Received: 23 October 2022 | Revised: 27 March 2023 | Accepted: 18 April 2023

DOI: 10.1002/aqc.3954

RESEARCH ARTICLE

WILEY

An environmental DNA assay for the detection of Critically Endangered angel sharks (*Squatina* spp.)

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Funding information
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Abstract

1. The three sympatric angel shark species occurring in the Mediterranean – *Squatina squatina* (the angelshark), *Squatina aculeata* (the sawback angelshark), and *Squatina oculata* (the smoothback angelshark) – are all classed as Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List. There is a clear need to better quantify their current status, using appropriate non-destructive methods, to help inform future conservation measures.
2. This study introduces an environmental DNA (eDNA) assay able to detect and distinguish *S. aculeata*, *S. oculata*, and *S. squatina* in the Mediterranean Sea by combining probe-based quantitative polymerase chain reaction (qPCR) technology and Sanger sequencing. The assay targets a 173-bp barcode in the mitochondrial cytochrome *c* oxidase I (*COI*) gene. It was tested *in silico*, *in vitro* on tissue-extracted DNA, and on eDNA extracted from filtration samples. This genus-specific assay was applied to detect the presence of *S. squatina* in eDNA samples collected in Corsica, France.
3. The target barcode was found in seven of 76 eDNA samples, revealing the presence of *S. squatina* in north-western Corsica, where the shark has never been observed, and confirming its existence on the eastern coast. The study also demonstrates that using eDNA sampling, based on 30 L of seawater filtered close to the substrate with a waterproof peristaltic pump, it was possible to detect the eDNA of this rare benthic species.
4. The results of detection can help identify critical areas for angel shark conservation and facilitate the development of local public awareness initiatives. This novel qPCR assay should be used for future applications in the Mediterranean and eastern Atlantic targeting angel sharks to better identify the remaining populations. In this study the qPCR assay was applied for *S. squatina* eDNA, but application to *S. aculeata* and *S. oculata* still needs to be validated in the field.

KEYWORDS
angel shark, *COI* mtDNA, eDNA, endangered species, quantitative PCR, *Squatina squatina*

1 | INTRODUCTION

Of the 77 Mediterranean chondrichthyan species, half are considered threatened (Dulvy et al., 2016; Dulvy et al., 2021), with the angel shark family (Squatinidae) being the second most endangered family after the Pristidae (Dulvy et al., 2014). The three sympatric angel shark species occurring in the Mediterranean, *Squatina squatina* Linnaeus, 1758 (the angelshark), *Squatina aculeata* Cuvier, 1829 (the sawback angelshark), and *Squatina oculata* Bonaparte, 1840 (the smoothback angelshark), are all classed as Critically Endangered on the International Union for Conservation of Nature (IUCN) Red List as a result of decreasing trends in their population sizes (Gordon et al., 2019). Of these three species, only *S. squatina* is still observed in the north-western Mediterranean basin (Lawson et al., 2020). Its range originally extended from Scandinavia to north-west Africa, including the whole of the Mediterranean and Black seas (Morey et al., 2019). In the Mediterranean, this shark was frequently observed during the 19th and early 20th centuries and was even targeted by coastal fisheries for its flesh, using specially designed nets (Ellis et al., 2020). As a result of this fishing pressure, its range has been greatly reduced (−58%; Lawson et al., 2020). Today, the Mediterranean populations of *S. squatina* appear to be depleted and severely fragmented (Gordon et al., 2019; Appendix S1), although it benefits from an increasing number of protective measures (Appendix S2). The island of Corsica appears to be the last refuge for this species in French waters, with adult and juvenile *S. squatina* still occasionally observed by fishers on the eastern coast, near the Réserve Naturelle de l'Étang de Biguglia (Lapinski & Giovos, 2019). In the same area, one individual was photographed *in situ* (Figure 1) in 2020 by a professional diver of Andromède Océanologie (https://www.andromede-ocean.com). These observations suggest that populations persist in some localized areas in the Mediterranean. Nevertheless, to help inform which conservation measures need to be implemented for this species, and potentially other sympatric *Squatina* species, data on their distribution are needed to evaluate their current status (Ellis et al., 2020). Ecological data (habitat use, nursery areas, and seasonal distribution) are mainly from the Canary Islands (Meyers et al., 2017; Jiménez-Alvarado et al., 2020) in the Atlantic Ocean, whereas information on the distribution of the species in the Mediterranean is limited to fisheries data (Lawson et al., 2020). But fishing (trawling, netting, and angling) is a disruptive and potentially fatal inventory method that should be avoided for a Critically Endangered species. Given the demersal lifestyle of this elasmobranch and its rarity, other traditional survey methods, such as underwater visual census (UVC) and baited remote underwater video station (BRUVS), are not suitable to rapidly assess the presence of the species over a wide area (Boussarie et al., 2018).

One alternative approach is environmental DNA (eDNA) barcoding (Thomsen & Willerslev, 2015; Muha et al., 2017). This non-invasive technique relies on DNA molecules left by organisms in their environment via the loss of their skin, scales, or blood, for example. By filtering water, it is possible to collect these DNA fragments, known as eDNA, and to detect the presence of a target species by eDNA barcoding (Taberlet et al., 2018). Single-species detections rely on the amplification of a specific barcode (i.e. a short DNA sequence) targeted by specific primers via conventional polymerase chain reaction (PCR), quantitative PCR (qPCR) or droplet digital PCR (ddPCR) (Nathan et al., 2014). The qPCR technique has been shown to be more sensitive than conventional PCR for the detection of eDNA from rare species, as it can reliably detect low DNA concentrations and it is less prone to false negatives (Xia et al., 2018; Klymus et al., 2019). The specificity of the qPCR assay can even be increased by the use of probe-based chemistries (Langlois et al., 2021), as the probe itself is barcode specific in addition to the primers (Taberlet et al., 2018). This has already been successfully applied to detect the presence of species of particular concern, such as invasive (Coster et al., 2021) and endangered species (Reyne et al., 2021), which require early detection for the implementation of an appropriate management measure. eDNA sampling is therefore particularly relevant for the study of chondrichthyans, as they are often present in low abundances. To date, targeted eDNA assays have been developed for different threatened chondrichthyan species, including sharks (Lafferty et al., 2018; Postaire et al., 2020; Schweiss et al., 2020; Budd et al., 2021; Cooper et al., 2021; van Rooyen et al., 2021; Jenrette et al., 2023) and rays (Simpfendorfer et al., 2016; Gargan et al., 2017; Weltz et al., 2017; Lehman et al., 2020). No eDNA barcoding studies have yet been conducted on the Mediterranean angel sharks, although there is a clear need to better assess their current status using appropriate non-destructive methods (Ellis et al., 2020).

This study therefore aims to: (1) develop a probe-based qPCR assay targeting the three Mediterranean angel sharks; and (2) test the assay on eDNA samples collected in Corsica from sea water filtration for clarifying the distribution of *S. squatina*. The results provide insight into the ability to detect this rare shark in its benthic environment using an adapted sampling design and eDNA barcoding. The methodology developed here should help future research that aims at better understanding the ecology of angel sharks and provide information for conservation stakeholders.

2 | METHODS

2.1 | Design of the angel shark barcode

A census of the mitochondrial sequences available from the National Center for Biotechnology Information (NCBI) database (NCBI

FIGURE 1 The angelshark (*Squatina squatina*). This individual was observed by the diver Laurent Ballesta at a depth of 40 m on the eastern coast of Corsica near Biguglia, south Bastia, in June 2020. Photo credit: Laurent Ballesta, Andromède Océanologie.

Resource Coordinators, 2016) was conducted for *S. squatina* and its two sympatric species (*S. aculeata* and *S. oculata*). Cytochrome *c* oxidase subunit I (*COI*) and *16S* sequences were available for all three *Squatina* species, and *12S* sequences (from three complete mitochondrial genomes) were available for *S. squatina* only. These sequences were extracted from NCBI and aligned using Geneious Prime 2021.0.3 to identify a primer pair and a probe to target a barcode for *S. aculeata*, *S. oculata*, and *S. squatina*. The probe and primers were designed using this software following the criteria set out in the protocol of Klymus et al. (2020) (see Appendix S3). This resulted in the following forward and reverse primer pair and probe targeting a 173-bp (with primers excluded) barcode of the *COI* region within the mitogenome of *S. aculeata*, *S. oculata*, and *S. squatina*:

F-primer, 5′-TACTTTTACTACTTGCCTCAGCCG-3′;
R-primer, 5′-GTGGTGTTTGATACTGGGAAATGGC-3′;
TaqMan™ probe, 5′-FAM-AGCAGGAGCCGGCACTGGTT-BHQ-3′.

This primer pair and probe were then tested *in vitro* by PCR on DNA extracted from tissues of three individuals of *S. squatina* obtained from fishers in Bastia, Corsica, France, and Valencia Oceanographic, Spain, and tissues of the two related species *S. aculeata* and *S. oculata* from the Natural History Museum of Comiso, Italy. DNA extraction was performed using the Qiagen DNeasy® Blood and Tissue kit, following the manufacturer's protocol (Qiagen, Hilden, Germany) and the GEMEX platform (Centre d'Ecologie Fonctionnelle et Evolutive (CEFE), Montpellier, France). Extracted DNA was then quantified using a Qubit fluorometer (ThermoFisher Scientific, Waltham, MA, USA). Amplification of the 173-bp target barcode was validated only after PCR products were run on agarose gel electrophoresis and Sanger sequenced at Eurofins (Lille, France). The characteristics of the designed primer pair and probe are detailed in Table 1.

TABLE 1 Characteristics of the primers and probe designed to amplify a 173-bp barcode of the *COI* gene of *Squatina aculeata*, *Squatina oculata*, and *Squatina squatina*.

	Sequence (5′→3′)	Length (bp)	GC content (%)	Melting temperature T_m (°C)	Hairpin T_m (°C)	Self-dimer T_m (°C)	Pair dimer T_m (°C)
Forward primer (AM-F)	TACTTTTACTACTTGCCTCAGCCG	24	45.8	60.6	38.7	None	None
Reverse primer (AM-R)	GTGGTGTTTGATACTGGGAAATGGC	25	48.0	62.6	None	None	None
Probe	AGCAGGAGCCGGCACTGGTT	20	65	66.7	39.7	35.3	None

Note: The barcode targeted by these primers is situated at base-pair position 5,803–5,975 of the *COI* region.

2.2 | Application to *S. squatina*: eDNA sampling and extraction

The fieldwork was carried out between 25 April and 6 May 2021 in Corsica, on board the catamaran of the company Andromède Océanologie (Carnon, France). The study area was chosen based on recent coastal marine biocenoses mapped by Andromède Océanologie (DONIA EXPERT, 2021) and information obtained from local fishers (Durieux & Bousquet, pers. comm.; Riutort, pers. comm.). Seventy-six non-overlapping 2.5-km-long transects were performed along the north-west and eastern Corsican coasts to collect eDNA samples for detecting the presence of angel sharks. One eDNA sample was taken at each transect by filtering 30 L of sea water (1 L per minute) along the whole transect. Each eDNA sample consisted of a sterile VigiDNA® cross flow filtration capsule (pore size, 0.2 µm; SpyGen, Le Bourget-du-Lac, France) through which sea water was filtered using a waterproof custom-designed peristaltic pump (Appendix S4). This filtration set-up was towed behind the catamaran at a depth of approximately 39 m following the 40-m depth isobath, corresponding to soft sea beds at the lower limit of *Posidonia oceanica* seagrass meadows. This set-up was designed to filter sea water as close as possible to the substrate to maximize the chance of collecting eDNA from this bottom-dwelling species. At this depth (40 m) and season (April), the temperature of the sea water was 15°C. At the end of each filtration, the sampling capsule was filled with an 80-ml CL1 conservation buffer (SpyGen) and stored in the dark at ambient temperature.

A field contamination control protocol was carried out including the use of disposable gloves and sterile single-use tubing for *in situ* filtration (Goldberg et al., 2016; Pont et al., 2018). The peristaltic pump was rinsed with fresh water and cleaned with a bleach wipe at the beginning of the fieldwork and at the end of each day (at each exit from the sea). As the transects follow each other in a continuous water mass and the pump did not come out of the water between the two transects, the pump was not cleaned between the two transects. The filters and hoses were changed on the sea surface at each new transect and never touched the pump. The extraction of eDNA was performed by SpyGen, following the protocol published by Pont et al. (2018). Negative extraction controls were carried out in parallel with each extraction of eDNA samples to monitor for contamination.

2.3 | qPCR assay development and deployment

The qPCR protocol was adapted from Secondi et al. (2016). The qPCR runs were performed on the CeMEB labex high-throughput qPCR platform (University of Montpellier), physically separated from the pre-qPCR laboratory (GEMEX platform, CEFE). The final reaction volume of 25 µl contained 3 µl of template DNA (eDNA extract), 12.5 µl of TaqMan™ Environmental Master Mix 2.0 (Life Technologies, now ThermoFisher Scientific), 6.5 µl of double-distilled water (ddH_2O), 1 µl of each primer (10 µM) and 1 µl of probe (2.5 µM). Samples were run on a LightCycler® 480 qPCR system (Roche, Basel, Switzerland) under the following thermal cycling conditions: 5 min at 50°C and 10 min at 95°C, followed by 55 cycles of 30 s at 95°C and 1 min at 62°C.

A standard curve was generated, made of a 13-fold dilution series of *S. squatina* tissue-extracted DNA (mixture of three individuals), with concentrations ranging from 19 to 6×10^{-8} ng µl^{-1}, with 3 µl of template DNA in a total reaction volume of 25 µl in each qPCR well. Three replicates for each concentration were run through qPCR to construct the standard curve. This serial dilution allowed the limit of detection (LoD) of the qPCR to be determined, defined as the lowest concentration returning at least one positive detection out of the three replicates (Agersnap et al., 2017). A second standard curve was constructed in a similar manner to the first to analyse the second set of eDNA samples, as they were run through a different qPCR machine. The first standard curve was used to quantify DNA concentrations in eDNA samples corresponding to transects 1–41 and the second standard curve was used for transects 42–77 (Appendix S5).

Preliminary qPCR tests were carried out on an eDNA sample collected in Corsica (June 2020) that was known to contain *S. squatina* DNA from metabarcoding analysis (Andromède Océanologie, unpubl. data). As only one well was positive in the 12 qPCR replicates tested for this first sample, the number of qPCR replicates was doubled for the analysis of each of the 76 eDNA samples to increase the probability of detecting the target eDNA. Thus, each eDNA sample was run in 24 replicates in a 384-well qPCR plate, with three technical blanks consisting of water and three positive control wells using *S. squatina* tissue-extracted DNA, separated from the eDNA-containing wells to minimize cross-contamination. In total, seven qPCR plates were required for analysing all 76 samples.

2.4 | Analysis of qPCR results

The results of each qPCR run were visualized and analysed with LightCycler® 480 (v.1.5; Roche, Basel, Switzerland). The standard curve was imported into the software to automatically determine the initial concentration of target barcode detected in the different qPCR wells, corresponding to each amplification curve. The standard curve also provided the efficiency (*E*) of qPCR, which was calculated by the software from the slope of the regression line ($E = 10^{-1/\text{slope}} - 1$). For qPCR results to be accepted, the following conditions should be met (Bustin et al., 2009; Budd et al., 2021).

1. Positive control DNA must have been amplified in at least two of the three qPCR replicates, with concentration values consistent with its actual standard concentration.
2. All replicates of the negative control must show unamplified curves. Ideally, the linear regression fitted to the qPCR standard curve data should have a slope of −3.32, meaning that the number of DNA copies exactly doubles with each cycle (100% efficiency), but the acceptable range of efficiency is 90–110%, and it should have a coefficient of determination, $r^2 > 0.99$ (Bustin et al., 2009).
3. Finally, for a detection to be considered positive, at least one qPCR replicate in one eDNA sample should be positive.

The qPCR assay was validated with *S. squatina* tissue-extracted DNA and the eDNA positive control showed complete amplification curves, which popped up at 24 and 42 amplification cycles, respectively. Therefore, when analysing the 76 eDNA samples, they were considered positive if at least one of the 24 qPCR replicates showed a complete amplification curve above the threshold level of fluorescence within 42 cycles; they were considered negative if no amplification curve occurred during the 42 cycles of qPCR.

2.5 | Sequencing of qPCR products

To confirm the specificity of the primers, all qPCR products (i.e. wells in which target DNA was successfully amplified) obtained from the analysis of eDNA samples were sent for Sanger sequencing in both forward and reverse directions at Eurofins (Lille, France). The sequences obtained were visualized and trimmed with CodonCodeAligner (https://www.codoncode.com/aligner) to remove low-quality bases and aligned in BioEdit (https://bioedit.software.informer.com) with the reference barcodes of the three angel shark species (GenBank accession numbers KY464954 for *S. squatina*, KY909582 for *S. oculata*, and KY909575 for *S. aculeata*). The sequences were also matched against the NCBI database (NCBI Resource Coordinators, 2016) for further taxonomic confirmation of the species (100% identity).

3 | RESULTS

3.1 | qPCR assay specificity

In silico, the designed primers amplified the *COI* sequence of 16 species of fish (Appendix S6), among which only *S. aculeata*, *S. oculata*, and *S. squatina* are present in the Mediterranean. None of the other 13 species occur within the range of *S. squatina*. This primer pair was the most suitable option to target all three Mediterranean angel sharks. *In vitro*, the 173-bp target sequence was successfully amplified by PCR for *S. aculeata*, *S. oculata*, and *S. squatina*. The sequenced PCR products returned a similarity of 100% pairwise identity against their respective reference sequences using BLAST (NCBI Resource Coordinators, 2016).

3.2 | qPCR assay sensitivity

For the first standard curve ($y = -3.46x + 23.98$, $r^2 = 1$, efficiency = 95%), tissue-extracted DNA was successfully amplified for 10 concentrations between 19 and 6×10^{-5} ng μl^{-1} (Appendix S5). For each of these 10 dilutions, DNA from all three replicates was correctly quantified, except for the sample with the lowest DNA concentration at 6×10^{-5} ng μl^{-1}, which had only one of three replicates amplified. Lower DNA concentrations were not detected by the qPCR assay as no fluorescence signals were observed for concentrations between 6×10^{-5} and 6×10^{-8} ng μl^{-1}. The LoD of this qPCR assay was therefore determined to be 6×10^{-5} ng μl^{-1}. The second standard curve ($y = -3.45x + 24.43$, $r^2 = 0.96$, efficiency = 95%; Appendix S5) presents the same qPCR efficiency and a slightly lower LoD (1.90×10^{-5} ng μl^{-1}) than the first standard curve, reflecting the higher sensitivity of the qPCR assay (Xia et al., 2018).

3.3 | Analysis of 76 eDNA samples

Seven of the 76 eDNA samples from Corsica showed positive genetic detection for the target barcode (Figure 2). In each qPCR run, the three technical blank controls were all negative, whereas the positive controls (*S. squatina* tissue-extracted DNA) presented the expected DNA concentration values, validating the qPCR assays. Sanger sequencing of the qPCR products corresponding to the seven positive transects confirmed that only *S. squatina* DNA was present in the eDNA samples (Table 2; Appendix S7). Five sequences returned a similarity of 100% pairwise identity against reference *S. squatina* sequences using BLAST (NCBI). The two other sequenced qPCR products (transects 45 and 66) did not produce readable sequences, and therefore it can only be affirmed that these sequences correspond to one of the three angel shark species.

Among the seven positive detections (Table 2), one corresponds to transect 5, which is located in the Parc Naturel Marin du Cap Corse et de l'Agriate (PNMCCA) (Figure 2). The target barcode was present in one

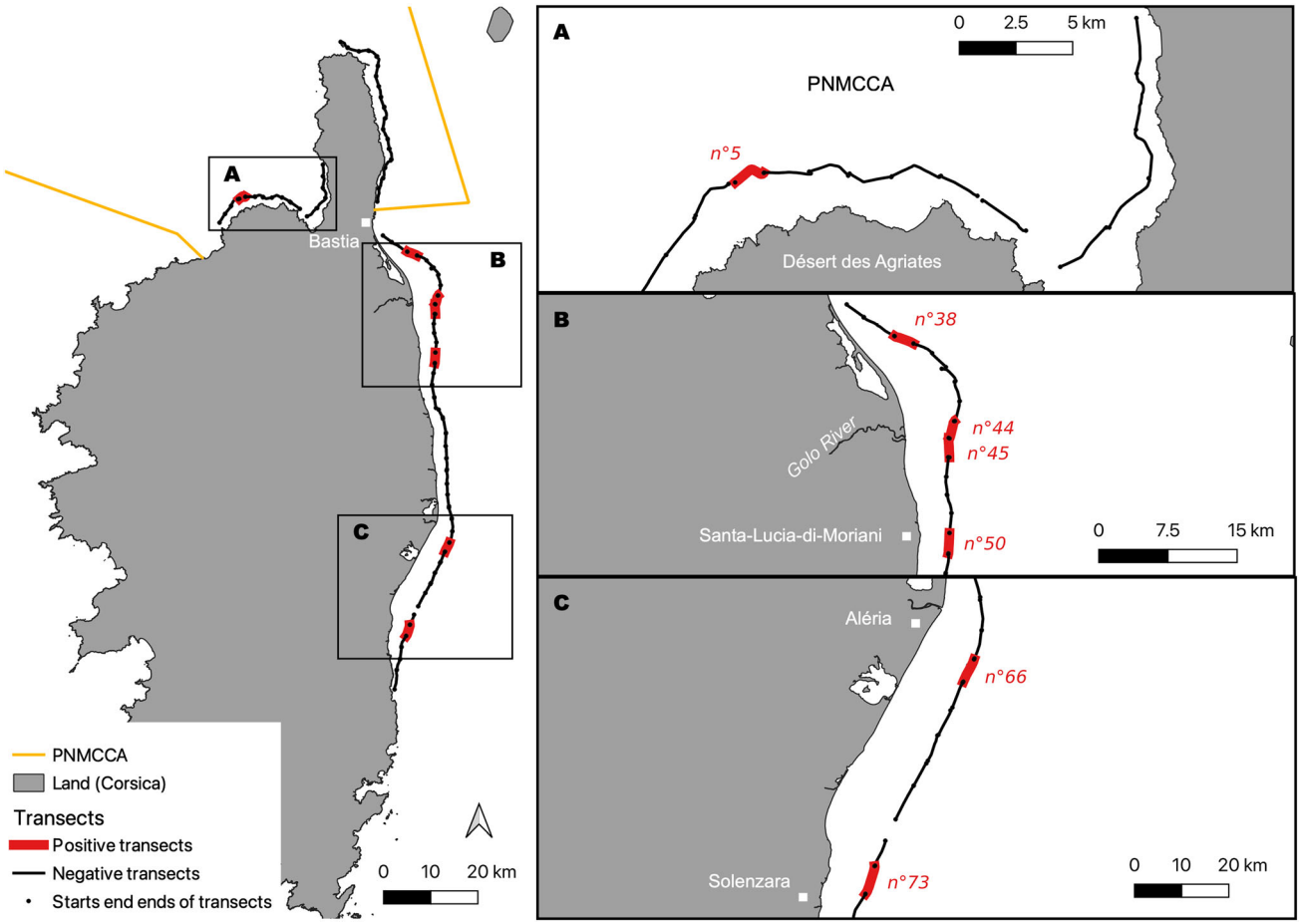

FIGURE 2 Location of the seven positive transects (bold and red) where the target angel shark barcode was detected by qPCR, following the sampling of a total of 76 eDNA samples from Corsican waters, France. Each transect measures 2.5 km and follows the 40-m depth isobath. Transect 5 is located along the Agriate coast, within the marine protected area Parc Naturel Marin du Cap Corse et de l'Agriate (PNMCCA). Transect 38 is south of Bastia, transects 44 and 45 are at the mouth of the Golo River, transect 50 is near Santa-Lucia-di-Moriani, transect 66 is at Aléria, and transect 73 is located in the waters of Solenzara. Target eDNA was not detected in the 69 other transects (black).

TABLE 2 Results of positive target eDNA detections obtained from qPCR analysis of eDNA samples.

Sampling site	Angel shark detection	Positive qPCR replicates	Target eDNA concentration (ng μl^{-1})	Mean target eDNA concentration (ng μl^{-1})	Cycle number detection threshold	Mean cycle number detection threshold	Identity of the sequenced qPCR product
Transect 5	Yes	1/24	2.20×10^{-5}	–	39.77	–	*S. squatina*
Transect 38	Yes	1/24	2.96×10^{-4}	–	35.88	–	*S. squatina*
Transect 44	Yes	3/24	Well 1: 2.27×10^{-4} Well 2: 7.67×10^{-4} Well 3: 3.26×10^{-4}	4.40×10^{-4}	38.05 35.98 37.44	37.16	*S. squatina*
Transect 45	Yes	1/24	1.07	–	24.05	–	Unreadable
Transect 50	Yes	1/24	2.72×10^{-04}	–	37.75	–	*S. squatina*
Transect 66	Yes	1/24	1.05×10^{-03}	–	35.58	–	Unreadable
Transect 73	Yes	1/24	7.30×10^{-05}	–	39.47	–	*S. squatina*

of the 24 qPCR replicates, with a concentration of 2.2×10^{-5} ng μl^{-1}. The qPCR analysis also revealed a detection near Bastia at transect 38 (Figure 2), with an eDNA concentration of 2.96×10^{-4} ng μl^{-1} in one of the 24 qPCR replicates. The highest eDNA concentration (1.07 ng μl^{-1}) was detected at transect 45, located at the mouth of the Golo River, south Bastia (Figure 2). The adjacent transect (44) also revealed the

presence of angel shark, with target eDNA detected in three of 24 qPCR replicates, leading to a mean eDNA concentration of 4.40×10^{-4} ng μl^{-1} (SD = 2.88×10^{-4} ng μl^{-1}). Additionally, target eDNA was present in one qPCR replicate for transect 50 near Santa-Lucia-di-Moriani, transect 66 at Aléria bay, and transect 73 in Solenzara, with eDNA concentrations of 2.72×10^{-4}, 1.05×10^{-3}, and 7.30×10^{-5} ng μl^{-1}, respectively.

4 | DISCUSSION

eDNA barcoding is a promising approach to provide important information for the conservation of rare species (Coutts et al., 2022), especially when used with an appropriate sampling design. Although the developed qPCR assay can amplify DNA of 13 other species of fish (Appendix S6), none overlap with the ranges of the three *Squatina* species in this study. Even though hybridization between primers and target sequences of *S. aculeata* and *S. oculata* present base-pair mismatches near the 3′ end of the primers, which has been shown to reduce extension efficiency (Wu, Hong & Liu, 2009; Wilcox et al., 2013), their tissue-extracted DNA was well amplified by the developed primers. By sequencing each qPCR product it was possible to confirm that the amplified eDNA sequence belongs to *S. squatina*, as its barcode slightly differs from the two other species (with nine mismatches between *S. aculeata* and *S. squatina* barcodes and 12 mismatches between *S. oculata* and *S. squatina* barcodes). The qPCR assay developed in this study could therefore be used in future research targeting *S. aculeata*, *S. oculata*, and *S. squatina*, with the addition of a mandatory sequencing step to identify the exact species. As all three species are in danger of extinction, targeting all three species at once is vital for their conservation, but application in the field to *S. aculeata* and *S. oculata* still needs to be validated on eDNA samples.

The qPCR assay reliably detected target eDNA concentrations of as little as 2.20×10^{-5} ng μl^{-1}. Improving the detection threshold of the assay (i.e. sensitivity) would allow detection of even lower concentrations of target eDNA, which could reduce false-negative results (Rees et al., 2014). To minimize false-positive results, synthetic DNA can be used for the positive control wells in the qPCR plate. Replacing control tissue-extracted DNA with synthetic DNA enables the detection of cross-contamination, as it is possible to distinguish synthetic DNA from true positive eDNA after Sanger sequencing, thanks to a unique synthetic insert (Wilson, Wozney & Smith, 2016).

The results confirmed the presence of *S. squatina* on the east coast of Corsica, where it is occasionally observed by local fishers (Lapinski & Giovos, 2019). They also revealed that the distribution area of *S. squatina* extends to the north-west coast of Corsica, within the PNMCCA, where it has never previously been observed. The lack of detection in 69 of 76 transects does not prove that angel sharks were not present at the sampling sites. There is potentially a large number of abiotic and biotic factors that can affect eDNA detectability at sea (Barnes & Turner, 2016). These non-detections could be explained by environmental parameters such as water currents that could have diluted the eDNA molecules (Wood et al., 2020), reducing the likelihood of collecting eDNA with the peristaltic pump. Also, the shedding of eDNA by organisms depends on the species and on various intrinsic parameters, such as the size of the individual, its diet, life stage, physiological stress, and metabolic rate (Rourke et al., 2021). For example, high water temperatures can generate higher metabolism and physiological activity of fish, increasing the loss of genetic material through the skin or mucus (Jo et al., 2019). Once released into sea water, eDNA gradually degrades (within hours and for up to several weeks; Collins et al., 2018; Salter, 2018; McCartin et al., 2022), mainly through abiotic factors such as salinity (Collins et al., 2018), sunlight (Andruszkiewicz, Sassoubre & Boehm, 2017), and temperature (Strickler, Fremier & Goldberg, 2015). Temperature is considered to be the most significant controlling factor of the eDNA degradation rate in sea water (Caza-Allard et al., 2022; McCartin et al., 2022), with a lower degradation rate below 16°C, compared with higher temperatures (Caza-Allard et al., 2022). The more intense activity of microbial communities at higher sea water temperatures (e.g. during summer) increases the breakdown of eDNA (Salter, 2018). Moreover, when inorganic phosphate is highly limited in the marine system, eDNA disappears in only 3 h because of the action of marine microbes using eDNA for its phosphorus, making eDNA scarce in the water column (Salter, 2018). Besides these environmental factors, the non-detection of target eDNA can also be explained by the number of biological replicates (Dickie et al., 2018). Only one eDNA sample was collected per transect, to cover a larger stretch of the Corsican coastline. Having more sample replicates per transect could have led to a higher detection rate of target eDNA. Xia et al. (2018) showed that three eDNA replicates were required to demonstrate rare species occurrence for field samples. However, they filtered only 100 ml of water per eDNA sample (cf. 30 L in current study) and performed only one qPCR replicate per eDNA sample (cf. 24 in current study).

5 | CONCLUSION AND CONSERVATION PERSPECTIVES

Improving knowledge on the distribution of *S. squatina* by detecting new occurrences is the first step to better understanding the ecology of this rare species in Corsica. Successful detections of *S. squatina* at 9% of the sites along the north-west and east coasts of Corsica provide an initial overview of the distribution of this Critically Endangered shark in one of its last refuges in the Mediterranean. The rapid results, obtained in only one week, allowed divers of the Andromède Océanologie team to visit the positive sites and two angelsharks (*S. squatina*) were observed near the mouth of the Golo River, where the highest target eDNA concentration had previously been detected. These two individuals were subsequently tagged to study their movements, which is still poorly documented for this species (Ellis et al., 2020). This illustrates the potential of eDNA barcoding for the instantaneous monitoring of imperilled species. However, this method must be rigorously used because false-positive

and false-negative detections could lead to costly management or ecological impacts (Langlois et al., 2021).

The methodology developed here will be applied to the west and south coast of Corsica (where the presence of angel sharks is unknown) in May 2023 to complete this present study, but with a more optimized sampling protocol (using two eDNA filters along each 5-km-long transect). It will also be used by PNMCCA stakeholders within this marine protected area to confirm the occurrence of the species. Results of detection can contribute to pinpointing critical areas for angel shark conservation in the region and facilitate the development of local public education and awareness initiatives. Further research should be carried out to study the spatiotemporal variability of angel shark presence along the Mediterranean coasts using this method, by filtrating sea water along transects parallel with the coast, at different depths and seasons. The results of such a study could help to inform the implementation of conservation measures such as limiting commercial and recreational fishing in particular zones during breeding (winter) and pupping (spring/summer) seasons (Meyers et al., 2017). The interest of using the eDNA approach is that one filter sample can provide information on both the presence of the target species, even in low abundance (by qPCR analysis), and the associated fish community (by metabarcoding analysis; Boulanger et al., 2021). Sea water eDNA can also offer important insights on genetic diversity, population structure, and connectivity (Dugal et al., 2022), which could help identify distinct angel shark populations that require separate management and conservation efforts.

AUTHOR CONTRIBUTIONS

Nadia Faure: Writing—original draft; investigation; writing—review and editing; methodology; visualization. **Stéphanie Manel:** Conceptualization; methodology; writing—review and editing; supervision. **Bastien Macé:** Investigation; methodology; writing—review and editing. **Véronique Arnal:** Investigation; methodology; writing—review and editing. **Nacim Guellati:** Investigation. **Florian Holon:** Investigation; methodology. **Adèle Barroil:** Investigation; visualization. **Franck Pichot:** Investigation. **Jean–Jacques Riutort:** Resources. **Gianni Insacco:** Resources. **Bruno Zava:** Resources. **David Mouillot:** Funding acquisition; conceptualization. **Julie Deter:** Conceptualization; funding acquisition; methodology; investigation; supervision; project administration; writing—review and editing.

ACKNOWLEDGEMENTS

This work was funded by the ANGE project led by the University of Montpellier and co-financed by Agence de l'eau Rhône Méditerranée Corse (French Water Agency), Office Français de la Biodiversité (OFB), Parc Naturel Marin du Cap Corse et de l'Agriate (PNMCCA), Andromède Océanologie, and Bastia Offshore Fishing. The fieldwork was part of the scientific expedition GOMBESSA CAP CORSE led by Andromède Océanologie and supported by Manufacture de Haute Horlogerie Suisse Blancpain, Blancpain Ocean Commitment, Prince Albert II de Monaco Foundation, Société des explorations de Monaco, OFB, PNMCCA, and Agence de l'eau RMC. The expedition was also supported by La Marine Nationale (French Navy) and Préfecture Maritime de la Méditerranée. We thank the CeMEB Labex high-throughput qPCR platform (University of Montpellier) and the GEMEX platform (CEFE) as well as Francis Poulat (IGH laboratory) and Manon Garcia (CEFE) for laboratory work. We also thank Laurent Ballesta (Andromède Océanologie) for the picture, Eric Durieux and Caroline Bousquet (Stella Mare) for sharing fishing data, and José Luis Crespo (Oceanographic Foundation, Valencia) for providing an angel shark tissue sample.

CONFLICT OF INTEREST STATEMENT

The authors have no conflicts of interest to declare.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/. GenBank accession numbers can be found in Appendix S7.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Faure, N., Manel, S., Macé, B., Arnal, V., Guellati, N., Holon, F. et al. (2023). An environmental DNA assay for the detection of Critically Endangered angel sharks (*Squatina* spp.). *Aquatic Conservation: Marine and Freshwater Ecosystems*, 1–10. https://doi.org/10.1002/aqc.3954