 L), (d) over extended timeframes (> 2 years), and (e) including nocturnal sampling. Such approaches hold great promise for revealing the hidden biodiversity of our oceans and informing conservation strategies for little-known taxa.

Author Contributions

Elena Valsecchi: conceptualization, methodology, data curation, investigation, formal analysis, supervision, validation, funding acquisition, project administration, writing – original draft, writing – review and editing, visualization, resources. **Alessia Rota:** data curation, samples collection and preparation. **Graziella Pupillo:** data curation, samples collection and preparation. **Natalia Fraija-Fernández:** resources, samples collection and preparation. **Juan-Antonio Raga:** supervision. **Fulvio Maffucci:** resources, supervision. **Paolo Galli:** resources, supervision. **Antonella Arcangeli:** project administration, writing – review and editing, funding acquisition, resources, data curation.

Acknowledgements

DNA metabarcoding library preparation and sequencing were carried out by AllGenetics & Biology SL (www.allgenetics.eu). We thank the following organisations for providing the control tissue samples from stranded specimens: SECAC (Canary Islands; Vidal Martin, Francesca Fusar Poli), Sociedade Portuguesa de Vida Selvagem (Portugal; Marisa Ferreira), the SMASS Unit at the University of Glasgow (Scotland; Andrew Brownlow, Mariel ten Doeschate, Nick Davison), CEMMA (Spain; Pablo Covelo), the Pelagis Observatory, CNRS/La Rochelle Université (France; Sarah Wund, Paula Méndez-Fernandez, Charlotte Dumortier), and the Mediterranean Marine Mammals Tissue Bank (MMMTB) at the University of Padua (Italy; Bruno Cozzi, Cinzia Centelleghe, Davide Trez). Open access publishing facilitated by Universita degli Studi di Milano-Bicocca, as part of the Wiley - CRUI-CARE agreement.

Funding

The massive effort for eDNA samples collection and analysis was fully covered by the EU funded project LIFE-CONCEPTU MARIS

(CONservation of CEtaceans and Pelagic sea TUrtles in Med: Managing Actions for their Recovery In Sustainability), reference LIFE20 NAT/IT/001371, funded by the European Commission (https://webgate.ec.europa.eu/life/publicWebsite/project/details/5707).

Disclosure

At the end of the review process (on acceptance day, 25/02/26), we learned of grey literature (Boutiba 2011; Lamouti et al. 2023) documenting a *Kogia breviceps* stranding on the Algerian coast in June 2001. This record supports our conclusion that the species has been present in the Mediterranean for a long time (at least 25 years) and should not be considered as vagrant in the Mediterranean. We thank Lea Davìd (member of the ACCOBAMS Scientific Committee) for bringing this cryptic report to our attention.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available on request from the corresponding author. All new DNA sequences detected and produced in this study have been deposited on GenBank, where they are publicly available.

References

Andrews, S. 2010. "*FastQC: A Quality Control Tool for High Throughput Sequence Data*. [Online]." https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

Arcangeli, A., M. Azzolin, L. David, et al. 2022. "First Draft Protocol Data Collection & Analysis." LIFE CONCEPTU MARIS CONservation of CEtaceans and Pelagic sea TUrtles in Med: Managing Actions for their Recovery In Sustainability - Deliverable A2. October 2022.

Arnason, U., A. Gullberg, S. Gretarsdottir, B. Ursing, and A. Janke. 2004. "Cetacean Mitochondrial Genomes: A Comparison Between Baleen and Toothed Whales and the Influence of Adaptive Evolution." *Gene* 333: 27–34. https://doi.org/10.1016/j.gene.2004.02.020.

Baccetti, N., F. Cancelli, and T. Renieri. 1991. "First Record of *Kogia simus* (Cetacea, Physeteridae) From the Mediterranean Sea." *Mammalia (Paris)* 55: 152–154.

Baird, R. W. 2026. "Dwarf and Pygmy Sperm Whales *Kogia sima* (Owen, 1866) and *Kogia breviceps* (de Blainville, 1838)." In *Ridgway and Harrison's Handbook of Marine Mammals*, edited by T. Jefferson, vol. 3. Elsevier.

Baird, R. W., S. D. Mahaffy, and J. K. Lerma. 2021. "Site Fidelity, Spatial Use, and Behavior of Dwarf Sperm Whales in Hawaiian Waters: Using Small-Boat Surveys, Photo-Identification, and Unmanned Aerial Surveys to Study a Difficult-To-Study Species." *Marine Mammal Science* 38: 326–348. https://doi.org/10.1111/mms.12861.

Barnes, M. A., and C. R. Turner. 2016. "The Ecology of Environmental DNA and Implications for Conservation Genetics." *Conservation Genetics* 17: 1–17. https://doi.org/10.1007/s10592-015-0775-4.

Benchoucha, S., M. N. Tamsouri, and F. El Aamri. 2018. "First Stranding Record of *Kogia sima* (Owen, 1866) in Morocco (Strait of Gibraltar)." *Journal of the Black Sea/Mediterranean Environment* 24, no. 2: 175–179.

Bloodworth, B. E., and D. K. Odell. 2008. "*Kogia breviceps* (*Cetacea: Kogiidae*)." *Mammalian Species* 819: 1–12. https://doi.org/10.1644/819.1.

Bohmann, K., A. Evans, M. T. P. Gilbert, et al. 2014. "Environmental DNA for Wildlife Biology and Biodiversity Monitoring." *Trends in Ecology & Evolution* 29, no. 6: 358–367. https://doi.org/10.1016/j.tree.2014.04.003.

Bortolotto, A., L. Papini, G. Insacco, et al. 2003. "First Record of a Dwarf Sperm Whale, *Kogia sima* (Owen, 1866) Stranded Alive Along the Coasts of Italy." In: 31st Symposium of the European Association for Aquatic Mammals, Tenerife, Spain. European Association for Aquatic Mammals, Brussels, Belgium. 1–8. March 14—17, 2003.

Boutiba, Z. 2011. "Peuplement des cétacés des eaux algeriennes." Numero special du Bulletin du CNDRB, 45–48.

Boyse, E., K. P. Robinson, M. Beger, et al. 2023. "Sampling From Commercial Vessel Routes Can Capture Marine Biodiversity Distributions Effectively." *Ecology and Evolution* 13: e9810. https://doi.org/10.1002/ece3.9810.

Boyse, E., K. P. Robinson, M. Beger, et al. 2024. "Environmental DNA Reveals Fine Scale Spatial and Temporal Variation of Prey Species for Marine Mammals in a Scottish Marine Protected Area." *Environmental DNA* 6: e587. https://doi.org/10.1002/edn3.587.

Chivers, S. J., R. G. Leduc, K. M. Robertson, N. B. Barros, and A. E. Dizon. 2005. "Genetic Variation of *Kogia* spp. With Preliminary Evidence for Two Species of *Kogia sima*." *Marine Mammal Science* 21, no. 4: 619–634. https://doi.org/10.1111/j.1748-7692.2005.tb01255.x.

Coll, M., C. Piroddi, J. Steenbeek, et al. 2010. "The Biodiversity of the Mediterranean Sea: Estimates, Patterns, and Threats." *PLoS One* 5, no. 8: e11842. https://doi.org/10.1371/journal.pone.0011842.

Collareta, A., F. Cigala Fulgosi, and G. Bianucci. 2019. "A New Kogiid Sperm Whale From Northern Italy Supports Psychrospheric Conditions in the Early Pliocene Mediterranean Sea." *Acta Palaeontologica Polonica* 64, no. 3: 609–626.

Dávila, C., P. Gauffier, F. Talita Alves Cruz, P. Verborgh, R. Domingues, and L. Freitas. 2026. "Evidence of Killer Whale Predation on Deep-DivingCetaceans in Madeira." *Marine Mammal Science* 42: e70103. https://doi.org/10.1111/mms.70103.

De Barba, M., C. Miquel, F. Boyer, et al. 2014. "DNA Metabarcoding Multiplexing and Validation of Data Accuracy for Diet Assessment: Application to Omnivorous Diet." *Molecular Ecology Resources* 14, no. 2: 306–323. https://doi.org/10.1111/1755-0998.12188.

di Notarbartolo Sciara, G., and A. M. Tonay, eds. 2021. *Conserving Whales, Dolphins and Porpoises in the Mediterranean Sea, Black Sea and Adjacent Areas: An ACCOBAMS Status Report 2021*. ACCOBAMS Permanent Secretariat.

Esteban, R., P. Verborgh, P. Gauffier, J. Giménez, C. Guinet, and R. de Stephanis. 2016. "Dynamics of Killer Whale, Bluefin Tuna and Human Fisheries in the Strait of Gibraltar." *Biological Conservation* 194: 31–38. https://doi.org/10.1016/j.biocon.2015.11.031.

Juhel, J. B., V. Marques, A. P. Fernández, et al. 2021. "Detection of the Elusive Dwarf Sperm Whale (*Kogia sima*) Using Environmental DNA at Malpelo Island (Eastern Pacific, Colombia)." *Ecology and Evolution* 11: 2956–2962. https://doi.org/10.1002/ece3.7057.

Lamouti, S., M. E. A. Bouhadja, S. Bensmail, et al. 2023. "Overview of Cetaceans' Strandings Records Along the Algerian Coastline." 6ème conference sur la conservation des cétacés dans les pays du sud de la Méditerranée. ACCOBAMS. November 13–15, 2023, Monastir, Tunisie.

Maggioni, D., G. Pupillo, A. Rota, et al. Submitted. "North-Eastern Atlantic Strandings Validate Genetic Patterns in Pygmy and Dwarf Sperm Whales." *Marine Mammal Science*.

Maio, N., T. Fioravanti, L. Latini, et al. 2024. "Genetic Characterization, Age and Reproductive Status of Dwarf Sperm Whales (*Kogia sima*) Stranded Along the Italian Coastlines." *Mediterranean Marine Science* 25, no. 2: 374–381. https://doi.org/10.12681/mms.35701.

Maio, N., F. Pollaro, A. Gasparro, et al. 2017. "New Record of Dwarf Sperm Whale *Kogia sima* (Owen, 1866) From the Mediterranean Sea (Cetacea Kogiidae)." *Biodivers Journal* 8, no. 4: 947–950.

Mansilla, S. P. 2007. "Distribución y abundancia de los géneros Kogia y Steno en la Bahía de Banderas y aguas adyacentes." Tesis, Universidad Nacional Autonoma de Mexico.

Martín, V., M. Tejedor, M. Carrillo, et al. 2021. "Strandings and At-Sea Observations Reveal the Canary Archipelago as an Important Habitat for Pygmy and Dwarf Sperm Whales." *Deep Sea Research Part II: Topical Studies in Oceanography* 190–191: 104961. https://doi.org/10.1016/j.dsr2.2021.104961.

Mousavi-Derazmahalleh, M., A. Stott, R. Lines, et al. 2021. "eDNAFlow, an Automated, Reproducible and Scalable Workflow for Analysis of Environmental DNA Sequences Exploiting Nextflow and Singularity." *Molecular Ecology Resources* 21, no. 5: 1697–1704. https://doi.org/10.1111/1755-0998.13356.

Rota, A., and E. Valsecchi. 2025. "The Floating Lab: Standard Operational Procedure for Collecting and Filtering Seawater Samples From Operating Ferries for Environmental DNA Analysis." *Journal of Visualized Experiments* 222: e67366. https://doi.org/10.3791/67366.

Sassoubre, L. M., K. M. Yamahara, L. D. Gardner, B. A. Block, and A. B. Boehm. 2016. "Quantification of Environmental DNA (eDNA) Shedding and Decay Rates for Three Marine Fish." *Environmental Science & Technology* 50, no. 19: 10456–10464. https://doi.org/10.1021/acs.est.6b03114.

Scott, M. D., and J. G. Cordaro. 1987. "Behavioral Observations of the Dwarf Sperm Whale (*Kogia simus*)." *Marine Mammal Science* 3, no. 4: 353–354. https://doi.org/10.1111/j.1748-7692.1987.tb00322.x.

Scott, M. D., A. A. Hohn, A. J. Westgate, J. R. Nicolas, B. R. Whitaker, and W. B. Campbell. 2001. "A Note on the Release and Tracking of a Rehabilitated Pygmy Sperm Whale (*Kogia breviceps*)." *Journal of Cetacean Research and Management* 3, no. 1: 87–94. https://doi.org/10.47536/jcrm.v3i1.905.

Shan, L., R. Tian, and Y. Liu. 2019. "The Complete Mitochondrial Genome of the Dwarf Sperm Whale *Kogia sima* (Cetacea: Kogiidae)." *Mitochondrial DNA Part B Resources* 4, no. 1: 72–73.

Stat, M., M. J. Huggett, R. Bernasconi, et al. 2017. "Ecosystem Biomonitoring With eDNA: Metabarcoding Across the Tree of Life in a Tropical Marine Environment." *Scientific Reports* 7, no. 1: 12240. https://doi.org/10.1038/s41598-017-12501-5.

Suarez-Bregua, P., M. Álvarez-González, K. M. Parsons, J. Rotllant, G. J. Pierce, and C. Saavedra. 2022. "Environmental DNA (eDNA) for Monitoring Marine Mammals: Challenges and Opportunities." *Frontiers in Marine Science* 9: 987774. https://doi.org/10.3389/fmars.2022.987774.

Taberlet, P., E. Coissac, M. Hajibabaei, and L. H. Rieseberg. 2012. "Environmental DNA." *Molecular Ecology* 21, no. 8: 1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x.

Thomsen, P. F., and E. Willerslev. 2015. "Environmental DNA – An Emerging Tool in Conservation for Monitoring Past and Present Biodiversity." *Biological Conservation* 183: 4–18. https://doi.org/10.1016/j.biocon.2014.11.019.

Turner, C. R., M. A. Barnes, C. C. Y. Xu, S. E. Jones, C. L. Jerde, and D. M. Lodge. 2014. "Particle Size Distribution and Optimal Capture of Aqueous Macrobial eDNA." *Methods in Ecology and Evolution* 5, no. 7: 676–684. https://doi.org/10.1111/2041-210X.12206.

Valsecchi, E., A. Arcangeli, R. Lombardi, et al. 2021. "Ferries and Environmental DNA: Underway Sampling From Commercial Vessels Provides New Opportunities for Systematics Genetic Surveys of Marine Biodiversity." *Frontiers in Marine Science* 8: 704786. https://doi.org/10.3389/fmars2021.704786.

Valsecchi, E., J. Bylemans, S. J. Goodman, et al. 2020. "Novel Universal Primers for Metabarcoding eDNA Surveys of Marine Mammals and Other Marine Vertebrates." *Environmental DNA* 2, no. 4: 460–476. https://doi.org/10.1002/edn3.72.

Yamada, M. 1954. "Some Remarks on the Pygmy Sperm Whale, Kogia." *Scientific Reports of the Whales Research Institute* 9: 37–58.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Location with detailed bathymetric profile of the site where was found the strongest pygmy sperm whale eDNA signal (sample 4GeBaTa60).