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Mestrado em Biologia Marinha

Supervisors:

Ester Serrão

Co-supervisor

Rima W. Jabado

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Declaração de autoria de trabalho

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Declaro ser a autora deste trabalho, que é original e inédito. Autores e trabalhos consultados estão devidamente citados no texto e constam da listagem de referências incluída.

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ABSTRACT

Sharks and rays are the most threatened group of marine vertebrates. Extinction risk is highest for species in tropical and subtropical areas, often associated with developing nations that generally lack baseline data on species diversity. Although considered a regional elasmbranch hotspot, species diversity in the Banc d'Arguin National Park (PNBA) in Mauritania has not been characterized. Here, a first description of species diversity is provided based on a combination of approaches. DNA barcoding was used to build a regional genetic reference database of species sampled at local processing and landing sites. Two potentially new species (genus *Gymnura* and *Torpedo*) were found and possible new lineages for *Sphyrna lewini* and *Aetomylaeus bovinus* were uncovered, however, results should be observed with caution and are pending further confirmation. *Mustelus punctulatus* and *Hypanus rudis* were confirmed from Mauritania for the first time, extending their known distribution range. Metabarcoding was used to explore species diversity in eDNA samples from the PNBA. Results confirmed the presence of 29 different species, 12 sharks and 17 rays of which 14 species had previously never been reported from the PNBA in the literature. Notably, *Mustelus punctulatus* was found in 77% of the eDNA samples, while the only locally reported smoothhound, *M. mustelus*, was absent in all samples. *Sphyrna lewini*, a species previously reported, was absent from all eDNA samples, however its presence was visually confirmed. Putative new *Gymnura* and *Torpedo* species were found throughout various eDNA samples. A total of 66.6% of shark and ray species in the PNBA are threatened with extinction (including *S. lewini*). Considering the high fishing pressure sharks and rays are exposed to, these results emphasize the importance of taxonomic identification for individual species management and provide a baseline to inform future studies and shark and ray specific conservation measures in the PNBA.

RESUMO

Os tubarões e as raias são o grupo de vertebrados marinhos mais ameaçado, principalmente devido a um aumento contínuo do esforço de pesca ao longo das últimas décadas. Os peixes cartilagíneos são inerentemente mais susceptíveis à pressão da pesca devido às características da história de vida que geralmente incluem crescimento lento, maturação tardia e baixa fecundidade. Actualmente, mais de um terço das espécies são de grande preocupação em termos de conservação. O risco de extinção é maior para espécies em áreas tropicais e subtropicais, frequentemente associadas a nações em desenvolvimento que geralmente carecem de dados de

base sobre diversidade de espécies e capacidade de monitorização adequada. Um desses países é a Mauritânia, localizada na África subsaariana ocidental, que alberga a maior área marinha protegida da África ocidental, o Parc National du Banc d'Arguin (PNBA), criado em 1976. Os tubarões e as raias eram fortemente explorados na área antes de 2003, quando a gestão do PNBA e a população de pescadores indígenas, aos quais foram concedidos direitos exclusivos de pesca em 2000, acordaram numa moratória sobre quase toda a pesca dirigida ao tubarão e às raias. No entanto, a pressão da pesca de tubarões e raias não parece ter diminuído e o grupo parece representar entre 35% e 45% dos desembarques no PNBA. Embora tenha sido considerado um hotspot regional de tubarões e arraias, a diversidade de espécies no PNBA não foi caracterizada anteriormente.

Aqui, a taxonomia das espécies locais é revista e é fornecida uma descrição detalhada da diversidade de espécies com base numa combinação de abordagens. A codificação de barras de ADN foi utilizada para construir uma base de dados de referência de sequência genética 12S específica de espécies amostradas em locais de processamento e aterragem de tubarões e raias locais entre Outubro de 2020 e Abril de 2021. Os genes COI e NADH2 foram utilizados para confirmar algumas identidades ambíguas de espécies que não puderam ser determinadas com confiança apenas através do 12S. Um total de 28 espécies foram codificadas com códigos de barras, das quais 12 eram tubarões e 16 eram raias. 14 espécies forneceram códigos de barras totalmente novos 12S à base de dados global e quatro espécies forneceram novos códigos de barras COI. Duas espécies potencialmente novas no género *Gymnura* e *Torpedo*, aqui referidas como *Gymnura* sp. e *Torpedo* sp., foram encontradas e preliminarmente corroboradas com base em resultados genéticos, bem como em diferenças morfológicas com outras espécies descritas dentro do seu género. Possíveis novas linhagens nas espécies *Sphyrna lewini* e *Aetomylaeus bovinus* foram descobertas, mas devem ser observadas com cautela e estão pendentes de confirmação genética e morfológica adicional. Foi encontrada uma *Rhinobatos rhinobatos* de forma anormal, que foi compatível com a espécie com base em sequências 12S, levando à conclusão preliminar de que o focinho arredondado dos espécimes é uma deformação morfológica. Da mesma forma, *R. rhinobatos* com padrões de coloração distintos semelhantes a *R. irvinei* foram atribuídos à espécie anterior com base em combinações de sequências 12S. No entanto, a confirmação para ambas está pendente de análise genética adicional, dado que as sequências 12S aqui codificadas mostram geralmente taxas de divergência mais baixas do que outros genes. *Mustelus punctulatus* e *Hypanus rudis* foram confirmados na Mauritânia pela

primeira vez, estendendo a sua conhecida área de distribuição a sul do Sahara Ocidental e a norte da Gâmbia, respectivamente. Uma espécie do género *Fontitrygon* não pôde ser resolvida com confiança ao nível da espécie e foi tratada como *F. margarita/margaritella*. No total, 19 das 28 espécies (20 se incluir *F. margarita*) são colocadas numa das três categorias de ameaça determinadas pela Lista Vermelha da IUCN, com seis espécies avaliadas como Criticamente Ameaçadas, cinco como Ameaçadas e oito (ou nove) como Vulneráveis. As espécies ameaçadas de extinção constituem 68% (ou 71%) das espécies de código de barras neste estudo.

Além disso, foi utilizado um ensaio de metabarcodificação específica de tubarões e raias para confirmar a presença de espécies no PNBA. Um total de 29 espécies, 12 tubarões e 17 espécies de raias, estiveram presentes em amostras de eDNA colhidas em locais espalhados pelas áreas norte, centro e sul do PNBA, incluindo uma espécie não identificada de *Myliobatis*, presumivelmente *M. aquila*. Uma única amostra colhida fora dos limites do PNBA detectou 12 espécies, contudo não mostrou diversidade adicional de espécies. Foram também recolhidas amostras de DNA ambiental em poços de salga em locais de processamento de tubarões e arraias para avaliar a utilidade do método na detecção de algumas espécies menos comuns e corroborar a sua utilidade como possível ferramenta de monitorização futura em locais de processamento.

Os resultados de metabarcodificação confirmaram a presença de 29 espécies diferentes no PNBA, das quais 12 são tubarões e 17 são raias, e das quais 14 espécies nunca tinham sido anteriormente registadas do PNBA na literatura, algumas das quais são tubarões de maiores dimensões, pelágicos ou de águas mais profundas, cuja presença no PNBA é presumivelmente transitória. Outras espécies incluem arraias da família Dasyatidae que são frequentemente reportadas apenas a nível familiar ou de género e que, por conseguinte, podem ter estado ausentes dos registos de desembarque anteriores. Notavelmente, *Mustelus punctulatus* foi encontrado em 77% das amostras de eDNA do PNBA, enquanto a única espécie *Mustelus* reportada localmente, *M. mustelus*, estava ausente em todas as amostras. *Squalus acanthias*, uma espécie comum em regiões de águas temperadas e frias, foi detectada em 69% das amostras, mas a sua presença ainda não foi confirmada visualmente. *Sphyrna lewini*, uma espécie comumente capturada no PNBA, esteve ausente em todas as amostras de eDNA, mas a sua presença foi confirmada visualmente. As putativas novas espécies de *Gymnura* e *Torpedo* foram ambas encontradas em várias amostras de eDNA. De um total de 29 espécies positivamente identificadas a partir de amostras recolhidas dentro dos limites do PNBA, seis espécies estão Criticamente em Perigo

(20,7%) se assumirmos a presença de *M. aquila*. Cinco espécies estão em Perigo (17,2%) e oito são Vulneráveis (27,6%) na Lista Vermelha da IUCN. As espécies listadas como ameaçadas de extinção no PNBA ascendem portanto a 65,5% e incluem as sete espécies mais abundantes em termos de abundância de leitura. Ao incluir *S. lewini*, o número de espécies de tubarões e raias ameaçadas no PNBA aumenta para 66,6%. Em amostras de eDNA dos poços de salga, um total de 26 espécies foram detectadas em apenas duas amostras, incluindo seis espécies não detectadas em amostras ambientais, nomeadamente *Galeocerdo cuvier, S. lewini, Sphyrna zygaena, Sphyrna mokarran, Torpedo marmorata* e *Pseudotriakis microdon* e três espécies que não foram observadas in situ (*G. cuvier, T. marmorata* e *P. microdon*). Apenas uma espécie confirmada in situ não foi detectada em amostras de eDNA (*Raja parva*).

Este estudo representa tanto o primeiro exaustivo esforço regional de codificação de barras como o primeiro levantamento de elasmobrânquios de eDNA na África Ocidental. É também o primeiro a caracterizar a diversidade de espécies de tubarões e raias no PNBA, utilizando ferramentas de levantamento molecular. Considerando que as espécies de alta pressão de pesca estão expostas no PNBA e o precário estado de conservação de uma elevada proporção de espécies locais, estes resultados enfatizam a importância da identificação taxonómica para a gestão individual das espécies e fornecem uma importante base de referência para futuros estudos e medidas de conservação específicas de tubarões e raias no PNBA.

STATE OF THE ART

1. Shark and Ray Conservation

1.1. Overview

Sharks and sharks, encompassed in the higher taxonomic category of elasmobranchs, date back around 400 million years in evolutionary history and therefore represent some of the oldest extant vertebrate lineages on the planet (Grogan & Lund, 2004). There are currently 1,239 species described including 554 sharks and 685 rays (Fricke et al., 2021). These two groups have diversified to adapt to and thrive in most aquatic conditions and habitats, from deep sea to coastal, pelagic, brackish and even fresh water environments as well as the freezing waters of the Arctic. They are found circumglobally in every major ocean and sea, where they play a fundamental regulatory role as meso- and apex predators within the ecosystems they occupy. Sharks and rays can hence be considered as keystone species in many ecosystems (Libralato et al., 2006) that regulate their environment mostly through top-down, mesopredator release effects (Myers et al., 2007; Ferretti et al., 2010) and through shaping prey behavior (de Vos et al., 2015). Their removal is thought to have important implications for overall ecosystem health (Duffy et al., 2002; Myers et al., 2007; Ferretti et al., 2010).

Research over recent decades have generated awareness on both the ecological importance of sharks and rays in marine ecosystems as top predators (Stevens et al., 2000) as well as on dwindling shark and ray populations due to human impact (Dulvy et al., 2021). Sharks and rays have experienced widespread population declines in recent decades due to a range of anthropogenically induced threats (Sala & Knowlton, 2006; Davidson et al., 2015). These include, but are not restricted to: large-scale targeted commercial fisheries, fisheries targeting other species where high amounts of bycatch are common, habitat destruction, climate change and pollution (Dulvy et al., 2021). Overexploitation from fishing is largely driven by the high demand of the Asian market for shark fins (Dulvy et al., 2008), mobulid (family Mobulidae) gill plates, and meat (Dent & Clarke, 2015), which is consumed in many countries, often unknowingly (Bornatowski et al., 2015). Shark fins have played a pivotal role in the exploitation of sharks, due to their economic high value (Dent & Clarke, 2015). In the past, this promoted wasteful practices such as “finning”, where fins were cut off from live animals and the severed body was thrown back in the water. While this practice has been outlawed in many places due to emerging conservation concerns, fins remain a valuable and highly traded item. However, the

shark and ray meat trade has been steadily increasing in recent decades and may currently be of more concern due to a more geographically widespread demand (Dent & Clarke, 2015).

Global shark and ray population declines have boosted efforts by the scientific and conservation community to tackle problems such as overfishing, high bycatch rates, finning and illegal, unreported and unregulated (IUU) fishing, as well as resolving distribution and abundance patterns and taxonomic uncertainties. Improving our understanding of species and addressing these issues is critical to ensure long-term survival of species.

1.2. Challenges to conservation

Sharks and rays as a group tend to be more susceptible to fishing pressure than many teleost species due to their inherent life history characteristics that generally include slow growth, late maturation and low fecundity, making them a more fragile resource with lower than average reproductive potential (Stevens et al., 2000; Simpfendorfer & Kyne, 2009). Shark and ray landings initially rose steadily from the 1950's (Dulvy et al., 2014; FAO, 2014) and animals were initially disregarded because they made up only a small percentage of the worlds' fisheries and had low economic value (Bonfil, 1994). Global reported landings however started decreasing after a peak in 2003, which is ascribed mainly to global population declines rather than the effects of positive management implementation, which are generally recent in most places (Davidson et al., 2015). Furthermore, fisheries often group sharks and rays into higher, more aggregated taxonomic categories compared to teleosts, with only 28% of landings identified to species level in 2011 (FAO, 2014). As a result, scientific baseline knowledge on the biology and ecology of many species as well as on accurate fisheries statistics are scarce. Lack of species-specific catch reports tend to prevail in developing countries, many of which account for some of the highest global shark and ray landings (FAO, 2014).

In addition to the scarcity of reporting, animals that are reported to species level are often misidentified by observers or fishermen. In an unprecedented study, Tillett et al. (2012) found that about 20% of sharks in a batch of morphologically similar carcharhinid sharks were misidentified by trained observers whereas genetic verification provided correct taxonomic placement for all species. Smart et al. (2016) obtained similar results where 14% of sharks were assigned an incorrect species name, potentially including significant errors into life history parameters of the studied species such as length-at-age and maturity estimates. These problems

emerge not only during landings, where sharks and rays are fresh and presumably easier to identify, but also upon entering the meat market where defining morphological features such as fins have usually been removed, making accurate identification more challenging. These studies point towards a general bias in the shark and ray trade that may skew abundance estimates of certain species and hinder accurate predictions on population viability under fishing pressure (Burgess et al., 2005). Misidentification of species occurs predominantly within clusters of morphologically similar species (Branstetter, 1982; Tillet et al., 2012) and leads to the collection of inaccurate biological data, in certain cases hindering proper conservation status assessments on species that are either over- or underrepresented (Tillet et al., 2012). This poses conservation issues for species in terms of management units.

Among other research priorities, to properly assess and implement successful management strategies it has been imperative to increase knowledge about the diversity and distribution of sharks and rays as well as the impact of fisheries on exploited species, among other research priorities. Therefore, emphasis had to be put on improving species identification, which in turn improves the specificity of catch reports. Overall, DNA barcoding has been a successful tool in addressing some of the existing problems concerning misidentification of species while environmental DNA (eDNA) collection has been shown to give insights into species diversity in places that are difficult to monitor and for species that are generally elusive.

2. Shark and ray taxonomy and diversity – molecular tools

2.1. Overview

Before the development of molecular techniques, the taxonomic classification of sharks and rays was based on morphometric and phenotypical studies (e.g. Heemstra, 1997). The phylogenetic relationships of sharks and rays were and are still the subject of scientific debate and have undergone a number of changes, specifically regarding the classification of rays in relation to sharks (reviewed in Naylor et al., 2005). Growing conservation concerns prompted a spike in interest in taxonomic resolution as a means for better promoting conservation and improving fisheries management of individual species and biodiversity (Crobe et al., 2021; Iglésias et al., 2010; Last et al., 2008; Veríssimo et al., 2017). Whilst the description of new species in the last two centuries saw increases in effort, the greatest peak in species descriptions is attributed to the last two decades, partly due to advances in molecular techniques (White et al., 2012). However, it

is important to note that shark and ray taxonomy remains dynamic and is regularly undergoing revisions and updates.

2.2.DNA barcoding

Genetic approaches to the taxonomy and phylogeny of sharks and rays have been advanced by the development of molecular techniques such as DNA barcoding. The use of DNA barcoding revealed the existence of cryptic species (Henderson et al., 2016; Griffiths et al., 2010; Quattro et al., 2006) and species complexes (Naylor et al., 2012), facilitating the identification and description of new taxa through the use of several genetic markers, some of which have been used more frequently for shark and ray research.

2.3.Genetic markers in shark and ray research

2.3.1. Cytochrome C oxidase subunit I

Cytochrome C oxidase subunit I (cox1 or COI) is a mitochondrial gene that is used as the standardized genetic marker for members of the entire animal kingdom (Hebert et al., 2003a; Hebert et al., 2003b). In light of its widespread success, the COI gene was chosen to establish a global reference library of DNA barcodes of all fish species in an international collaborative research effort through The Fish Barcode of Life Initiative (FISH-BOL; www.fishbol.org). This global effort in DNA barcoding of marine fishes is based on the amplification of an approximately 650bp long COI region for which Ward et al., (2005) created a set of universal primers (FISH F1&2/R1&2).

Ward and Holmes (2007) analyzed the DNA barcode region in 388 species of fishes, including 61 sharks and rays, showing that barcoding could distinguish between 98 – 99% of the examined species. Other studies that have focused specifically on the identification of sharks and rays through the barcoding of the COI gene have also shown it to be an effective marker with a species level success rate of between 83 – 100 % (Velez-Zuazo et al., 2015; Ward et al., 2008; Holmes et al., 2009; Sembiring et al., 2015) and a mean interspecific sequence divergence of 7.48% (Ward et al., 2008). Most studies using the COI marker have focused on the use of DNA barcoding for the identification of processed shark and ray products such as fins (Fields et al., 2015) or meat (Marchetti et al., 2020) in an attempt to uncover misidentifications or mislabeling of these products, while other studies focused on creating a species inventory for certain countries or regions (Jabado et al., 2014; Moftah et al., 2011).

Despite the previous widespread use of the universal COI primers for sharks and rays, distinct primer sets have been necessary for more specific aims. As the 650 bp sequence is relatively long, it often fails to amplify heavily processed products where DNA is too degraded or DNA from environmental samples that is usually too fragmented. Leray et al. (2013) designed a primer that would target a 313 bp region of the COI gene and performed well across different metazoan taxa when used for Next-Generation Sequencing. Fields et al. (2015) developed an even smaller barcode of 110 – 130 bp based on partial COI sequences in order to identify species listed on the Convention on International Trade in Endangered Species of Flora and Fauna (CITES) from processed shark fins. Cardeñosa et al. (2017) on the other hand developed a multiplex PCR mini-barcode assay based on two short fragments of the COI gene with which they were able to identify all CITES listed sharks to species level.

Even though COI barcoding is an effective tool for shark and ray species identification, it is not without its flaws. First, no shark and ray-specific primers have been developed for this genetic marker in its full length, as it was intended for a widespread and standardized use. Furthermore, sequences can be deposited in the database indiscriminately, possibly sacrificing quality over quantity at times. As was shown by Tillet et al. (2012) and Smart et al. (2016) species are often misidentified, even by professional observers. Hence, it can be expected that genetic sequences may sometimes be erroneously assigned to certain species and made public without further verification. This may be problematic as COI has amassed the largest barcode database and hence remains the most used genetic marker. Second, while COI sequence divergences between species usually exceed the 2% threshold used as criterion for species delimitation (Hebert et al., 2003a), it cannot reliably discriminate among closely related species with low interspecific sequence divergence such as some species in the "*Carcharhinus* spp." complex (Jabado et al., 2014; Ward et al., 2008) or the "*Raja* spp." complex (Ball et al., 2016) and often provides unclear matches. Therefore, other markers that display a faster rate of evolution and have primers specifically designed for sharks and rays may be more useful in resolving some of the taxonomic uncertainties that are problematic with COI.

2.3.2. NADH dehydrogenase subunit 2

Naylor et al. (2005; 2012) proposed the mitochondrial gene NADH dehydrogenase subunit 2 (NADH2) as an alternative to COI for molecular identification of sharks and rays due to its faster-evolving nature and longer amplicon of around 1041 – 1047 bp. A set of universal primers

(ILEM & ASNM) and additional genus- and species-specific primers were developed (Naylor et al., 2012) based exclusively on a large selection of existing shark and ray genetic mitogenomes. The study, which resulted in a highly curated and verified genetic reference database, included a total of 468 species of sharks and rays, about a third of the known biodiversity. Intraspecific sequence divergences ranged between 0 – 2.12% with an average intraspecific divergence of 0.27% and an average interspecific divergence of 10.81%, supporting the hypothesis that the NADH2 marker would be more successful at distinguishing between recently evolved sister species than the COI barcode fragment. This was demonstrated further by Henderson et al. (2016) whose exhaustive taxonomic assessment of sharks, rays and guitarfishes of the Arabian Peninsula resulted in distinct clustering of specimens such as the grey reef shark (*Carcharhinus amblyrhynchoides*), the Australian blacktip shark (*C. tilstoni*), the blacktip shark (*C. limbatus*) and the spinner shark (*C. brevipinna*), which belong to the problematic *Carcharhinus* spp. complex. However, the sandbar shark, *C. plumbeus*, and the bignose shark, *C. altimus*, as well as the shortfin devil ray, *Mobula kuhlii*, and the longhorned devil ray, *M. eregoodootenkee* (now *M. eregoodoo*), displayed very low genetic distances, supposedly due to recent speciation, resulting in a failure to distinguish between species during phylogenetic analyses despite clear morphological differences.

Many recent studies have resorted to making use of both markers, COI and NADH2, to achieve higher taxonomic resolution or to fill in gaps through the use of shorter sequences of both markers when processed products do not allow for the amplification of the full barcode (Marchetti et al., 2020; Feitosa et al., 2018).

2.3.3. 12S ribosomal RNA

Certain cases warrant the use of genetic markers with very short regions of less than 200 bp that can reliably be amplified to identify individuals to species level when DNA is too degraded, as for example, the relatively conserved mitochondrial 12S rRNA gene. While Cawthorn et al. (2012) found that average inter-species distances were 33 times higher than intra-species distances when using 12S as a genetic marker, the overlap in range of both sequence divergences was high and was attributed to the close relationship among certain congeners, giving rise to doubts about the marker as a reliable barcoding tool. However, Miya et al. (2015) developed new universal primers (MitoFish-U) to target a short, hypervariable region (163-185bp) of the 12S rRNA gene that is flanked by two highly conservative regions. Where other studies failed to

achieve high species resolution or to detect shark or ray species through the use of the 12S marker (Kelly et al., 2014), Miya et al. (2015) were able to detect 93.3% of species out of a broad selection of taxonomically diverse fishes, proving the efficacy of this marker in studies involving metabarcoding of large fish assemblages. Additionally, the development of shark and ray specific primers (MitoFish-E) was an important step towards improving eDNA metabarcoding for shark and ray research purposes (Miya et al., 2015). The 12S sequences allow better species identification than COI, but lack the extensive reference libraries already available for COI, therefore updated reference libraries are urgently needed (Collins et al. 2019).

As a final consideration, and although all three genetic markers perform relatively well individually, when it comes to reliable species identification of sharks and rays, it is advisable to a) verify the genetic information obtained through one marker with others, at least where doubt exists, and b) complement genetic information with morphological considerations.

2.4. Environmental DNA (eDNA)

DNA barcoding of shark and ray species goes hand in hand with the recent development of environmental DNA (eDNA) techniques involving the amplification and parallel sequencing of DNA fragments present in the water column. Similar to other living organisms, sharks and rays shed genetic material in the form of skin cells, urine, tissue and other biological material as they interact with their environment, leaving behind small trace amounts of DNA that can be detected through molecular techniques. These can be used to detect single target species through the design of species-specific primers, commonly done when looking for rare and threatened species (Simpfendorfer et al., 2016; Budd et al., 2021). Other studies have focused on characterizing entire fish communities or shark and ray biodiversity in different habitats based on eDNA (Bakker et al., 2017; Yamamoto et al., 2017; Boussarie et al., 2018; Lafferty et al., 2019; Mariani et al., 2021).

However, the amount and detectability of eDNA present in any particular environment is linked to the rate of organismal production, degradation and physical transport (Hansen et al., 2018). Collins et al. (2018) reported that eDNA could be detected for approximately 48h, but that its degradation rate was 1.6 times higher in inshore than in offshore environments. Due to the rapid degradation of DNA in the environment, primers need to fulfil certain requirements in order to produce results: a) primers need to amplify short genetic sequences of maximum a few hundred

basepairs and, b) the resulting amplicon needs to allow for good species resolution. Most eDNA studies involving sharks and rays have used primers targeting various smaller fragments of the COI gene, with the advantage of having a taxonomically wide and diverse database already at their disposal. In spite of this obvious advantage, Deagle et al. (2014) questioned COI as a suitable marker for metabarcoding, arguing that primer-binding sites within the gene are not sufficiently conserved for most amplicon-based metabarcoding applications. Unlike in traditional barcoding, where protocols can be adjusted to recover failed amplifications, metabarcoding usually yields amplicons from many different taxa and hence masking the absence or failed amplification of others, which hampers protocol optimization (Deagle et al., 2014). Yet some studies have made use of shark and ray specific COI "mini-barcoding" primers with good to moderate success, encountering problems mostly with phylogenetically complicated groups such as the *Carcharhinus* spp. complex, but also other genera such as *Rhizoprionodon* spp. and *Negaprion* spp. (Bakker et al., 2017). It has also been documented that species within the family Ginglymostomatidae (nurse sharks), among others, fail to amplify with these primers due to their non-degenerate sequences containing mismatches with the binding regions (Bakker et al., 2017), leading to false negative results.

Alternatively, many metabarcoding studies that focused on teleost fishes and/or sharks and rays have worked with 12S as their marker of choice due to the higher specificity of its primer-binding sites and its good species resolution (Kelly et al., 2014; Miya et al., 2015; Stat et al., 2017; Yamamoto et al., 2017; Budd et al., 2021). Miya et al. (2015) developed an shark and ray specific primer that succeeded in detecting all shark and ray species contained in eDNA samples from a source of known species composition and that has shown to cover a phylogenetically diverse array of species. Budd et al. (2021) successfully designed 12S primers aimed specifically at detecting the Critically Endangered scalloped hammerhead shark (*Sphyrna lewini*) in Micronesian waters for the first time in five decades, proving the efficacy of the method at bio-monitoring elusive species. The same is exemplified by the study conducted by Yamamoto et al. (2017), who in a single eDNA sample detected more than 60% of the fish species that had been recorded in 14 years of Underwater Visual Censuses (UVCs). When comparing eDNA metabarcoding to other well established shark survey methodologies such as baited remote underwater video surveys (BRUVS) and UVCs, eDNA revealed the presence of several species that had not been recorded through traditional methods in spite of a considerably greater sampling effort (Boussarie et al., 2018). The uses of eDNA are manifold and also include

comparing species diversity across regions of different levels of anthropogenic impact (Bakker et al., 2017), the study of population genetics (Sigsgaard et al., 2016) or the estimation of species abundance (Mariani et al., 2021), among others.

The use of eDNA shows great potential as a non-invasive, time and resource efficient survey technique with high detection rates that appears to reflect species composition more or as accurately as other methods, given the availability of thorough genetic reference databases. However, results should always be interpreted with caution as sample contamination is frequent and certain species may fail to amplify and hence provide incomplete or misleading results. The method also does not readily replace other monitoring methods that are used to inform fisheries as it only counts species as present or absent, but does not provide any further biological information. Nonetheless, it has shown promising results that will assist in informing management decisions for vulnerable species or groups of sharks and rays.

3. Mauritania and the Banc d'Arguin National Park

As mentioned above, accurate statistics on fisheries and basic taxonomic baselines are often scarce and largely missing in lesser developed regions of the world. This can become a problem when these regions are resource-rich but also highly economically dependent on these same resources. One such region is West Africa, where countries such as Mauritania have been largely overlooked by the scientific community when investigating the diversity of sharks and rays.

3.1. The location

Mauritania is a country located in western sub-Saharan Africa that borders the North-East Atlantic Ocean, south of Western Sahara and north of Senegal. The country's vast and unproductive desert landscape stands in contrast to the highly productive nature of its marine counterpart, which bears some of the richest waters in the world (Belhabib et al., 2012). The high amount of productivity stems mainly from a permanent upwelling zone around Cap Blanc in the north (M'Bareck & Mahfoudh, 1996; Wolff et al., 1993) and the desert dust (Michel et al., 2009), which serves as an additional source of nutrients enriching coastal waters (Fig. 1).

Occupying more than a third of the approximately 750 km of coastline and encompassing an area of 12.000 km² split almost equally between land and sea, is the Parc National du Banc d'Arguin (PNBA), the largest marine protected area in western Africa. The PNBA was created in 1976 and

has since been recognized as a wetland of international importance by the RAMSAR Convention in 1982 and as a UNESCO World Heritage Site in 1989.

The Banc d'Arguin is a shallow water bay composed mainly of intertidal sandbanks, mudflats, intricate channels and several dispersed islands (Wolff et al., 1993). Fuelled by cold, nutrient-rich water from the north, high rates of evaporation and without any continental freshwater inlets, the wetland area shows large variability of abiotic factors such as temperature, salinity and nutrient concentrations throughout the year (Ould Dedah, 1993; Wolff et al., 1993), allowing for a wide variety of biotopes and biodiversity. Although the area is primarily known for its importance as a foraging, nesting and wintering ground for various local and migratory bird species (Campredon, 2000), its ecological value stems from the dense seagrass meadows as well as scattered mangrove forests, which support a rich invertebrate fauna and function as feeding and nursery areas for a variety of fish species, including sharks and rays (Jager, 1993; Valadou et al., 2006), and others marine organisms (e.g. Schaffmeister et al., 2005).

3.2.Fishing culture

The Mauritanian Exclusive Economic Zone (EEZ) extends over 200 miles and covers an estimated area of 234,000 km². A prominent continental shelf and favorable hydro-climatic variables contributes to the high primary productivity of Mauritanian water (M'Barek & Mahfoud, 1996) and have made the country one of the most sought after African fishing ground for international commercial fishing fleets in the last few decades (Belhabib et al., 2012). Through fisheries agreements with the European Union, China and Turkey, among others, levels of fishing and marine resource exploitation have intensified substantially to the point of over-exploitation of several fish stocks (Meissa & Gascuel, 2014). Fishing contributes to almost 5% of the GDP in Mauritania and economic contributions from the fishing sector to Mauritanian export revenues amount to 43% and are therefore substantial, but only small amounts of it originate from locally operated, small-scale fisheries (Diop & Dossa, 2011).

The Banc d'Arguin however is off limits to commercial fishing vessels since the establishment of Law 2000-024 in January of 2000, which granted exclusive fishing rights to the indigenous Imraguen fisher community to protect and preserve the Park's natural resources. The Park contains nine villages that are home to over 1000 Imraguen, nomadic tribesmen whose lifestyle has been closely linked to fishing, using traditional techniques as well non-motorized sail boats

since the 1960's (Boulay, 2013; Ducrocq et al., 2004). Severe droughts in the 1970's pushed nomadic communities to settle down by the coast, increasing the demand for marine resources (Boulay, 2013). While the Imraguen took active part in commercial fishing ventures prior to the establishment of this law (Diop & Dossa, 2011; Ducrocq et al., 2004), it restricted all non-traditional and commercial activities within the Park, allowing only subsistence fishing with restrictions placed on fishing gear and quota imposed. Regulations were necessary to preserve dwindling fish stocks and ecosystem services provided by the Park.

Imraguen fishers primarily target fish based on their seasonal migrations including mainly the Meagre (*Argyrosomus regius*), the Mullet (*Mugil* spp.), the Tollo (*Mustelus* spp.) and the Sole (*Solea* spp.) However, Trégarot et al. (2020) found that approximately 257 different species were landed in the PNBA throughout their study period every year (2006 - 2017). According to their calculations the PNBA would contribute 15% to the gross added value of national fishing operations, demonstrating that revenues from catches within the PNBA were substantial in spite of restrictions placed on fishing for commercial purposes. Additionally, annual catches appeared to steadily increase, however only about 2% were used for self-consumption. These numbers are in conflict with the law established in 2000 under which Imraguen are granted the right to fish solely for subsistence. The fishing pressure exerted on sharks and rays inside (Barham et al., 2011; Trégarot et al., 2018) as well as outside the PNBA (Leurs et al., 2021) is likely to affect the status of local species under conservation threat.

3.3.The status of sharks and rays

Due to its oceanographic and topographic characteristics, Mauritania constitutes a natural boundary for southern and northern distribution limits of various shark and ray species (Ebert et al., 2013; Last et al., 2016a). Hence, the region hosts a high amount of shark and ray diversity in unique species combinations and is also thought to be an important pupping and nursery area for many species. This applies especially to the Banc d'Arguin whose sheltered and shallow nature and vast seagrass beds (Trégarot et al., 2018) offer the protection that many sharks and rays seek in their juvenile stages (Valadou et al., 2006).

Since the start of soaring demands for shark fins in the 1980's, extensive international fishing operations as well as artisanal fishing vessels have continuously diminished shark and ray stocks (Diop & Dossa, 2011). While shark and ray fishing was almost nonexistent in Mauritania in the

1970´s and catches were usually discarded, the arrival of Senegalese fishers targeting sharks in the country in 1978 marked the beginning of shark and ray targeted fisheries, exporting meat to neighboring countries and to Europe (Diop & Dossa, 2011). Driven by the steep increase in shark fin prices in Asian markets in the late 1980´s that led to migratory movements of fishers from neighboring countries looking for more resource-rich waters, and the high fishing effort from commercial vessels, shark and ray fishing developed rapidly into a state of overexploitation by the 2000´s (Diop & Dossa, 2011).

Before 2003, the Imraguen fishermen targeted sharks and rays with specially designed nets, particularly the Blackchin guitarfish (*Glaucostegus cemiculus*), whose fins fetch high prices in the market (Barham et al., 2011). However, the shark and ray fishery in the PNBA started showing signs of over-exploitation by the end of the century. In a report by the Institut Mauritanien de Recherches Océanographiques et de Pêches (IMROP), Barham et al. (2011) concluded that catches and average size of landed *G. cemiculus* were decreasing. Milk sharks (*Rhizoprionodon acutus*), one of the most commonly caught sharks in the PNBA, also showed yearly decreases in average length of 3% and higher amounts of juvenile sharks were being caught in the late 1990´s, with some species becoming increasingly rare and yields getting significantly smaller (Walker et al., 2005). In response to these issues, the PNBA and the Imraguen put forth a joint management plan as well as a system of co-management of small-scale fisheries in 2003 that resulted in a moratorium on targeted shark and ray fishing within the protected area. Exempt from this ban are the common smoothhound (*Mustelus mustelus*) and the barbeled houndshark (*Leptocharias smithii*), which require a minimum landing size of 60 cm (FAO, 2018). Shark and ray nets were repurchased from fishers and burned and their use was forbidden. However, the incidental catch of sharks and rays was not included in the ban, which prompted a shift of strategy from the outlawed shark and ray nets designed to catch meagre and tollo (Westlund et al., 2017). Between the years 2003 and 2017, shark and especially ray catches would experience a significant increase, comprising between 35% and 50% of landings (Trégarot et al., 2020; Westlund et al., 2017) and lead to the development of an illegal trade in sharks and rays in the PNBA (Diop & Dossa, 2011), in spite of efforts by the authorities to halt such activities. Among the most commonly fished ray species are the Lusitanian cownose ray (*Rhinoptera marginata*) and *G. cemiculus*, which made up 95% of ray catches in 2009 (Barham et al., 2011). Shark catches were dominated by *R. acutus*, the scalloped hammerhead shark, the nurse shark (*Gynglimostoma cirratum*) and in lesser quantities, the Atlantic weasel shark

(*Paragaleus pectoralis*) and various requiem shark species (*Carcharhinus* spp.) (Barham et al., 2011; Trégarot et al., 2020). The high monetary value of fins, the difficult access and the limited monitoring capacity in the PNBA fishing villages as well as at sea continue to be serious hindrances to the effective implementation of the ban (FAO, 2018).

Many of the most commonly landed species in the PNBA are assessed as threatened (Critically Endangered, Endangered, or Vulnerable) or of conservation concern on the IUCN Red List of Threatened Species (IUCN, 2021). However, conservation status is based on global assessments and may not represent the degree of threat to regional or local populations of the Banc d'Arguin. Additionally, no comprehensive taxonomic studies of sharks and rays have been published from the region, in spite of their ecological importance and the considerable local and international fishing effort.

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DNA barcoding as a tool to explore elasmobranch diversity in environmental DNA off the Banc d|Arguin (Mauritania)

Authors: Carolina de la Hoz Schilling[1], Rima W. Jabado[2], Ana Veríssimo[3], Luca Caminiti[1], Gareth A. Pearson[1], Ebaye Sidina[4], Khallahi Brahim[5], Mamadou A. Ba[4], Cheikhna Gandega[5], Alioune Niang[5], Ester Serrão[1]

[1] CCMAR—Center of Marine Sciences, CIMAR, University ofAlgarve, Campus de Gambelas, 8005-139 Faro, Portugal

[2] Elasmo Project, PO Box 29588, Dubai, United Arab Emirates

[3] CIBIO – U.P. – Research Center for Biodiversity and Genetic Resources, Vairão, Portugal

[4] Parc National du Banc d'Arguin (PNBA), Nouakchott, Mauritania

[5] Institut Mauritanien de Recherches Océanographiques et des Pêches (IMROP), Nouadhibou, Mauritania

***Corresponding author:** Carolina de la Hoz Schilling

E-mail: cdelahozschilling@gmail.com

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ABSTRACT

Sharks and rays are the most threatened group of marine vertebrates. Extinction risk is highest for species in tropical and subtropical areas, often associated with developing nations that generally lack baseline data on species diversity as well as monitoring capacity. Although considered a regional shark and ray hotspot, species diversity in the Banc d'Arguin National Park (PNBA) in Mauritania has not been characterized. Here, a first description of species diversity is provided based on a combination of approaches. DNA barcoding was used to build a region-specific genetic reference database of species sampled at local processing and landing sites. Two potentially new species in the genus *Gymnura* and *Torpedo* were found and possible new lineages in the scalloped hammerhead *Sphyrna lewini* and the duckbill eagle ray *Aetomylaeus bovinus* were uncovered. However, these results should be observed with caution and are pending further genetic and morphological confirmation. The blackspotted smoothhound *Mustelus punctulatus* and the smalltooth stingray *Hypanus rudis* were confirmed from Mauritania for the first time, extending their known distribution range. Furthermore, A shark and ray specific metabarcoding assay was used to explore species richness from water samples taken in the PNBA using the custommade genetic reference database. Results confirmed the presence of 29 different species, 12 shark and 17 ray species, of which 14 had previously never been officially reported in the PNBA. Notably, *Mustelus punctulatus* was found in 77% of the eDNA samples from the PNBA, while the only locally reported smoothhound, *M. mustelus*, was absent in all samples. *Sphyrna lewini*, a commonly caught species in the PNBA, was absent from all eDNA samples, however its presence was visually confirmed. The putative new *Gymnura* and *Torpedo* species were both found throughout various eDNA samples. Based on these results, 66.6% of shark and ray species in the PNBA are threatened with extinction according to the IUCN Red List of Threatened Species, when including *S. lewini*. Considering the high fishing pressure species are exposed to in the PNBA, these results emphasize the importance of taxonomic identification for individual species management and provide a baseline to inform future studies and shark and ray specific conservation measures in the PNBA.

INTRODUCTION

Elasmobranchs (sharks and rays) are currently assessed as the most threatened group of vertebrates with over one third of species considered to be threatened with extinction (Dulvy et al., 2021). Overexploitation through fishing has been identified as the main threat to species and

the leading cause of global population declines (Dulvy et al., 2021). The susceptibility of sharks and rays to high fishing pressure stems from their inherent biological traits, which generally include slow growth, late maturity and low fecundity, putting sharks and rays at higher risk of unsustainable exploitation relative to other commercially fished species (Simpfendorfer & Kyne, 2009; Stevens et al., 2000). However, sharks and rays continue to be extracted from the oceans in large numbers (Dulvy et al., 2021; FAO, 2014), a practice that is largely fueled by the trade in their products such as fins and meat (Dent & Clarke, 2015; Dulvy et al., 2008). In order to regulate shark and ray fishing and trade, an understanding of species diversity, abundance and distribution is essential.

Baseline data on species diversity and abundance at national levels, especially in developing countries (FAO, 2014), are often sparse due to landings mostly going unreported or reported catches not being taxonomically resolved (Burgess et al., 2005; FAO, 2014). To implement effective conservation and management strategies for sharks and rays, accurate species-specific data are required. Indeed, when species identifications are erroneous, data collected on size, maturity or other life history traits, as well as the abundance of taxa become potentially skewed (Smart et al., 2016), directly affecting fisheries management (Burgess et al., 2005). However, shark and ray species can be difficult to distinguish morphologically, even by trained observers (Smart et al., 2016; Tillett et al., 2012). Unreliable identification therefore directly interferes with conservation efforts designed to improve the status of individual species.

Molecular techniques based on the barcoding of genetic sequences has allowed the identification of species that are phenotypically similar (Hebert et al., 2003a). Mitochondrial genes combine a number factors making them suitable for species identification, including a desirable balance between conserved and fast-evolving, variable regions as well as a large barcoding gap, where intraspecific variability is low and interspecific variability is high enough to accurately assign taxonomy (Hebert et al., 2003b; Miya et al., 2015; Naylor et al., 2012). Although this does not apply to all taxa, as some species complexes (e.g. *Carcharhinus* spp.) display very low interspecific genetic divergence (Fields et al., 2015; Naylor et al., 2012), several genetic markers have been successfully used for the phylogenetic disentanglement of shark and ray species (Naylor et al., 2012; Ward et al., 2005, 2008). It has also lead to the discovery of cryptic diversity (Griffiths et al., 2010; Naylor et al., 2012; Quattro et al., 2006) and/or the revision of taxonomic assignments (Crobe et al., 2021; Henderson et al., 2016; Naylor et al., 2012).

In additional to the taxonomic resolution of individual shark and ray species, effective management also relies on knowledge on habitat specific or regional species diversity and distribution to support population assessments. Commonly used survey methods such as Underwater Visual Census (UVC), Baited Remote Underwater Visual Surveys (BRUVS) and fisheries-independent surveys can be time, effort, and resource intensive and are often inefficient at detecting rare and elusive species (Boussarie et al., 2018; Budd et al., 2021; Simpfendorfer et al., 2016; Thomsen et al., 2012). Molecular survey methods have emerged that can amplify and sequence DNA particles shed by marine organisms in the form of skin cells, urine, tissue and other biological material as they interact with their environment. Environmental DNA (eDNA) decay is subject to varying biotic and abiotic influences impacting degradation rates (Barnes et al., 2014; Strickler et al., 2015). However, eDNA usually degrades in a matter of days in marine ecosystems (Collins et al., 2018; Thomsen et al., 2012) and is hence able to reflect local species composition as or more efficiently than traditional methods (Boussarie et al., 2018; Yamamoto et al., 2017). Different studies have proven its efficacy when targeting threatened species in their natural environment, like the largetooth sawfish (*Pristis pristis*) (Simpfendorfer et al., 2016), the scalloped hammerhead (*Sphyrna lewini*) (Budd et al., 2021) or the whale shark (*Rhincodon typus*) (Sigsgaard et al., 2016). The primary advantage, however, lies in the ability of this method to simultaneously detect a large amount of taxa, which has led to a variety of studies assessing community-level biodiversity throughout different marine (Bakker et al., 2017; Boussarie et al., 2018; Lafferty et al., 2021; Yamamoto et al., 2017) and freshwater (Fernández et al., 2018) environments. Although cost effective and time efficient, results depend largely on the quality of the primers used and available public reference databases with large taxon coverage. While the COI gene has been used as the standardized genetic marker for members of the entire animal kingdom (Hebert et al., 2003a; Hebert et al., 2003b), Collins et al., (2019) argue that COI, although typically the marker of choice due to its universality and the availability of a comprehensive database, compromises metabarcoding results through non-specific amplification and is outperformed by the 12S gene. Furthermore, Miya et al. (2015) designed fish- and shark and ray specific primers targeting a short, hypervariable region of the 12S gene that succeeded in detecting all shark and ray species contained in eDNA samples from a source of known species composition, covering a phylogenetically diverse array of species. These results indicate that the 12S marker is indicated for community level assessments of sharks and rays, a generally less abundant and elusive group of animals. These assessments are especially important in regions

where sharks and rays are undergoing high fishing pressure but lack proper catch data, previous taxonomic studies and/or bio-monitoring and management resources (Dulvy et al., 2017).

One such region is the Banc d'Arguin, a large shallow water bay covering one third of the Mauritanian coastline in West Africa. A permanent upwelling zone at its northern border funnels nutrients into the bay and is the primary driver of high regional productivity, attracting large fleets of commercial fishing vessels over the past decades (Chavance, 2004; Gascuel et al., 2007). It is inhabited by an indigenous population, the Imraguen, whose livelihood is closely linked to fishing and the marine resources of the region. Once described as the largest sanctuary for sharks and rays in Africa due to its species richness (Ducrocq et al., 2004), the Banc d'Arguin was declared a national park (the Parc National du Banc d'Arguin or PNBA) in 1976 and became the largest marine protected area in West Africa. However, fisheries targeting sharks and rays that had started developing outside as well as inside the PNBA's borders since the 1980's started showing signs of over-exploitation by the end of the century (Diop & Dossa, 2011). Average sizes of landed specimens reportedly started getting smaller and some species became increasingly rare (Barham et al., 2011; Walker et al. 2005). In response to these issues, the PNBA management authority and the Imraguen put forth a joint management plan as well as a system of co-management of small-scale fisheries in 2003 that resulted in a moratorium on targeted shark and ray fishing within the protected area, including all but two species, the common smoothhound (*Mustelus mustelus*) and the barbeled houndshark (*Leptocharias smithii*) (FAO, 2018). In order to further preserve the PNBA's natural resources and ecological value, a law was established in 2000 granting exclusive fishing rights to the Imraguen and outlawing non-traditional fishing equipment and motorized vessels. However, fishing pressure on sharks and rays has continued to increase over time (Failler et al., 2009; Barham et al., 2011; Trégarot et al., 2020) due to the lucrative nature of their products (fins and meat) and a lack of monitoring capacity (FAO, 2018). Anecdotal evidence suggests that some species are already regionally extinct (Diop & Dossa, 2011), such as sawfishes (Leeney & Downing, 2016) and the endemic false shark ray, *Rhynchorhina mauritaniensis* (Kyne et al., 2020). Furthermore, a large number of species reportedly use the Banc d'Arguin as a feeding, pupping and/or nursery ground (Jager, 1993; Valadou et al., 2006). Many other species are considered to face high levels of extinction risk at the global level (IUCN 2021), yet make up a large part of the regional and international trade in Mauritania (Trégarot et al., 2020). It is therefore increasingly important to obtain up to

date and accurate knowledge of local shark and ray diversity in order to inform policy on the conservation of sharks and rays in the PNBA and broader region.

This goal of this study is to characterize the species diversity of sharks and rays in the PNBA. Specifically, we aim to a) create a genetic reference database of local shark and ray species using 12S, COI and NADH2 genes to validate and/or correct taxonomy based on morphological assessments; b) investigate the presence of cryptic lineages or unexplored diversity; c) assessing the feasibility of eDNA metabarcoding from samples obtained from the PNBA to estimate the occurrence of shark and ray species using the genetic sequence reference database produced in a), and d) explore the "unseen" species diversity through eDNA metabarcoding. Results from this study provide a framework for future efforts towards better informed shark and ray population assessments and improving fisheries management policies within the PNBA.

MATERIALS AND METHODS

Sampling location and collection

Mauritania is located in western sub-Saharan Africa that borders the Central-Eastern Atlantic Ocean, south of Western Sahara and north of Senegal. Occupying more than a third of the approximately 750 km of coastline and encompassing an area of 12.000 km² split almost equally between land and sea, is the Parc National du Banc d'Arguin (PNBA). The Banc d'Arguin is a shallow water bay composed mainly of intertidal sandbanks, mudflats, intricate channels and several dispersed islands (Wolff et al., 1993), fuelled by cold, nutrient-rich water from a permanent upwelling zone in the north (M'Bareck & Mahfoudh, 1996). Nine Imraguen villages are dispersed along the PNBA's coastline with over 1000 inhabitants that operate an artisanal fisheries with a maximum of 114 traditional fishing sailboats (Boulay, 2008). Landings from industrial fisheries are generally offloaded at the main port in Nouadhibou, at the far north of the country, or in Nouakchott, the countries' capital city. On the other hand, artisanal landings occur along the coast of the country and are often transported to select processing and trading sites. The largest such sites are in Nouadhibou and Belawakh where both industrial and artisanal landings can be found aggregated for processing (salting, drying, and packing).

Shark and ray tissue samples (n = 217) were collected between October 2020 and April 2021 from processing sites in Nouadhibou (n = 159) and Belawakh (n = 35) (Fig. 1). Additional samples were also taken from freshly landed specimens at Iwik (n = 17), an individual found

dead in Arkeiss, and from live specimens (n = 5) caught during surveys for different projects in the PNBA, which were released back alive (Compain; Serrao, unpubl. data). Most animals found at the processing sites had their fins removed, were gutted, and subsequently processed in salting wells and laid out to dry in the sun. Photographic vouchers were retained for each sampled individual to aid with species identification, with the exception of samples 159-166 and 401-404 (Table S1). The former were taken from fresh landings at Iwik that were being prepared for transportation, placing a time constraint on the sampling process, and the latter were live specimens caught during surveys associated with a different project that were released immediately after sampling. Each of these samples were preliminarily assigned to a species in the field. Photographs of the remaining samples were later preliminarily identified to the highest taxonomic level possible using morphological identification keys (Ebert et al., 2013; Last et al., 2016a). For genetic analysis, tissue samples of 1-2 cm^2 size were only taken from pelvic fins to avoid sampling the same specimen twice. In cases where pelvic fins were absent, any available fin tissue from the right side of the body was collected. Depending on the presence of species, three to five samples per species were collected (range 1-40 per species). Samples were stored in 96% ethanol and kept at 4°C upon arrival at the laboratory facilities until DNA extraction.

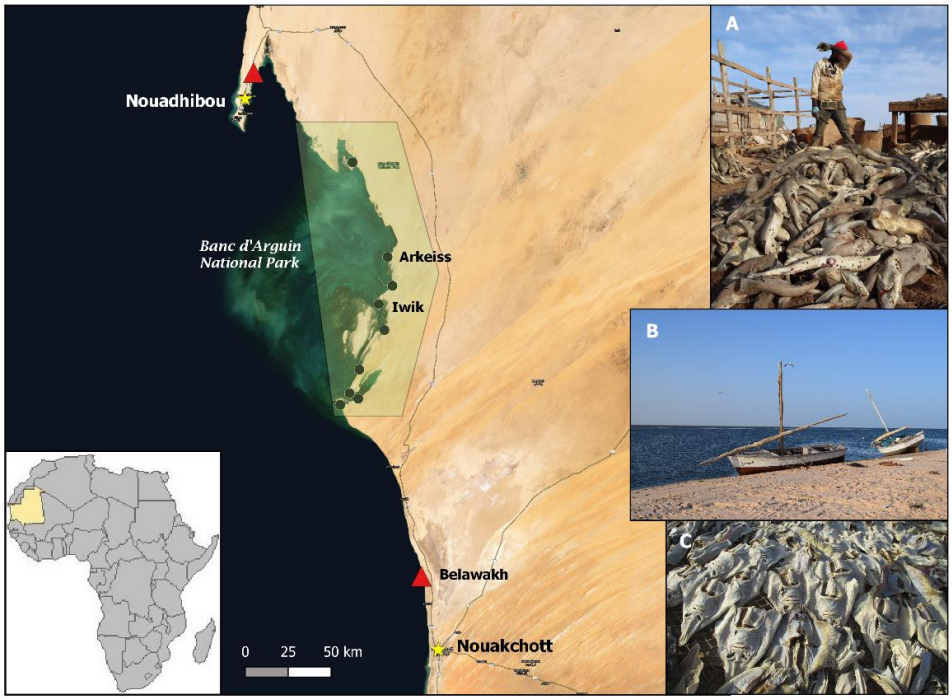


Figure 1. Map of the northern Mauritanian coastline showing the PNBA area (green polygon), major cities (yellow stars), shark and ray processing sites (red triangles) and Imraguen villages (black dots). **A** Worker at Nouadhibou processing site preparing sharks for salting **B** Traditional Imraguen sailboats at Iwik **C** Critically Endangered guitarfishes laid out to dry at Nouadhibou processing site.

DNA barcoding

DNA was extracted using a NaCl protocol with a single ethanol washing step (Sambrook & Russell, 2001). A small subset of samples was re-extracted to yield better quality DNA with a slightly amended protocol. This consisted of removing excess salt from samples of processed individuals by soaking them overnight in autoclaved Milli-Q water prior to extraction and adding two additional ethanol and a final isopropanol washing step. DNA stained with Gelred (Biotium, Inc) was visualized in 0.8% agarose gel electrophoresis and quantified in Nanodrop 1000 (ThermoFisher). To minimize interferences of potential PCR inhibitors such as salt, extracted DNA was diluted to 1-5 ng/µl. The MiFish-E universal primer pair (Miya et al., 2015) was used for PCR amplification of a small region (~200bp) of the 12s mitochondrial gene. Each PCR reaction included 2X Colorless GoTaq Flexi Buffer, 8 mM MgCl2, 320µM dNTP, 0.2 µM of each primer and 1.25 U GoTaq G2 Flexi DNA polymerase (Promega) on a 25µl volume. PCR conditions consisted of an initial 2 minute denaturation phase at 95°C followed by 30 cycles of 30 seconds of denaturation at 95°C, 30 seconds of annealing at 55°C and 1 minute of extension at 72°C with a final extension phase of 5 minutes at 72°C and were run on a GeneAmp® PCR System 9700 Thermal Cycler (Applied Biosystems). Final PCR products were visualized on 2% agarose gel under UV light and bi-directionally sequenced at CCMAR's Sequencing Platform, with an Applied Biosystems 3130xl Genetic Analyzer, BigDye®Terminatorv3.1 chemistry and POP7 polymer.

Sequence ends were trimmed and the quality of pair-wise assembled sequences was assessed and checked for consistency using Geneious Prime® 2021.1.1. Samples were identified using the Basic Local Alignment Research Tool (BLAST), which compared new sequences to sequences deposited in GenBank (National Center for Biotechnology Information - NCBI) and resolved taxonomic doubts relative to some species based on similarity percentages. Sequences with matches of <98% or where no reference sequence exists for a non-identified species were noted.

In order to improve taxonomic resolution for unresolved species, partial regions of mitochondrial COI (~650bp) and NADH2 (~1050bp) genes were targeted for a subset of samples (COI = 95, NADH2 = 23). The COI region was amplified using universal primer pair Fish-F1&R1, or Fish-F2&R2 (Ward et al., 2005) when the former did not yield an amplicon. Universal primer pair ILEM & ASNM (Naylor et al., 2012) as well as genus specific primers for *Mustelus* spp. (Naylor

et al., 2005) (Table 1) were employed to amplify the NADH2 region. Each 25µl PCR mix was comprised of 1X Colorless GoTaq Flexi Buffer, 1.5 mM (COI) or 2.5 mM (NADH2) MgCl2, 200 µM dNTP, 0.1 µM (COI) or 0.2 µM (NADH2) of each primer and 1 U (COI) or 0.25 U (NADH2) GoTaq G2 Flexi DNA polymerase (Promega). Amplification conditions for COI consisted of 2 minutes at 94°C for initial denaturation, 35 cycles of 30 seconds at 94°C, 30 seconds at 55°C and 1 minute at 72°C followed by 10 minutes of final extension time at 72°C. The PCR for NADH2 was set for 3 minutes of denaturation at 94°C and 35 cycles of 30 seconds at 94°C, 30 seconds at 48°C and 90 seconds at 72°C with a final extension of 5 minutes at 72°C. Due to the larger size of both markers and the highly fragmented nature of the DNA extracts, amplification was successful only for approximately one third of the targeted samples, with both markers showing similar amplification success rates (COI = 41% & NADH2 = 43.5%, respectively). PCR products were purified with a sodium acetate precipitation protocol and bi-directionally sequenced. Trimming and quality assessment of sequences was performed as described above and samples were identified using BLAST. Results were cross-checked with species identification based on the 12s marker (Table 2).

Table 1. List of primers used for amplification and sequencing.

Target gene	Primer	Sequence	Reference
12s	MiFish-E-F	5'- GTTGGTAAATCTCGTGCCAGC -3'	Miya et al., 2015
	MiFish-E-R	5'- CAAACTAGGATTAGATACCCTACTATG -3'	
COI	Fish F1	5'- TCAACCAACCACAAAGACATTGGCAC -3'	Ward et al., 2005
	Fish R1	5'- TAGACTTCTGGGTGGCCAAAGAATCA -3'	
	Fish F2	5'- TCGACTAATCATAAAGATATCGGCAC -3'	
	Fish R2	5'- ACTTCAGGGTGACCGAAGAATCAGAA -3'	
NADH2	ILEM	5'- AAGGAGCAGTTTGATAGAGT -3'	Naylor et al., 2012
	ASNM	5'- AACGCTTAGCTGTTAATTAA -3'	
	Ile-Mustelus	5'- AAGGACCACTTTGATAGAGT -3'	Naylor et al., 2005
	Asn-Mustelus	5'- AACGCTTAGCTGTTAATTAA -3'	

A multiple alignment was performed with the software package MUSCLE (Edgar, 2004) using the default settings. Intra- and intergeneric distances were calculated using p-distances in MEGA X (Kumar et al., 2018). A neighbor-joining tree was built for 12S sequences of all sampled species on the same software using the p-distance model (Nei & Kumar, 2000) with pairwise deletion and 1,000 bootstrap replicates for statistical support of the tree nodes. The spiny dogfish, *Squalus acanthias* (NC_002012), was used as outgroup to root the tree.

eDNA sample collection and extraction

Environmental DNA samples (n = 41) were collected from 16 locations (Fig. 9a), with two or three replicates per location, during several expeditions (n = 4) between February 2020 and April 2021 (Table S3). One set of (oceanographic) samples, taken under the scope of a different (non-elasmobranch specific) project aimed at contrasting three benthic habitats of the PNBA (outside, near the border, and at the inner coastline), were integrated into this study. These samples were collected from the sea bottom at depths between 1 – 18.3 m using 1L Niskin bottles operated from an oceanographic vessel, released several times at each site for flushing before retrieving the final three sampling replicates. Two samples were also collected from salting wells at the shark and ray processing site in Nouadhibou to verify whether it would be suitable to identify species treated and sold at the site without visual confirmation. The remaining eDNA samples (n = 30) were obtained from inshore waters nearby Imraguen villages or offshore waters at sites within the PNBA in which Imraguen fishers operate. Except for the first sample set described above, all seawater samples were collected at surface level, covering habitats with different depth and vegetation profiles. Samples were collected in replicates of three, with the exception of Belkeiznaya, Kiji and Nair samples, which had two replicates each (table S3).

Polyethylene terephthalate (PET) plastic bottles with a 750 ml volume were used to obtain water samples. All samples were filtered (750ml) using Sterivex™ Filter Units (Merck Millipore, 0.2 µm pore size for oceanographic samples and 0.45 µm pore size for eukaryote eDNA samples) immediately upon collection on board of the vessel (oceanographic samples) or within 2 to maximum 48 hours of sample collection. Samples were preserved either in Longmire buffer solution (0.1 M Tris, 0.1 M EDTA, 10 mM NaCl, 0.5% (w/v) SDS) (Longmire et al., 1997) or Silica beads to prevent DNA degradation. Filters were stored at -20°C until DNA extraction in the lab. DNA extractions were performed with the DNeasy® Blood & Tissue kit (Qiagen) following a modified environmental DNA (eDNA) extraction protocol from Spens et al. (2017). Extractions were visualized on 0.8% agarose gels and further quantified on Nanodrop 1000 (ThermoFisher). Hygiene control protocols were strictly enforced during all laboratory stages to prevent the occurrence of contamination and extraction blanks were performed to check for possible contamination during the extraction process.

Library preparation and sequencing

Sample extractions were sent to the Research Centre in Biodiversity and Genetic Resources (CIBIO) for PCR metabarcoding, library preparation and sequencing, where the elasmobranch specific MiFish-E primer set was used for the amplification of eDNA metabarcoding markers. A total of 48 samples were run on a parallel sequencing MiSeq platform (Illumina, San Diego, CA, USA) alongside samples from a different project, following a modified protocol from Miya et al. (2015) as described below.

A two-step PCR approach was used to build paired-end libraries based on the MiFish-E primer set used along with Illumina- compatible overhangs to amplify the desired target region during the first round. In order to increase the specificity of PCRs, a touchdown PCR approach was used. The 20 μL-reaction mixture contained 12.5 μL Qiagen Multiplex PCR Kit mastermix (Qiagen), 0.5 μL of each primer (10 μM), 6.5 μL of sterile distilled water and 2.5-5.0 μL eDNA template (5ng/μL). The PCR profile consisted of an initial 15 min denaturation step at 95°C followed by 11 denaturation cycles of 20s each at temperatures between 95-98°C. A 15s annealing phase started at 65°C and decreased by 0.5°C each cycle. The extension phase lasted 15s at 72°C. Another 29 cycles of 20s of denaturation at 98°C were followed by 15s of annealing at 60°C, 15s of extension at 72°C and a final extension phase of 5min at 72°C.

During the second round PCR, indices and adapter sequences were added to the barcode templates created during first round PCR. PCR amplifications were carried out in a 17 μL reaction mixture containing 7 μl of 2X Kapa HiFi Hot Start, 0.7μl of each of two indexes (P7 and P5, 10 μM), 2.8 μl of autoclaved water and 2.8 μl of cleaned PCR products. Thermocycling conditions included an initial denaturation phase of 3min at 95°C followed up by 10 cycles of a 30s denaturation phase at 95°C, a 30s annealing phase at 55°C and a 30s extension phase at 72°C and a final extension phase of 5min at 72°C. Three PCR replicates per physical sample were performed to avoid missing rare sequences and to check for consistency. PCR blanks were also obtained and sequenced as described for the samples. The quality of purified PCR products was assessed by electrophoresis using 2% agarose gel.

All sample replicates and blanks were subsequently pooled together in a single library and normalized to 15nM. The library concentration was estimated using Nanodrop 1000 Spectrophotometer v3.8.1 (Thermo Fisher Scientific Inc.) and the quality was determined using

Agilent TapeStation. In the interest of validating and quantifying the pooled libraries, a quantitative PCR (qPCR) was performed and the final pool was sequenced on a single Illumina MiSeq run using the v2 250PE kit at a concentration of 12 pM and 25% of PhiX for sequencing quality control.

To avoid contamination throughout the entire process, all lab working spaces and equipment were sterilized and single-use filtered pipette tips were used for all procedures. To further rule out contamination during later stages, PCR blanks were created and processed alongside field samples.

Bioinformatic and Statistical Analysis

Analysis of demultiplexed raw reads was performed with the Anacapa Toolkit (Curd et al., 2019), a module based pipeline which integrates a simple method for creating custom made reference libraries into its toolkit under the *Creating Reference libraries Using eXisting tools* (CRUX) module (Curd et al., 2019). A genetic sequence reference database was tailored to this study including 12S barcodes built upon the MiFish-E primer set (Miya et al., 2015). The database was created from newly generated barcodes for the species from this study as well as elasmobranch sequences deposited in NCBI, excluding unverified entries as well as *Chimaera* species. Lastly, these barcodes were concatenated with a preexisting 12S database based on the MiFish-U primer set (Miya et al., 2015) provided by the Anacapa Toolkit. This step was included in order to avoid the incorrect assignment of amplified sequences belonging to other taxa, to sharks and rays. The final database consisted of 38,127 sequences of which 1,370 sequences belonged to Chondrichthyes, amounting to a total of approximately 396 elasmobranch and chimaera species.

Two other modules performing quality control and taxonomy assignment were run on default settings with the custom made 12S reference database. The Quality Control and ASV parsing module include a series of steps to remove primers, adapters and low quality bases via *cutadapt* (Martin, 2011) and *FastX-toolkit* (Gordon & Hannon, 2010). Classified reads are denoised and dereplicated, paired reads merged and chimeric sequences removed through *DADA2* (Callahan et al., 2016). Amplicon Sequence Variant (ASV) tables are generated, which are used as input files for the Anacapa Classifier module. Here, taxonomy is assigned using *Bowtie2* and the Bayesian Lowest Common Ancestor algorithm (BCLA) (Gao et al., 2017).

Only the class Chondrichthyes was considered for analysis. In order to limit the probability of including false positive results from potential contamination, species represented by a single sequence read across all 16 samples were left out of the analysis. All taxonomic assignments above species level were excluded except for ASVs assigned to the genus *Myliobatis*, which was only identified to genus level. The taxonomic assignment was presumed unambiguous, as only a single species within that genus is described from the East Atlantic (the common eagle ray: *Myliobatis aquila*). Furthermore, Pacific or West Atlantic species were assumed to be erroneously assigned or be the product of amplification, sequencing or reference database errors and were removed from the final dataset. Four species had high read counts (> 300 reads) across two extraction blanks and were therefore excluded from the corresponding environmental samples. PCR replicates with no or only a single read were discarded and remaining PCR replicates and sample replicates were pooled together into a single unit per site. Last, samples from the salting wells were excluded from further analysis and described separately, as results are not comparable with environmental (water) samples and cannot be applied to describe regional species diversity, as the specific origin of processed animals is unknown.

Taxon diversity, community composition and read abundance were explored through α- and β-biodiversity indices using presence/absence data and abundance data. Community composition across sites was described using species richness (S), Simpson's (D) and Shannon's (H) diversity index to explore possible differences when placing more weight on richness or evenness within communities. Differences between samples were assessed for significance using a Kruskal-Wallis test for non-parametric datasets. Sample based species accumulation curves were created to assess the completeness of sampling. Non- metric multidimensional scaling (nMDS) was used to further analyze differences in community composition among samples based on Bray-Curtis dissimilarity index and Jaccard similarity index. All analysis were conducted using the vegan package on R v.4.1.1. (https://www.R-project.org/).

PNBA species list

In order to monitor the integrity of the genetic sequence reference database created for the PNBA, a custom list of shark and ray species either recorded or suspected to occur in the PNBA was compiled (Table 3) through the use of different resources, including shark and ray identification books (Ebert et al., 2013; Last et al., 2016a), records from online open-access databases such as the Global Biodiversity Information Facility (GBIF, www.gbif.org) and the

Ocean Biogeographic Information System (OBIS, www.obis.org) as well as peer reviewed literature (Diop & Dossa, 2011; Ducrocq et al., 2004; Jager, 1993; Séret & Naylor, 2016; Valadou et al., 2006) and reports from the Institut Mauritanien de Recherches Océanographiques et de Pêches (IMROP) (Barham et al., 2011).

Species that were included had a documented distribution in Mauritania with habitat preferences encompassing depths between 0 – 50 m, considering the shallow nature of the Banc d'Arguin. The presence of the smalltooth stingray, *Hypanus rudis*, whose known distribution has not been previously confirmed north of The Gambia (Moore et al., 2019), was confirmed through personal observations of catch landings in Iwik and added to the list.

RESULTS

Species identification and DNA barcoding

A total of 217 samples were collected, from which 31 species were initially identified based on morphological features using photographs. Although all samples were amplified for 12S, 14 sequences were discarded due to poor quality. After running all sequences through BLAST, some taxonomic assignments were corrected both at species and at genus level. The final 12S dataset included 203 sequences from 26 confirmed species and two putative new species (Table S1) from 24 different genera, 16 families and eight orders. Twelve shark species were present from 11 genera, nine families and four orders. Sixteen ray species (including two putative new species) were present from 13 genera, seven families and four orders. Sequence lengths ranged between 163 and 241 bp. Each species was represented by a minimum of one and a maximum of 40 samples. None of the sequences discarded refer to putative species for which only a single specimen was sampled, and thus it is not expected to have affected the species representation in the final dataset. Out of 39 and 10 samples that were amplified for COI and NADH2, respectively, 32 COI sequences (606 – 655 bp) and 5 NADH2 sequences (824 – 1339 bp) had good enough quality to be used in molecular identification. Most taxa with existing references were readily identified based on 12S alone, with some exceptions and special cases presented below. A detailed account of the DNA barcoding results for each sampled individual is available in Supplementary Table S1.

Sharks

Photographs were used to confirm genetic species identification when possible, however photographs alone were not enough to support species identification in many cases due to the physical state of the sampled animals. Nonetheless, photographs were instrumental in confirming the assignment of species to genus or at least family level.

The only *Carcharhinus* species present in the sample set, the dusky shark *Carcharhinus obscurus*, was confirmed through all three markers. Whilst 12S sequences were not sufficient to confidently distinguish between *C. obscurus* (99.1%) and *C. brachyurus* (98.7%) and COI sequences had 100% matches with both *C. obscurus and C. galapagensis,* NADH2 sequences were unequivocally assigned to *C. obscurus.*

Scalloped hammerhead (*Sphyrna lewini*) specimens were easily identified based on the shape of the cephalofoil from photographs, however 12S sequence matches with existing database entries were insufficiently accurate to confidently assign them to the species (97.4%), leaving questions open about potential divergent genetic lineages (Fig. 2). Attempts at amplifying COI and NADH2 to provide further genetic insight were not successful. Two detached heads thought to belong to the great hammerhead, *Sphyrna mokarran*, were also sampled, however failed to amplify any of the genetic barcodes and were not included in the results.

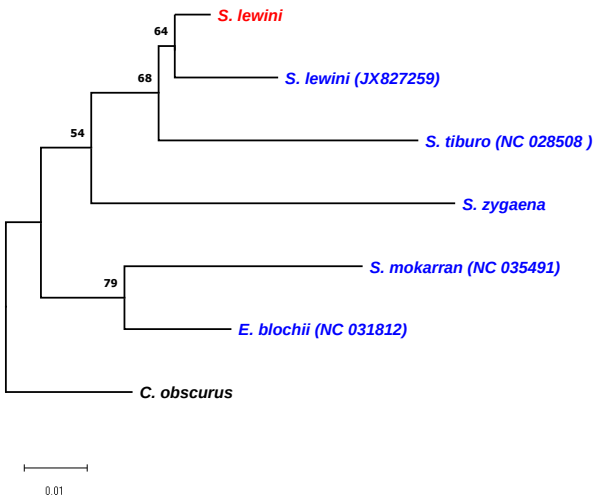


Figure 2. Neighbor-joining tree for Sphyrnidae based on 12S barcodes. P-distance model with 1000 bootstrap replicates. Bootstrap values above 50% are displayed. Species displayed in red is the *S. lewini* genotype from this study in relation to other sphyrnid species. Accession number is given for sequences retrieved from NCBI. *C. obscurus* was included as outgroup.

The only species within the family Triakidae, the blackspotted smoothhound *Mustelus punctulatus,* was first identified as the common smoothhound *Mustelus mustelus,* as the known distribution of *M. punctulatus* is believed to be limited to the Mediterranean and East Atlantic coastal regions north of Cap Blanc and the presence of *M. mustelus* in the area is well established. However, 12S sequences from *Mustelus* samples matched most closely with *M. griseus and M. manazo* (both 94.3%) instead of *M. mustelus* (92.6%) and were only able to place the specimens at genus level. One specimen also successfully amplified for the NADH2 sequence, which confirmed a 99.9% match with *M. punctulatus,* pointing towards a possibly wider range southwards of the species than previously recorded. A 12S reference barcode was previously unavailable for this species, explaining the lack of matches for its query.

Rays

Five juvenile specimens of the common guitarfish, *Rhinobatos rhinobatos,* were initially believed to be the spineback guitarfish *Rhinobatos irvinei,* a rare guitarfish species with a distinct pattern of dark-rimmed blotches on its dorsal side. Some *R. rhinobatos* specimens appear to display similar patterns, markedly around the head area, which can confound identification in dead specimens (Fig. 3a,b).

However, 12S sequence similarity was 100% among all samples, leading to the preliminary conclusion that all sampled rhinobatid specimens belonged to the same species. Furthermore, a guitarfish specimen was found with a distinctly shortened, round snout shape (Fig. 4), as opposed to the pointy shape typical of guitarfishes in the *Rhinobatos* genus (Fig. 3). The specimen could not be identified morphologically, but barcodes confirmed a 100% sequence similarity of 12S sequences with *R. rhinobatos,* however COI and NADH2 sequences did not successfully amplify for any of the rhinobatid specimens.

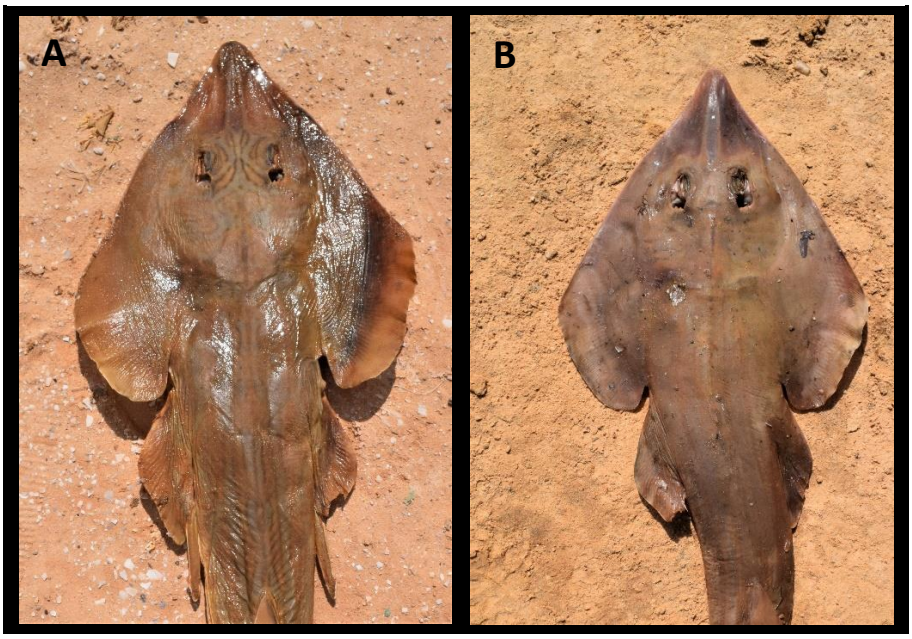


Figure 3. Color patterns of specimens identified as *R. rhinobatos* based on 12S sequences **A** Specimen with spot patterns similar to *R. irvinei* **B** *R. rhinobatos* specimen without spot pattern

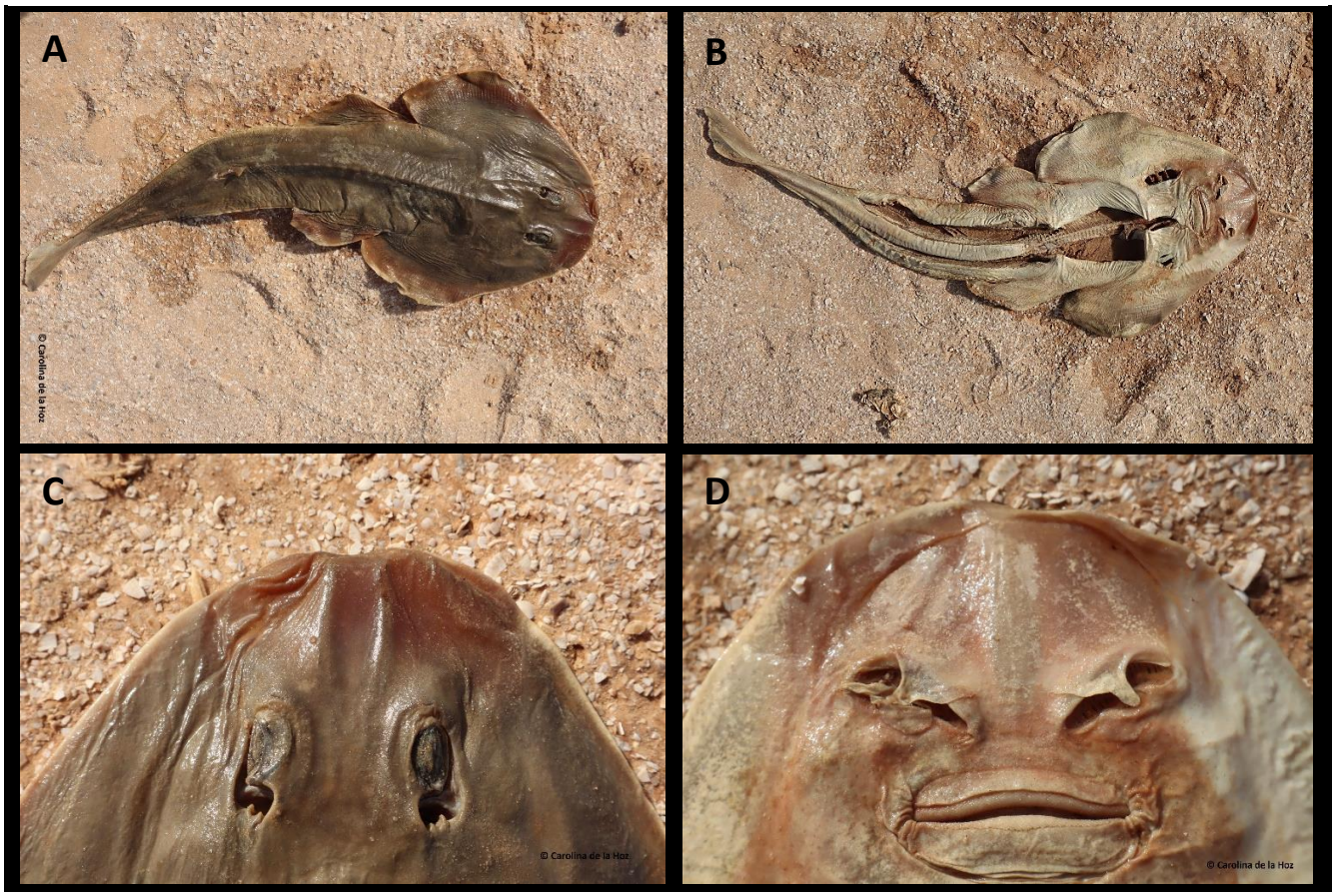


Figure 4. *R. rhinobatos* found at Belawakh processing site with rounded snout **A** Dorsal view **B** Ventral view **C** Head region dorsal view **D** Head region ventral view

The duckbill eagle ray, *Aetomylaeus bovinus*, showed clear intra-specific genetic divergences (D = 1.25 ± 0.89%) displayed on the neighbor-joining tree (Fig.8), where each one of two distinct 12S barcodes forms a separate lineage in its cluster, supported by high bootstrap values. Each of these sequences is represented by a minimum of three specimens (Fig. 8). This divergence could not be confirmed for the other markers due to negative amplification.

Within the genus *Gymnura* (D = 5.66 ± 1.88%), several specimens were initially thought to be the newly described Seret´s butterfly ray, *G. sereti*, one of only two described species from northern West Africa. However, the species did not match the species description of *G. sereti* when contrasting key morphological features (e.g. presence of spiracular tentacles in specimens observed). The species had no existing records neither for 12S and COI (NADH2 amplification was unsuccessful) and its closest match on GenBank was its congener the spiny butterfly ray, *G. altavela*, with 93.8% and 91.2% similarity for 12S or COI, respectively. The species is suspected to be new to science (Fig. 5) and is referred to in this study as "*Gymnura* sp.".

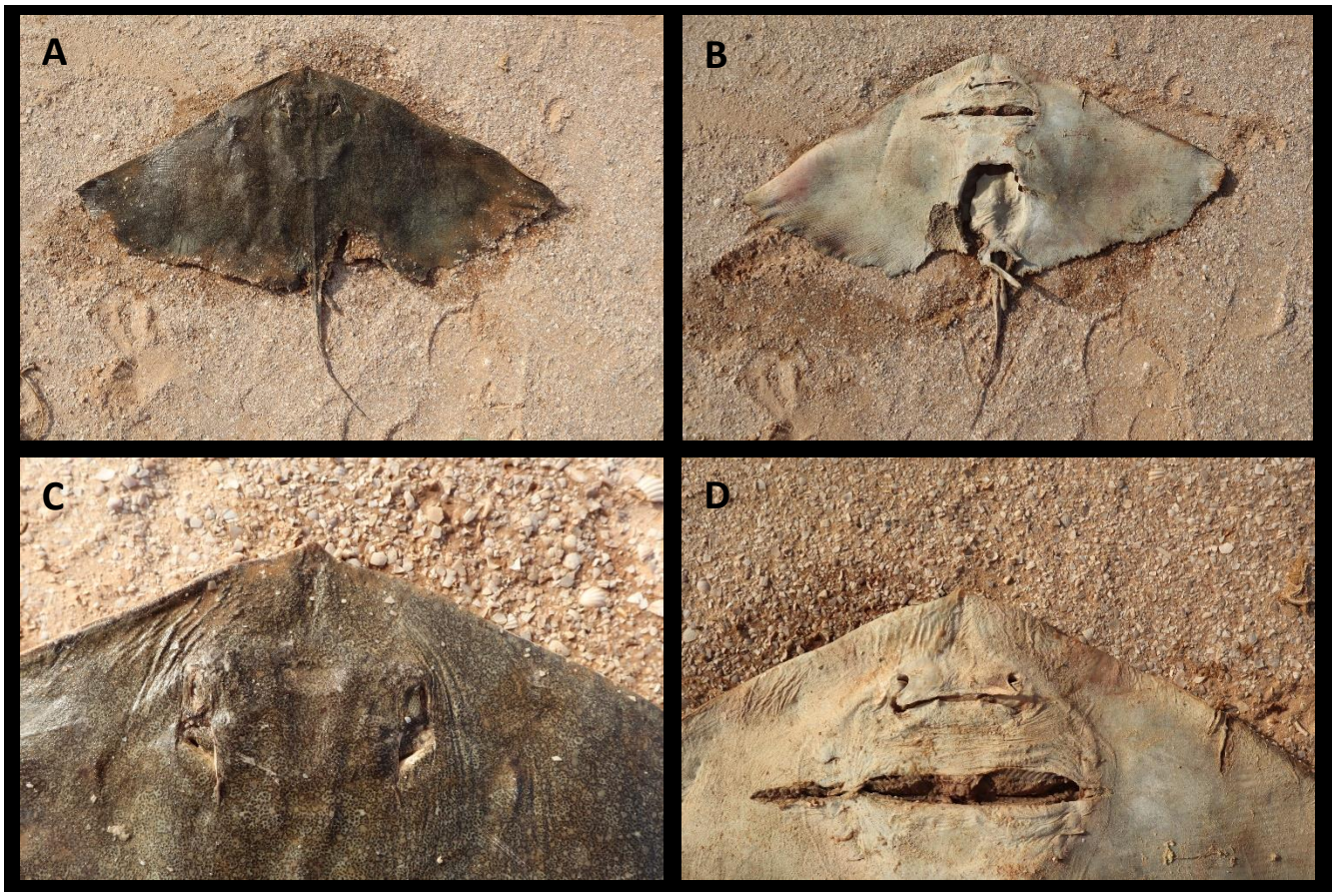


Figure 5. Unidentified *Gymnura* sp. **A** Full body dorsal view **B** Full body ventral view **C** Head region dorsal view **D** Head region ventral view

Two *Dasyatis* species were sampled (D = 3.16 ± 1.41%). The marbled skin patterns of specimens of the marbled stingray *D. marmorata* were not recognizable on dried animals, therefore leading to initial misidentifications of several individuals as the common stingray, *D. pastinaca*. However, 12S sequences were similar for all *Dasyatis* specimens, except for a single individual that matched *D. pastinaca* (99.5%) and was recorded as such. For some morphologically ambiguous specimens, COI sequences showed that they had perfect matches to *D. marmorata* (100%). Also in the family Dasyatidae, *Hypanus rudis* was confirmed to occur in Mauritania and the PNBA for the first time through visual records at processing sites and at Iwik (Fig. 6). No reference sequences exist for the species, however, its well-studied Western Atlantic sister species *H. americanus* was a close match at 98 – 99.5%. Also within the same family, one group that remains contested is the genus *Fontitrygon*, where differences between the daisy whipray, *F. margarita*, and the pearl whipray, *F. margaritella*, could not confidently be resolved neither based on morphological characteristics nor on DNA barcoding of 12S and COI markers. NADH2, which holds the only available sequence records for these species, could not be successfully amplified. The species complex *F. margarita/margaritella* was therefore treated as one for the purpose of this study.

Figure 6. Dorsal view of the smalltooth stingray, *Hypanus rudis*, sampled at Iwik

Within the genus *Raja* (D = 5 ± 1.68%), all specimens collected were dried, rigid and with coloration patterns often faded. Specimens initially believed to be the biscuit skate, *Raja straeleni*, were confirmed as the undulate skate, *R. undulata*, based on GenBank matches of 12S

(99.1%) and COI (100%) sequences. The African brown skate, *Raja parva*, has no existing sequence entries on public databases in order to confirm species identification, yet 12S sequences were instrumental in excluding *R. miraletus* (95.6%) as a match, the only other species with similar coloration patterns and regional overlap.

Lastly, one *Torpedo* spp. specimen was caught during a beam trawl survey in the PNBA and could not be placed on species level, as no record of it exists (Fig. 7). Matches to other torpedo ray species in the 12S database ranged from 91.2 – 92.9%, with the closest match being *T. marmorata.* It is presumed to be a new, undescribed species, henceforth referred to as "*Torpedo* sp.*"*.

Figure 7. Dorsal view of unidentified *Torpedo* sp.

Conservation status

Overall, 19 out of 28 species (20 if including *F. margarita*) representing 68% (or 71%) of the recorded species in this study, are placed in one of the threatened categories determined by the IUCN Red List (Table 2), with six species assessed as Critically Endangered, five as Endangered and eight (or nine) as Vulnerable. Five species (six if including *F. margaritella*) are considered Near Threatened (18% or 21%) and only one is considered Least Concern (3.5%). As of September 2021, none of the species evaluated herein are considered Data Deficient but (at least) two of the species in the sample set are potentially new to science and their status is unknown.

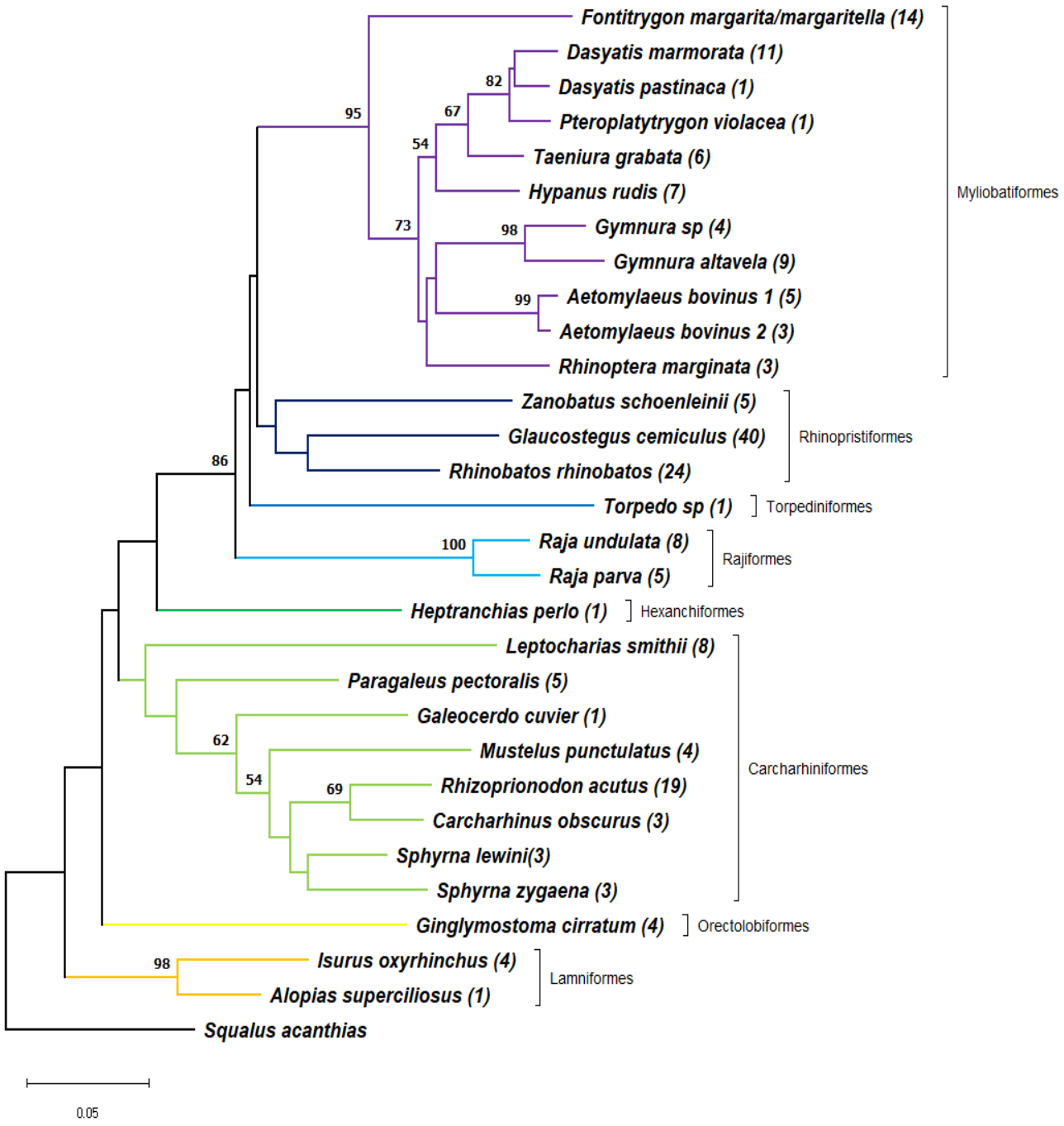


Figure 8. Neighbor-joining tree for shark and ray species sampled in Mauritania based on 12S barcodes. P-distance model with 1000 bootstrap replicates. Bootstrap values above 50% are displayed. Species are colored by order to display lineage diversity within the region. *Squalus acanthias* (NC_002012) is used as outgroup to root the tree. Numbers behind taxa indicate the number of samples included in the construction of the tree.

Table 2. Shark species sampled in Mauritania including their common name and global conservation status based on the IUCN Red List. DD – Data Deficient, LC – Least Concern, NT - Near Threatened, VU – Vulnerable, EN – Endangered, CR – Critically Endangered, NE – Not Evaluated. Mitochondrial markers with existing barcodes (YES/NO) or new barcodes from present study (PS) for all species.

Order	Family	Species	Common name	IUCN	Mitochondrial markers		
					12S	COI	NADH2
Carcharhiniformes	Carcharhinidae	*Carcharhinus obscurus*	Dusky shark	EN	YES	YES	YES
		Rhizoprionodon acutus	Milk shark	VU	YES	YES	YES
		Galeocerdo cuvier	Tiger shark	NT	YES	YES	YES
	Triakidae	*Mustelus punctulatus*	Blackspotted smoothhound	VU	PS	YES	YES
	Hemigaleidae	*Paragaleus pectoralis*	Atlantic weasel shark	EN	PS	PS	YES
	Leptochariidae	*Leptocharias smithii*	Barbeled houndshark	VU	PS	NO	YES
	Sphyrnidae	*Sphyrna lewini*	Scalloped hammerhead	CR	YES	YES	YES
		Sphyrna zygaena	Smooth hammerhead	VU	YES	YES	YES
Lamniformes	Alopiidae	*Alopias superciliosus*	Bigeye thresher	VU	YES	YES	YES
	Lamnidae	*Isurus oxyrinchus*	Shortfin mako	EN	YES	YES	YES
Orectolobiformes	Ginglymostomidae	*Ginglymostoma cirratum*	Atlantic nurse shark	VU	YES	YES	YES
Hexanchiformes	Hexanchidae	*Heptranchias perlo*	Sharpnose sevengill shark	NT	YES	YES	YES
Rhinopristiformes	Rhinobatidae	*Rhinobatos rhinobatos*	Common guitarfish	CR	PS	YES	YES
		Zanobatus schoenleinii	Striped panray	VU	PS	YES	YES
	Glaucostegidae	*Glaucostegus cemiculus*	Blackchin guitarfish	CR	YES	YES	YES
Myliobatiformes	Myliobatidae	*Aetomylaeus bovinus*	Duckbill eagle ray	CR	PS	YES	YES
		Rhinoptera marginata	Lusitanian cownose ray	CR	PS	YES	NO
	Gymnuridae	*Gymnura altavela*	Spiny butterfly ray	EN	YES	YES	YES
		Gymnura sp.*	-	-	PS	PS	-
	Dasyatidae	*Dasyatis pastinaca*	Common stingray	VU	YES	YES	YES

		Dasyatis marmorata	Marbled stingray	NT	PS	YES	YES
		Hypanus rudis	Smalltooth stingray	CR	PS	PS	YES
		Taeniura grabata	Round stingray	NT	PS	YES	YES
		Pteroplatytrygon violacea	Pelagic stingray	LC	YES	YES	YES
		*Fontitrygon margarita / margaritella***	Daisy / Pearl whipray	VU/ NT	PS	PS	YES
Rajiformes	Rajidae	*Raja parva*	African brown skate	NT	PS	NO	NO
		Raja undulata	Undulate skate	EN	YES	YES	YES
Torpediniformes	Torpedinidae	*Torpedo* sp.*	-	-	PS	-	-

* Potential new species

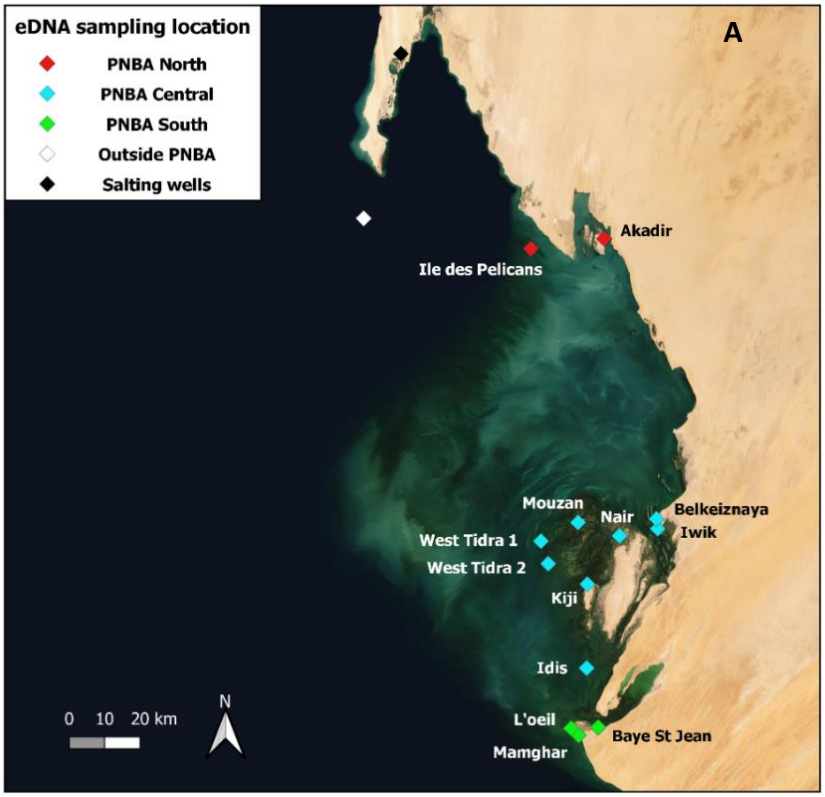
** Closely related species could not be confidently differentiated based on morphological characteristics nor barcoding

Elasmobranch species diversity in eDNA

A total of 5,941,500 raw reads were extracted from 16 samples as well as two PCR blanks and five extraction blanks. After completing quality control protocols and assigning taxonomy to filtered sequence reads, 2,233019 reads were retained and appointed to one of 429,183 distinct ASVs. Sharks and rays accounted for 11.5% of total filtered reads (n = 256,675) and bony fish made up the largest proportion of reads with 21.7% (n = 484,199) assigned to teleost taxa, indicating a high overlap in specificity of the MiFish-E primer binding regions of both groups. Mean read depth per field sample was 17,235 and taxa from six eukaryotic taxonomic groups, including Actinopterygii, Amphibian, Aves, Chondrichthyes, Mammalia and Petromyzonti were identified. However, only sharks and rays were considered for this study. After removing singletons and ASVs presumed to be erroneously assigned to geographically improbable species, a total of 35 species were identified, of which 17 were sharks and 18 were rays. Total number of species varied between sample locations, with the highest amount of species (n = 26) found in the two samples from the salting wells. All 13 samples from inside the PNBA recovered 29 species combined, including an unidentified *Myliobatis* species. A single sample taken outside the PNBA boundaries detected 12 species, however showed no additional species diversity compared to PNBA samples and was not used for further analysis in this study. Five species were detected exclusively at the salting wells while nine species were present only in environmental samples

(Table 3). Shark and ray taxa were recovered in 100% of the samples, with samples collectively displaying an average species richness (S) of 16. The most diverse sites were West Tidra 1 (S = 20) and West Tidra 2 (S = 19), with similar species composition dominated by *R. rhinobatos* and the Lusitanian cownose ray, *R. marginata*, as well as L'oeil (S = 19), with *R. acutus* dominating in terms of relative abundance. The least diverse site (Nair) still contained 11 species (Fig. 9B).

Three species were present across all samples, namely *G. altavela*, *R. acutus* and the striped panray, *Zanobatus schoenleinii*, however relative read abundance and species composition varied across all samples with no distinct patterns emerging across northern, central and southern regions (Fig. 10a,b). Site diversity was generally high among sample locations with the exception of Belkeiznaya (Fig. 9), which was dominated by the blackchin guitarfish, *Glaucostegus cemiculus,* in terms of relative read abundance. Differences in community structure were not apparent between sampling locations (North/Centre/South) using presence/absence data (r^2 = 0.23, p = 0.24) or abundance data (r^2 = 0.22, p = 0.29) and species overlap between northern, central and southern locations was high (Fig 10).



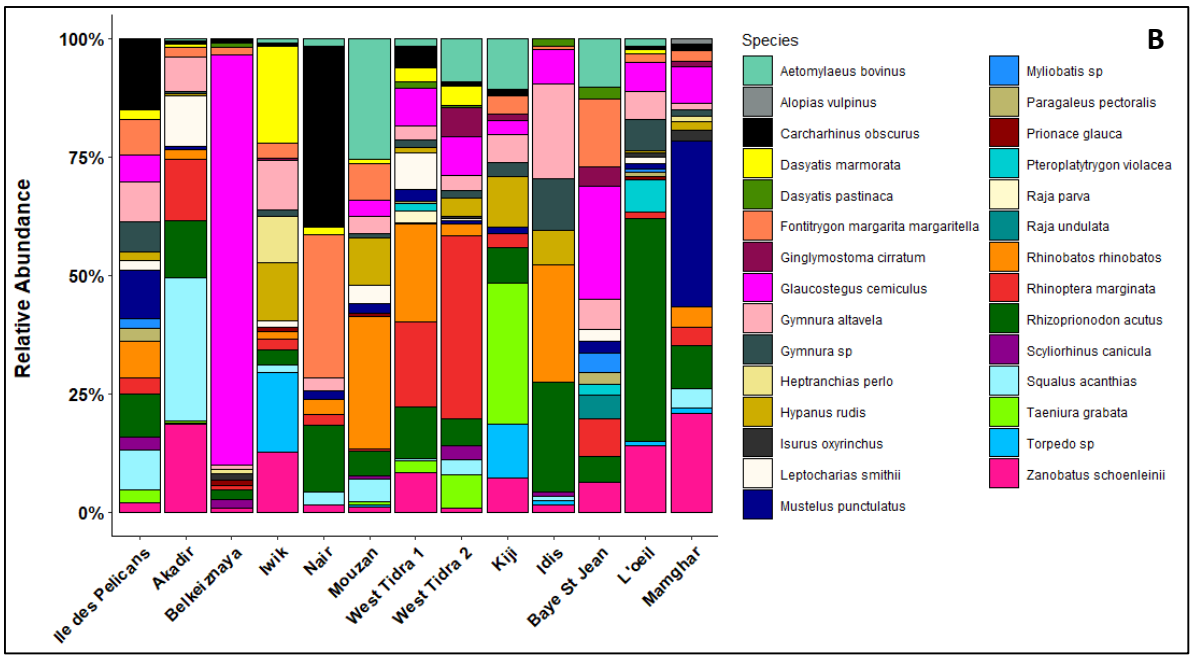


Figure 9. A Map displaying sample collection points inside and outside the PNBA and at salting wells **B** Bar plot showing relative read abundance (square root transformed) for every shark and ray species across samples inside the PNBA. Sample locations are ordered from North to South and species are ordered alphabetically.

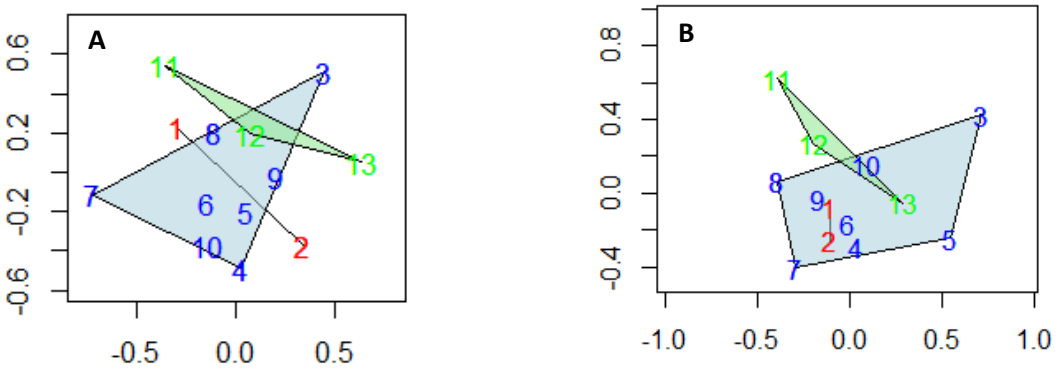


Figure 10. nMDS plots based on **A)** Bray Curtis dissimilarity index (stress = 0.19) and **B)** Jaccard similarity index (stress = 0.13). Sample sites are represented by numbers and color indicates location within the PNBA (red = North, blue = Centre, green = South).

Previous catch records from the PNBA have documented the presence of 31 species of sharks and rays, although many may presently be very rare or regionally extinct. However, up to 58 species could potentially inhabit the area either permanently or intermittently based on the habitat preferences and depth distribution of regionally recorded species (Table 3). Out of 31 previously recorded species (counting *Raja* sp. as one taxonomic unit as recorded in reports), 15 species were detected in eDNA and six non-detected species are missing a reference sequence. Fourteen

species are new (official) records (Table 3), such as the smallspotted catshark, *Scyliorhinus canicula* (47 reads), *Hypanus rudis* (1,044 reads) and the pelagic stingray, *Pteroplatytrygon violacea* (158 reads). The former has been sighted under a different project (Jabado, unpubl. data) and the latter two had been collected as a physical sample at the Nouadhibou processing site and were therefore retained as true positives. Large pelagic sharks such as the common thresher, *Alopias vulpinus* (2 reads), the shortfin mako, *Isurus oxyrinchus* (8 reads), and the blue shark, *Prionace glauca* (7 reads), as well as species associated with deeper depth ranges such as the sharpnose sevengill shark, *Heptranchias perlo* (248 reads), and the spiny dogfish, *Squalus acanthias* (5,547 reads), had not been previously recorded in the literature but were retained as true positives as they are a widespread species in the Atlantic. One of the most common species in the region, *M. mustelus*, was not detected, instead, the less common *M. punctulatus* was recorded in 77% of the samples with 877 reads. *Carcharhinus obscurus* was the only representative of its genus, while other previously commonly reported congeners were notoriously absent (e.g. *C. brevipinna*). *Sphyrna lewini* and the smooth hammerhead, *S. zygaena*, were recorded solely in salting well samples although their presence in the PNBA is well recorded and visual observations during a visit in May 2021 provided additional confirmation.

Species accumulation curves are plotted to show shark and ray diversity as a function of the number of eDNA samples taken inside the PNBA (Fig. 11). The curve flattens after approximately eight samples, suggesting that a higher sampling effort would likely not significantly increase the observed diversity as the species accumulation curve has reached saturation.

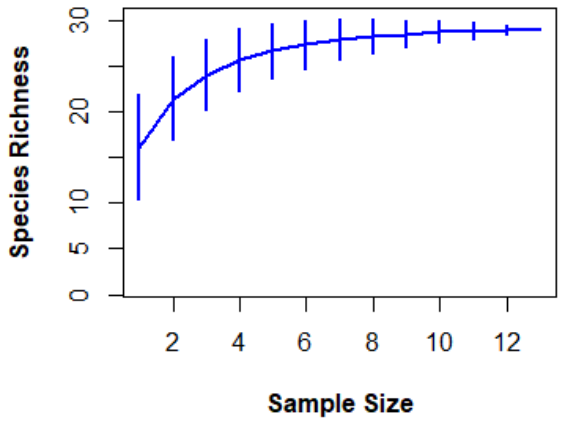


Figure 11. Sample based shark and ray species accumulation curve for samples inside the PNBA displayed with standard error bars after 100 permutations.

Conservation status

From a total of 29 species positively identified from samples taken inside the PNBA boundaries, six species are Critically Endangered (20.7%) if assuming the presence of *M. aquila*. Five species are Endangered (17.2%) and eight are Vulnerable (27.6%) according to the IUCN Red List (IUCN 2021). Species listed as threatened with extinction in the PNBA therefore amounts to 65.5% of all species recorded in eDNA samples across the area and includes the seven most abundant species in terms of read abundance (*A. bovinus, G. altavela, G. cemiculus, R. acutus, R. marginata, R. rhinobatos* and *S. acanthias*) (Table S4). Five out of six Critically Endangered species, *A. bovinus, G. cemiculus, H. rudis, R, marginata* and *R. rhinobatos*, account for ~56% of the reads from PNBA samples (5, 17, 2, 18 and 14%, respectively). The relative contribution to read abundance of species in all three threat categories combined amounts to 93% (excluding *F. margarita*). Out of the remaining species, five species are Near Threatened (17%), two species are Least Concern (7%) and two putative new species from this study have not been evaluated.

Salting wells

The salting wells at the shark and ray processing site in Nouadhibou are wells in which processed animals are placed next to and on top of each other. Each animal layer is covered in salt in order to dry the meat (Fig. 12). Logically, fluid samples collected from two empty salting wells were expected to contain high amounts of elasmobranch DNA, which would be comparable with in-situ observational data on species occurrences. Environmental DNA could potentially function as a tool to record rare species that might otherwise go unnoticed where thousands of individuals are processed on a weekly basis. A total of 26 species were detected in only two samples, including six species not detected in any of the environmental samples, namely the tiger shark, *Galeocerdo cuvier, S. lewini, S. zygaena, S. mokarran,* the marbled torpedo ray *Torpedo marmorata* and the false catshark, *Pseudotriakis microdon.* The latter, although not reported in the literature review from the region, was retained as a potential true positive identification as its known distribution falls into the studied geographical range. *Galeocerdo cuvier* and *S. mokarran*, both species easy to identify morphologically, were not detected on site, same as *P. microdon* and *T. marmorata. Rhizoprionodon acutus* (56,112 reads) and *G. altavela* (47,146 reads) contributed 47% and 39.5% respectively to the total read abundance (119,313 reads), which roughly aligns with visual

observations of species abundance on site (beginning of November, 2020). When adding reads from *Gymnura* sp. (7,191), *M. punctulatus* (3,085), *G. cemiculus* (1,027) and *R. rhinobatos* (968), the six species combined account for approximately 97% of the read abundance. Notably, all these species, with the exception of the undescribed *Gymnura* sp., are placed in one of three extinction threat categories of the IUCN Red List.

Figure 12. Worker at shark and ray processing site in Nouadhibou laying out sharks in a salting well.

Table 3. Shark and ray species diversity (possible and/or expected) in the PNBA reported from literature records or based on geographical range as well as depth and habitat preferences of species, and sampled diversity based on eDNA results from inside the PNBA, outside the PNBA and at salting wells. Red dots indicate species that were visually confirmed at the processing site or PNBA during the sampling period but were not detected in eDNA samples.

Species	Possible / Expected	PNBA	Outside PNBA	Salting wells	IUCN
Sharks					
Rhizoprionodon acutus	●	●	●	●	VU
Paragaleus pectoralis	●	●	●	●	EN
Leptocharias smithii	●	●	●	●	VU
Pseudotriakis microdon				●	LC
Mustelus mustelus	●				EN
Mustelus punctulatus		●	●	●	VU
Sphyrna lewini	●	●		●	CR
Sphyrna zygaena	●			●	VU
Sphyrna mokarran	●			●	CR
Carcharhinus brevipinna	●				VU

Carcharhinus limbatus	•				VU
Carcharhinus plumbeus	•				EN
Carcharhinus obscurus	•	•	•		EN
Carcharhinus signatus[2]	•				EN
Carcharhinus falciformis	•				VU
Carcharhinus brachyurus	•				VU
Carcharhinus leucas	•				VU
Galeocerdo cuvier	•			•	NT
Prionace glauca		•		•	NT
Negaprion brevirostris[1]	•				VU
Scyliorhinus canicula	•	•			LC
Scyliorhinus stellaris[1]	•				VU
Ginglymostoma cirratum	•	•	•	•	VU
Carcharias taurus[1]	•				CR
Alopias vulpinus		•			VU
Isurus oxyrinchus		•			EN
Squalus acanthias		•	•	•	VU
Heptranchias perlo		•			NT
Squatina aculeata[2]	•				CR
Squatina oculata[2]	•				CR
Squatina squatina[2]	•				CR
Rays					
Pristis pectinata[3]	•				CR
Pristis pristis[3]	•				CR
Glaucostegus cemiculus	•	•		•	CR
Rhinobatos rhinobatos	•	•	•	•	CR
Rhinobatos albomaculatus[2]	•				CR
Rhinobatos irvinei[2]	•				CR
Rhynchobatus luebberti[3]	•				CR
Rhynchorhina mauritaniensis[2]	•				CR
Zanobatus schoenleinii	•	•	•	•	VU
Rhinoptera marginata	•	•	•	•	CR
Aetomylaeus bovinus	•	•		•	CR
*Myliobatis aquila**	•	•			CR
Mobula birostris	•				EN

Mobula hypostoma	●				EN
Mobula tarapacana	●				EN
Mobula thurstoni	●				EN
Gymnura altavela	●	●	●	●	EN
*Gymnura sereti***	●				EN
Gymnura sp.		●		●	-
Dasyatis marmorata	●	●		●	NT
Dasyatis pastinaca	●	●	●	●	VU
Taeniura grabata	●	●		●	NT
Hypanus rudis[4]		●	●	●	CR
Bathytoshia lata[5]	●				VU
Pteroplatytrygon violacea	●	●			LC
*Fontitrygon margarita****	●	●	●	●	VU
*Fontitrygon margaritella****	●	●	●	●	NT
Leucoraja naevus	●				LC
Raja straeleni	●				NT
Raja brachyura[1]	●				NT
Raja clavata[1]	●				NT
Raja microocellata[1]	●				NT
Raja parva	●	●		●	NT
Raja undulata	●	●	●	●	EN
Torpedo torpedo	●				VU
Torpedo marmorata	●			●	VU
Torpedo sp.		●			-

* eDNA results resolved only to genus level "Myliobatis"
** Previously recorded as *Gymnura micrura*, distributional range now limited to Southwestern Atlantic. *G. sereti* found in Eastern Central Atlantic
*** Taxa not resolved to species level. At least one of two species is present in the PNBA
[1] Presence is possible, but has not been confirmed
[2] Presence is possible, but species considered to be very rare at present
[3] Species considered locally extinct
[4] Known distributional range limited to Eastern Central Atlantic, but presence confirmed on site.
[5] Previously recorded as *Bathytoshia centroura*

DISCUSSION

To the best of our knowledge, this study represents both the first exhaustive regional barcoding effort as well as the first eDNA shark and ray survey in West Africa. It is also the first to

characterize the species diversity of sharks and rays in the PNBA using molecular survey tools. Limited current in-depth information was previously available on shark and ray diversity in the PNBA other than few catch and landing reports (e.g. Ducrocq et al., 2004; Barham et al., 2011) and thus far, biodiversity monitoring strategies in the protected area have relied solely on landings records. Based on in-situ observational data, species may not always be identified correctly and a taxonomic overhaul of shark and ray species has given an insight into potentially undiscovered species diversity and a slightly modified species composition than previously recorded in the PNBA.

Taxonomic identification based on DNA barcoding

This study produced 12S barcodes for at least 26 recognized species, with the taxonomic and genetic placement of one *Gymnura* and one *Torpedo* species yet undetermined, for which further morphological and genetic research is needed.

Most samples were collected from specimens that had undergone processing involving the removal of fins, lengthwise slicing of the body, salting and subsequent drying of the flesh, which often made in-situ identification difficult. Genetic barcoding proved crucial for the identification of several specimens and species that would have otherwise been challenging to identify given their physical state after processing. Most species encountered were expected based on fisheries catch and landings data (Barham et al., 2011; Jager, 1993; Valadou et al., 2006; Trégarot et al., 2020) or could be inferred from current knowledge on geographical distribution (Ebert et al., 2013; Last et al., 2016a). Some exceptions were *M. punctulatus* and *H. rudis*, and for which further research is needed to determine its actual distribution in the Eastern Atlantic and whose previously known distribution was limited from Sierra Leone (Last et al., 2016a) to the Gambia (Moore et al., 2019), respectively.

The species identity of *M. punctulatus* could be confirmed through the additional amplification of the NADH2 molecular marker for a single specimen, however, taking into account that 12S reference sequences for *M. mustelus* are available and not a match (92.6%), all specimens were inferred to belong to the same species. *Mustelus punctulatus* is known to occur northwards of Western Sahara (Ebert et al., 2013) and has not been included in local available landings reports (Barham et al., 2011; Trégarot et al., 2020; Ducrocq et al., 2004). Morphological differences between the more common *M. mustelus* and *M. punctulatus* are subtle and are commonly

determined by the presence of black spots and/or dark margins on the dorsal fins of *M. punctulatus* (Ebert et al., 2013). However, Marino et al., (2018) found these traits to be unreliable for species identification due to their frequent absence. Instead, the shape of dermal denticles and inter-nostril distance in relation to nostril width were deemed as the most reliable morphological traits to differentiate both species (Marino et al., 2018). While *M. mustelus* is a commonly reported species in landings records off the Mauritanian coast, misidentification and/or mislabeling of *Mustelus* spp. seems to occur to a currently unknown extent. Guardone et al., (2017) used COI and 16S to barcode different species from non-European countries and found that species labeled as *M. mustelus* originating from Mauritania were, in fact, *M. punctulatus*. These findings suggest that the distribution of *M. punctulatus* may range more southwards in the Central-East Atlantic than previously postulated and that the species is frequently misidentified.

Aetomylaeus bovinus 1 and 2 were treated as separate entities to construct the neighbor-joining tree as two distinct sequences emerged across several samples. Although Hebert et al. (2003) set a 2% threshold for species delineation, this is often challenged as certain well described congeneric species show lower genetic divergences (Naylor et al., 2012; Ward et al., 2008). Additionally, sharks and rays have been found to display lower rates of molecular evolution when compared to mammals (Martin et al., 1992) or other fish (Inoue et al., 2010), which can differ across lineages. Cawthorn et al., (2012), for example, found a mean interspecific distance of 1% for a 543bp fragment of the 12S gene, indicating that low genetic distances do not necessarily prove lack of speciation, especially if considering only a single genetic marker. The genus *Aetomylaeus*, however, is notoriously absent from the 12S sequence database in NCBI, providing no references in the genus for sequences from this study. From a morphological viewpoint, sampled specimens, which were mostly sun-dried and sliced up, did upon superficial inspection not show any obvious differences. However, morphological similarity may also not be an unambiguous sign of lack of speciation (e.g. Quattro et al., 2013). Further exploration involving other genetic markers and the collection of biometric data may grant clarification on whether it is a case of cryptic speciation or high intraspecific divergence, which could potentially have implications for the management of this Critically Endangered species.

Similarly, in the case of the *Rhinobatos* specimen with the rounded snout, further investigation would be advised to determine the cause of the morphological abnormality. Unfortunately, it could not be retained as voucher specimen. However, morphological abnormalities in free-

swimming sharks and rays appear to often go unreported, likely due to a lower survival rate , however, viable deformations of the snout, fins (Moore, 2015) and skeleton (Heupel et al., 1999) have been recorded and sometimes linked to environmental pollution (Moore, 2015). In this case, the specimen is genetically identical to *R. rhinobatos,* based on a short 12S fragment. However, the choice of genetic marker in molecular barcoding techniques can influence the taxonomic resolution of species. Collins et al., (2019) recommended the use of 12S in pointing out the flaws of COI in terms of specificity and reproducibility. These flaws are usually outweighed by the availability of an extensive public reference database for COI. The 12S primer set designed by Miya et al. (2015) offers an elasmobranch-specific alternative to COI with high amplification rates and adequate interspecific variability, observed also in this study, but it also occasionally appears to lack enough potential to distinguish between certain species. This can be verified when comparing 12S sequence similarity and overlap of various closely related congeneric species in public genetic databases (e.g. genus *Mobula, Pristis, Rhinoptera,* etc.). While this could potentially be related to misidentifications of deposited specimens, high identity similarity (> 98%) with sister species was also found in several species in this study, such as *D. marmorata (D. tortonesei), C. obscurus (C. brachyurus)* and *R. marginata (R. brasiliensis)*. Furthermore, certain genera within the order Rhinopristiformes, such as *Rhynchobatus,* have 100% sequence similarity for 12S between some closely related species (*R. australiae, R. djiddensis* and *R. laevis*), however differ on COI and NADH2 level. Therefore, it should not be ruled out that the specimen may be a recently diverged sister species of *R. rhinobatos*. The same principle applies to specimens believed to be *R. irvinei.* It has been hypothesized that juvenile *R. rhinobatos* may (sometimes) display similar blotch patterns as adult *R. irvinei* specimens that fade throughout different ontogenetic stages, however, the specimen in Fig. 3a is an adult male (large claspers surpassing length of pelvic fins). It is also possible that coloration patterns vary in intensity throughout the species, nonetheless, further genetic verification is advised in both cases to clear up any existing taxonomic ambiguities, as guitarfishes in general are one of the most threatened group of cartilaginous fish and many species are believed to be regionally very rare (e.g. *R. irvinei*) or extinct.

As for *Gymnura* sp. and *Torpedo* sp., both species were distinctly different on a genetic level from their closest relatives in the NCBI database. Although genetic distinction may stem from a deficiency of available 12S reference sequences, other species identities were excluded based on morphological attributes. Out of ten officially recognized *Torpedo* species, 12S sequences are

currently only available for four species, possibly explaining the lack of close matches. Also four out of ten species fall into the approximate studied geographical range (Last et al., 2016a), from which only *T. marmorata* had available reference sequences. Average pairwise identities for 12S of existing reference species (n = 4) is 93.9%, which is only slightly higher than the pairwise distance between the studied specimen and *T. marmorata* (92.9%), supporting its provisional status as new species. Additionally, their coloration patterns and body proportions were not congruous with the referenced specimen herein. However, different morphotypes could exist and the group requires further taxonomic work. Similarly, *Gymnura* sp. is proposed as a potentially new species based on three factors: a) sequence matches for 12S placed the species within the genus *Gymnura* with no available close species matches (*G. altavela*: 93.8%). COI sequence matches were even lower, with *G. altavela* still being the best match (91.2%). The genus currently contains 12 valid species (Last et al., 2016a) including two newly described species (*G. lessae* & *G. sereti* by Yokota & De Carvalho, 2017) previously contained in the *G. micrura* species complex. Two species fall into the studied geographical region, namely *G. altavela* and *G. sereti* (previously reported as *G. micrura*) and are regularly reported from fisheries in the PNBA (Barham et al., 2011). However, b) available COI sequences for *G. micrura* and *G. lessae* had pairwise identities with *Gymnura sp* of 85.4% and 84.9%, respectively, placing it genetically closer to *G. altavela.* Furthermore, c) *Gymnura sp* can be distinguished from *G. sereti* based on a series of conspicuous physical characters, e.g. the presence of spiracular tentacles and a distinctly long tail (± 45% of disc width). *G. sereti* is a medium sized ray with disc width averaging 38 cm (range between 18 – 75 cm) (Yokota & Rodrigues de Carvalho, 2016), whereas specimens of *Gymnura sp* were visually estimated to attain larger disc widths. However, no voucher specimens could be retained for both *Torpedo* sp. and *Gymnura* sp., and 12S databases lack sufficient species representation within their respective genera. Further data collection on both species is essential to confirm current findings.

The same applies to *S. lewini*, where 12S markers provided inconclusive sequence matches. The cosmopolitan and highly migratory species is known to show genetic divergences between oceans and across ocean basins (Duncan et al., 2006; Quattro et al., 2006) and the occurrence of a cryptic lineage (*S. gilberti*) was recently found in the western North Atlantic (Quattro et al., 2006, 2013). Large bodied and widely distributed species like the scalloped hammerhead are usually associated with high gene flow and low population genetic structure. However, in the case of *S. lewini*, studies have consistently observed clear population subdivisions linked to restricted

genetic connectivity, which has been associated with their reproductive behavior and coastal dependency for parturition (Duncan et al., 2006; Pinhal et al., 2020). None of these studies included the full range of *S. lewini* populations, and West African samples originated either from Ivory Coast (Quattro et al., 2006) or undefined West African countries south of Senegal (Duncan et al., 2006). Tagging data from a study based on mark and recapture in eastern USA indicated that median traveling distances of scalloped hammerheads were less than 100 km, with few exceptions (Kohler & Turner, 2001), meaning that local Mauritanian populations that supposedly use the PNBA as nursery grounds (Ducrocq et al., 2004) may be genetically distinct from other regional populations. Although Duncan et al. (2006) observed population structures congruent with genetic connectivity between close by nursery populations that are connected through continuous coastline, further insight into local *S. lewini* population genetic structure needs to be gained to determine whether it could represent a discrete population or lineage. Given the sharp population declines of scalloped hammerheads globally (reviewed in Gallagher & Klimley, 2018) and high catch numbers of juveniles in Imraguen fisheries (Ducrocq et al., 2004; Barham et al., 2011), sorting out the genetic architecture of local populations is essential in order to inform on adequate management strategies. Additionally, the scalloped hammerhead is listed on Appendix II in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES), yet it is traded without permits, at least in the PNBA, creating serious conservation concerns for the species on a regional level.

All species discussed are to some degree threatened or potentially threatened with extinction. In their recent revision of the global status of sharks and rays, Dulvy et al. (2021) found that species were disproportionally threatened in tropical and subtropical regions, including Mauritania, a finding that is corroborated in this study where more than two thirds of barcoded species are in one of three threat categories. Taxonomic certainty regarding species discussed herein would provide a more accurate baseline on local diversity to consider in local shark and ray directed conservation measures.

PNBA species diversity in eDNA

The previous creation of 12S barcodes from sampled species was instrumental to the success of the metabarcoding effort. This study established their usefulness in assigning taxonomy at high confidence levels to sharks and rays detected in eDNA samples taken under sub-optimal conditions. This is, transport and preservation of samples could not comply with recommended

sampling standards due to contextual restrictions, such as the lack of sterile conditions on the wooden sailboats and the lack of electricity and refrigerated storage possibilities. Yet, every species barcoded in this study was retrieved in at least one sample (with the exception of *Alopias superciliosus*), hence proving the viability and efficacy of 12S as a metabarcoding marker for sharks and rays. Some extraction blanks contained high read counts for several species while PCR blanks showed no amplification, which points towards contamination occurring during the DNA extraction process. However, several factors are to be considered: a) no samples from other studies were treated and/or extracted at the same time, b) no other on-going projects involving sharks and rays were taking place during the extraction period, c) gel electrophoresis showed no signs of DNA amplification and d) the samples most heavily contaminated were not extracted at the same time as the samples from the salting wells or the oceanographic sample taken outside the PNBA. Therefore, taking into account that the taxa detected in the extraction blanks were taxa common to most samples and have previously been recorded in the PNBA (with the exception of *S. acanthias*), it does not affect diversity estimates from eDNA results. The same is true for potential field contamination on the boats, as expeditions were undertaken on traditional Imraguen sail boats, which only operate inside the PNBA. Hence, any contamination that could have occurred would affect abundance and site diversity estimates, but not overall species diversity.

A current inconvenience of 12S compared to COI as a universal genetic marker is the lack of an extensive reference library (Collins et al., 2019). This study has produced 48% of the 12S barcodes belonging to sharks and ray species detected in PNBA eDNA samples and 26% of barcodes of species previously recorded in PNBA landings records. This has greatly increased probabilities of accurately determining species diversity in the PNBA, however, many rare species thought to be locally extinct could not be sampled and lack a corresponding reference sequence, potentially leading to false negative results. However, ASV´s of such species, if present, would be expected to be assigned to a genus level that is represented by other species in the database (e.g. *Pristis*, *Squatina*, *Negaprion*), but this was not the case. Otherwise, factors such as collected water volume, sampling depth, strong tidal fluctuations and seasonality (most samples were collected at sea surface between November and April) may have an influence on detection rates of rare, bottom-dwelling species or migratory species (Hansen et al., 2018).

Hammerhead species are known to pup near shore in relatively shallow areas during warmer months (Duncan & Holland, 2006) and juvenile *S. lewini* have been reported consistently as one of the two most frequently landed shark species in the PNBA (Ducrocq et al., 2014, Barham et al., 2011; Trégarot et al., 2020). In May 2021, a juvenile *S. lewini* was visually confirmed among a group of fresh catches at Arkeiss. Surprisingly however, no hammerhead species were present in eDNA samples. Its lack of detection could be explained by factors related to seasonal migration patterns of both *S. lewini* and *S. zygaena* or insufficient coverage of samples, however is not expected to be due to amplification failure, since the species is present in salting well samples and has successfully amplified in other studies targeting the same 12S fragment (Mariani et al., 2021).

Most newly recorded species in the PNBA from this study are larger, pelagic sharks with wide-ranging distribution limits such as *P. glauca, I. oxyrinchus* and *A. vulpinus* or species associated with deeper habitats, such as *H. perlo,* or colder waters, such as *S. acanthias* whose presence is likely transitory as they would not heavily rely on the PNBA as an essential habitat. The latter two species were present in higher relative read abundances, with *S. acanthias* making up 8.7% of the total reads. Inferences on species abundance in eDNA have historically been controversial, however some studies have found positive correlations between read and species abundance (Lafferty et al., 2021; Mariani et al., 2021; Muri et al., 2020). Applying their results to this study, the species with the highest proportion of reads, namely *R. marginata* (18%), *G. cemiculus* (17%), *R. acutus* (14%) and *R. rhinobatos* (14%) coincide with the species that make up the largest proportion of reported elasmobranch catches inside the PNBA (Barham et al., 2011; Trégarot et al., 2020), with the exception of *S. lewini*. By deduction, *S. acanthias*, present as well in nine out of 13 PNBA samples, should be relatively frequent, yet it has never been noted in official reports. *Squalus acanthias* is usually associated with relatively cool temperatures (Sagarese et al., 2014; Shepherd et al., 2002) and its distribution is limited to cold, temperate regions (Ebert et al., 2013), which could potentially explain why highest reads were recorded in locations near northern PNBA borders where the Canary Current and consistent upwelling produce a cold water influx. However, the genus has recently undergone genetic-level revisions, which have highlighted issues concerning unresolved *Squalus* taxonomy (Ferrari et al., 2021; Veríssimo et al., 2017), and while ASV´s for *Squalus acanthias* provided 99.5% sequence matches, sequence similarity for 12S among congeneric species is not uncommon, as commented above. Its presence and accurate identification remain to be confirmed visually.

Generally, the incidence of most previously unrecorded species is low (<65 reads/species from four or less samples) and would presumably belong to highly mobile and transient shark or ray species, with the exception of *S. canicula* and the two skate species, *R. parva* and *R. undulata,* which are smaller, demersal and mainly bottom-feeding species (Ebert et al., 2013; Last et al., 2016a). *Scyliorhinus canicula*, although not having been reported in the PNBA, is a fairly abundant species in the North-East Atlantic (Ebert et al., 2013). *Raja undulata* is known for its patchy distribution around the continental shelf of the north-east Atlantic Ocean, where it is most commonly found in shallow waters below 50 m and often close to estuaries or bays (Ellis et al., 2012; Serra-Pereira et al., 2014) and not much is known about the biology of the newly described *R. parva* (Last & Séret, 2016). This study expands the geographic range previously confirmed for both poorly studied species. Unrecorded species in the literature with higher read counts (>150) mainly belonged to taxa placed in the Dasyatidae family (*T. grabata, P. violacea* and *H. rudis*). The paucity of records can be explained by difficulties of local observers in assigning taxonomy, as evidenced in reports where catches are often grouped as "Dasyatidae" (Barham et al., 2011), however, access to databases from the IMROP is restricted. Therefore, data may often be collected but not made accessible. Also, considering numerous recent changes in the taxonomy of the family Dasyatidae (Last et al., 2016b), local observers may be prompted to record species in higher taxonomic categories. It was also observed in-situ that *H. rudis* was locally identified as *D. pastinaca* (pers. obs.), possibly explaining the total absence of this species in regional landings records. Also, *Torpedo* sp. does not figure in official statistics probably due to the fact that electric rays, although commonly caught as bycatch throughout their range, are generally not kept for consumption or trade. Therefore, albeit the species might have been known to local fishers, it remains unidentified.

Lastly, and most intriguing, is the absence *M. mustelus* throughout all eDNA samples, including samples taken outside the PNBA and at the salting wells, although it is reportedly a commonly caught species by both small-scale fisheries (Ducrocq et al., 2004; Barham et al., 2011) as well as commercial vessels (Gascuel et al., 2007). Although the smooth-hound species sampled at the processing sites were identified as the less common *M. punctulatus* through DNA barcoding, the specific origin of the animals at these sites is unknown (Jabado, unpubl. data). Supplies arrive from all nearby national landing sites, including the PNBA (processing site staff, pers. comm.). Environmental DNA results however confirm the presence of *M. punctulatus* throughout the PNBA but did not detect *M. mustelus*, a species associated with vertical migrations, where local

shifts to coastal areas follow decreasing sea temperatures during cold season from January to May (Khallahi, 2004). Several samples were taken during given time frame, all of which detected only *M. punctulatus*, including the samples taken at salting wells. This poses the question whether *M. punctulatus*, a species often recorded as *M. mustelus* (Ebert et al., 2013; Marino et al., 2018) is in fact predominant in the region and misidentified or whether both species co-occur and other factors (e.g. sample size, sampling area) are driving the absence of *M. mustelus* throughout the sample period and range. If both species do co-exist in the PNBA, where *M. mustelus* is one of two shark species Imraguen are allowed to fish and that are targeted through specific houndshark ("tollo") nets on a seasonal basis, fisheries management needs to be revisited to assess individual species populations in terms of management units, especially as both species are under extinction threat.

Although species accumulation curves indicate that an increased sampling effort would not yield significantly more species diversity, it may be worth including more variables into future eDNA monitoring surveys to cover the entire spectrum of species diversity occurring in the PNBA, considering also the notorious absence of *S. lewini*. Considering that results were based on only two limited sampling campaigns and resulted in two putative new species, the study highlights the need for further research. The focus should be on including a wider range of habitats and depth profiles as well as covering different locations in the PNBA throughout every season to explore causes of variability and to further explore the presence of rare, threatened species, for which detection could have important implications in terms of management. Additionally, results from eDNA samples from the salting wells indicated that metabarcoding could be an efficient monitoring method to determine what species are being landed and impacted by fisheries.

Recent global species assessments concluded that sharks and rays represent the most threatened group of marine vertebrates, with between 32.6 and 37.5% of species in one of three threat categories (Dulvy et al., 2021). It was noted that the level of threat increases towards tropical and subtropical areas, which is congruent with our findings, where 65.5% of species currently found in the PNBA are threatened with extinction. This number rises to 66.6% when including *S. lewini*. These numbers are alarming considering that, in spite of attempts at regulating shark and ray fisheries in the PNBA, landings have not seemed to significantly decrease since the ban on shark and ray fishing (Trégarot et al., 2020). A large proportion of shark and ray species that rely on the PNBA for refuge, parturition and/or food are affected throughout different ontogenetic

stages, but the extraction of a large proportion of juvenile animals seems prevalent for many species (Ducrocq et al., 2004) and is likely unsustainable in the long term. However, imposing blanket bans on shark and ray fishing usually encounters local resistance and leads to the development of illegal and covert activities (e.g. Carr et al., 2013; Diop & Dossa, 2011; Trégarot et al., 2020; Vianna et al., 2016), counteracting conservation efforts. This study has provided the first consolidated species checklist in the PNBA, which should be used as basis to improve knowledge on species distributions and seasonality as well as it should contribute towards individual species management, helping managers to make better informed decisions to contribute to their conservation.

CONCLUSION

Data collection for this study was limited due to temporal and spatial constraints, however, results illustrate the importance of taxonomic and molecular survey studies in uncovering previously overlooked and hidden diversity, showing that the region still holds a lot of unexplored potential. We provide a first list of shark and ray species in the PNBA confirmed through multiple approaches, however, more work is necessary. Preliminary results point to the PNBA being an important region in terms of species diversity. A high amount of species detected are threatened with extinction, and rare species (e.g. some guitarfishes, wedgefishes and sawfishes) were not detected in our samples, which possibly points to their regional disappearance, in line with previous suggestions from anecdotal reports. This should be urgently addressed in light of the dire current conservation status of sharks and rays as a whole, but also of local species specifically.

In spite of shark and ray fishing bans, the enforcement of such regulations is limited by a lack of resources and monitoring capacity. This is a serious concern in light of our results, considering a limited sampling effort resulted in two putative new species, potential cryptic diversity of others and taxonomic corrections, such as the previously unknown presence of *Mustelus punctulatus*, indicating that the tollo (*M. mustelus*) fisheries needs to be managed with urgency. More research is warranted to expand on our study, however, taking into account the continuing fishing pressure sharks and rays are exposed to in and outside the PNBA and their generally low recovery potential, current monitoring and regulatory strategies concerning shark and ray species in the PNBA should be reexamined immediately.

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SUPPLEMENTARY INFORMATION

Table S1. All samples used for amplification and sequencing ordered by sample ID and collection date. Sample area is presented. Initial species identification is compared to NCBI sequence matches for 12S, COI and NADH2, where blank cells represent samples that were not amplified for the specific genetic marker.

ID	Sampling date	Sampling location	Initial ID	12S match	COI match	NADH2 match	Voucher
001	31-Oct-20	Nouadhibou	*R. rhinobatos*	New barcode			Y
002	31-Oct-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
003	31-Oct-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
004	31-Oct-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
005	31-Oct-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
006	31-Oct-20	Nouadhibou	*R. acutus*	-			Y
007	31-Oct-20	Nouadhibou	*M. mustelus*	New barcode		*M. punctulatus*	Y
008	31-Oct-20	Nouadhibou	*M. mustelus*	New barcode		-	Y
009	31-Oct-20	Nouadhibou	*M. mustelus*	-			Y
010	31-Oct-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
011	31-Oct-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
012	31-Oct-20	Nouadhibou	*A. bovinus*	New barcode			Y
013	31-Oct-20	Nouadhibou	*A. bovinus*	-			Y
014	31-Oct-20	Nouadhibou	*R. marginata*	New barcode			Y
015	31-Oct-20	Nouadhibou	*R. marginata*	New barcode			Y
016	31-Oct-20	Nouadhibou	*H. rudis*	-			Y
017	31-Oct-20	Nouadhibou	*G. altavela*	*G. altavela*	*G. altavela* *G. natalensis*		Y
018	31-Oct-20	Nouadhibou	*G. altavela*	*G. altavela*	*G. altavela* *G. natalensis*		Y
019	31-Oct-20	Nouadhibou	*D. pastinaca*	*New barcode*			Y
020	31-Oct-20	Nouadhibou	*D. pastinaca*	New barcode			Y
021	31-Oct-20	Nouadhibou	*S. lewini*	-			Y
022	31-Oct-20	Nouadhibou	*G. cirratum*	*G. cirratum*			Y
024	31-Oct-20	Nouadhibou	*G. cirratum*	*G. cirratum*			Y
025	31-Oct-20	Nouadhibou	*R. acutus*	-			Y
026	31-Oct-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
030	31-Oct-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
031	31-Oct-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
033	31-Oct-20	Nouadhibou	*Z. schoenleinii*	New barcode			Y
034	31-Oct-20	Nouadhibou	*Z. schoenleinii*	New barcode			Y
035	31-Oct-20	Nouadhibou	*Z. schoenleinii*	New barcode			Y
036	31-Oct-20	Nouadhibou	*Z. schoenleinii*	New barcode			Y
037	31-Oct-20	Nouadhibou	*Z. schoenleinii*	-			Y
038	31-Oct-20	Nouadhibou	*R. straeleni*	*R. undulata*			Y
039	31-Oct-20	Nouadhibou	*R. straeleni*	*R. undulata*	-		Y
040	31-Oct-20	Nouadhibou	*R. straeleni*	*R. undulata*	*R. undulata*		Y

041	31-Oct-20	Nouadhibou	*R. straeleni*	*R. undulata*			Y
042	31-Oct-20	Nouadhibou	*Dasyatis sp*	New barcode			Y
043	31-Oct-20	Nouadhibou	*Dasyatis sp*	New barcode			Y
044	31-Oct-20	Nouadhibou	*Dasyatis sp*	New barcode	*D. marmorata* *D. pastinaca*[3]		Y
045	31-Oct-20	Nouadhibou	*Dasyatis sp*	New barcode			Y
046	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode	New barcode		Y
047	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode			Y
048	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode			Y
049	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode			Y
050	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode			Y
051	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode	New barcode		Y
052	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode	New barcode		Y
053	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode	New barcode		Y
054	31-Oct-20	Nouadhibou	*R. parva*	New barcode			Y
055	31-Oct-20	Nouadhibou	*R. parva*	New barcode			Y
056	31-Oct-20	Nouadhibou	*R. parva*	New barcode			Y
057	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode	New barcode		Y
058	31-Oct-20	Nouadhibou	*R. straeleni*	*R. undulata*			Y
059	31-Oct-20	Nouadhibou	*F. margarita / margaritella*	New barcode			Y
060	31-Oct-20	Nouadhibou	*R. parva*	New barcode			Y
061	31-Oct-20	Nouadhibou	*S. lewini*	*S. lewini*[2]			Y
062	31-Oct-20	Nouadhibou	*S. lewini*	-			Y
063	31-Oct-20	Nouadhibou	*Carcharhinus sp*	*C. obscurus* *C. brachyurus*[1] *C. brevipinna*[1] *C. longimanu*[1] *C. amboinensis*[1]			Y
064	31-Oct-20	Nouadhibou	*S. zygaena*	*S. zygaena*[1]			Y
065	31-Oct-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
068	31-Oct-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
069	31-Oct-20	Nouadhibou	*R. rhinobatos*	New barcode			Y
072	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
073	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
074	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
075	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
076	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
082	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y

083	1-Nov-20	Nouadhibou	*R. rhinobatos*	New barcode			Y
084	1-Nov-20	Nouadhibou	*R. rhinobatos*	New barcode			Y
089	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
092	1-Nov-20	Nouadhibou	*S. zygaena*	*S. zygaena*[1]			Y
093	1-Nov-20	Nouadhibou	*R. straeleni*	*R. undulata*			Y
094	1-Nov-20	Nouadhibou	*R. straeleni*	*R. undulata*			Y
095	1-Nov-20	Nouadhibou	*R. straeleni*	*R. undulata*			Y
096	1-Nov-20	Nouadhibou	*H. rudis*	New barcode			Y
097	1-Nov-20	Nouadhibou	*R. acutus*	-			Y
098	1-Nov-20	Nouadhibou	*R. acutus*	*New barcode*[4]			Y
099	1-Nov-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
100	1-Nov-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
101	1-Nov-20	Nouadhibou	*R. acutus*	-			Y
102	1-Nov-20	Nouadhibou	*M. mustelus*	New barcode			Y
103	1-Nov-20	Nouadhibou	*L. smithii*	New barcode			Y
104	1-Nov-20	Nouadhibou	*D. marmorata*	New barcode			Y
105	1-Nov-20	Nouadhibou	*D. marmorata*	New barcode			Y
106	1-Nov-20	Nouadhibou	*D. marmorata*	New barcode			Y
107	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
109	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
110	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
111	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
112	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
113	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
114	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
115	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
116	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
117	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
118	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
119	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
120	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
121	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
122	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
123	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
124	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
125	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
126	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
127	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
128	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
129	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
130	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
131	1-Nov-20	Nouadhibou	*S. lewinii*	*S. lewini*[2]		-	Y
132	1-Nov-20	Nouadhibou	*R. rhinobatos*	New barcode		-	Y
133	1-Nov-20	Nouadhibou	*R. rhinobatos*	New barcode			Y

134	1-Nov-20	Nouadhibou	*R. rhinobatos*	New barcode			Y
135	1-Nov-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
136	1-Nov-20	Nouadhibou	*R. acutus*	-	New barcode	*P. pectoralis*	Y
137	1-Nov-20	Nouadhibou	*T. grabata*	New barcode			Y
138	1-Nov-20	Nouadhibou	*R. acutus*	*R. acutus*			Y
139	1-Nov-20	Nouadhibou	*M. mustelus*	-			Y
140	1-Nov-20	Nouadhibou	*L. smithii*	New barcode			Y
141	1-Nov-20	Nouadhibou	*G. altavela*	*G. altavela*			Y
142	1-Nov-20	Nouadhibou	*T. grabata*	New barcode			Y
143	1-Nov-20	Nouadhibou	*T. grabata*	New barcode			Y
144	1-Nov-20	Nouadhibou	*H. rudis*	New barcode			Y
146	1-Nov-20	Nouadhibou	*G. cemiculus*	*G. cemiculus*			Y
147	1-Nov-20	Nouadhibou	*R. marginata*	New barcode	*R. marginata* *R. brasiliensis*		Y
148	1-Nov-20	Nouadhibou	*A. bovinus*	New barcode			Y
150	3-Nov-20	Iwik	*H. rudis*	New barcode	-		Y
151	3-Nov-20	Iwik	*G. cirratum*	*G. cirratum*	*G. cirratum*		Y
152	3-Nov-20	Iwik	*Torpedo sp*	New barcode			Y
153	3-Nov-20	Arkeiss	*L. smithii*	New barcode			Y
155	3-Nov-20	Iwik	*G. cemiculus*	*G. cemiculus*			Y
156	4-Nov-20	Iwik	*H. rudis*	New barcode			Y
157	4-Nov-20	Iwik	*P. pectoralis*	New barcode	New barcode	*P. pectoralis*	Y
158	4-Nov-20	Iwik	*P. pectoralis*	New barcode		*P. pectoralis*	Y
159	4-Nov-20	Iwik	*A. bovinus*	New barcode	*A. bovinus*		N
160	4-Nov-20	Iwik	*A. bovinus*	New barcode	*A. bovinus*		N
161	4-Nov-20	Iwik	*A. bovinus*	New barcode	*A. bovinus*		N
162	4-Nov-20	Iwik	*H. rudis*	New barcode	New barcode (*H. americanus*)		N
163	4-Nov-20	Iwik	*H. rudis*	New barcode			N
164	4-Nov-20	Iwik	*H. rudis*	New barcode			N
165	4-Nov-20	Iwik	*A. bovinus*	New barcode			N
166	4-Nov-20	Iwik	*G. cirratum*	*G. cirratum*			N
200	5-Nov-20	Belawakh	*S. zygaena*	*S. zygaena*			Y
201	5-Nov-20	Belawakh	*Gymnura sp*	New barcode	New barcode		Y
202	5-Nov-20	Belawakh	*A. bovinus*	New barcode	-		Y
203	5-Nov-20	Belawakh	*R. acutus*	*R. acutus*			Y
204	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
205	5-Nov-20	Belawakh	*G. altavela*	*G. altavela*	*G. altavela* *G. natalensis*		Y
206	5-Nov-20	Belawakh	*G. cemiculus*	*G. cemiculus*			Y
207	5-Nov-20	Belawakh	*A. bovinus*	New barcode	-		Y
208	5-Nov-20	Belawakh	*R. acutus*	*R. acutus*			Y
209	5-Nov-20	Belawakh	*R. acutus*	*R. acutus*			Y
210	5-Nov-20	Belawakh	*R. acutus*	*R. acutus*			Y

211	5-Nov-20	Belawakh	*L. smithii*	New barcode			Y
212	5-Nov-20	Belawakh	*L. smithii*	New barcode			Y
213	5-Nov-20	Belawakh	*L. smithii*	New barcode			Y
214	5-Nov-20	Belawakh	*Gymnura sp*	New barcode	New barcode		Y
215	5-Nov-20	Belawakh	*Gymnura sp*	New barcode	New barcode		Y
216	5-Nov-20	Belawakh	*S. lewini*	*S. lewini*[2]			Y
217	5-Nov-20	Belawakh	*G. cemiculus*	*G. cemiculus*			Y
218	5-Nov-20	Belawakh	*R. rhinobatos or R. irvinei*	New barcode	-		Y
219	5-Nov-20	Belawakh	*R. rhinobatos or R. irvinei*	New barcode			Y
220	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
221	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
222	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
223	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
224	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
225	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
226	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
227	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
228	5-Nov-20	Belawakh	*R. rhinobatos*	New barcode			Y
300	28-Mar-21	Nouadhibou	*M. mustelus*	New barcode		-	Y
301	28-Mar-21	Nouadhibou	*G. altavela*	*G. altavela*			Y
302	28-Mar-21	Nouadhibou	*I. oxyrinchus*	*I. oxyrinchus*			Y
303	28-Mar-21	Nouadhibou	*T. grabata*	New barcode			Y
304	28-Mar-21	Nouadhibou	*T. grabata*	New barcode			Y
305	28-Mar-21	Nouadhibou	*I. oxyrinchus*	*I. oxyrinchus*			Y
306	28-Mar-21	Nouadhibou	*I. oxyrinchus*	*I. oxyrinchus*			Y
307	28-Mar-21	Nouadhibou	*R. rhinobatos or R. irvinei*	New barcode			Y
308	28-Mar-21	Nouadhibou	*R. rhinobatos or R. irvinei*	New barcode			Y
309	28-Mar-21	Nouadhibou	*R. acutus*	*R. acutus*			Y
310	28-Mar-21	Nouadhibou	*R. acutus*	*R. acutus*			Y
311	28-Mar-21	Nouadhibou	*R. acutus*	*R. acutus*			Y
312	28-Mar-21	Nouadhibou	*R. acutus*	*R. acutus*			Y
313	28-Mar-21	Nouadhibou	*I. oxyrinchus*	*I. oxyrinchus*			Y
314	28-Mar-21	Nouadhibou	*P. violacea*	*P. violacea*	*P. violacea*		Y
315	28-Mar-21	Nouadhibou	*G. altavela*	*G. altavela*	*G. altavela* *G. natalensis*		Y
316	28-Mar-21	Nouadhibou	*Z. schoenleinii*	New barcode			Y
317	28-Mar-21	Nouadhibou	*R. parva*	New barcode			Y
318	28-Mar-21	Nouadhibou	*D. marmorata*	New barcode	*D. marmorata* *D. pastinaca*[3]		Y
319	28-Mar-21	Nouadhibou	*H. perlo*	*H. perlo*[1]			Y
320	28-Mar-21	Nouadhibou	*A. superciliosus*	*A. superciliosus*			Y
321	28-Mar-21	Nouadhibou	*Carcharhinus sp*	-			Y

322	28-Mar-21	Nouadhibou	*C. signatus*	*New barcode*[4]	New barcode		Y
323	28-Mar-21	Nouadhibou	*R. acutus*	*R. acutus*			Y
324	29-Mar-21	Nouadhibou	*S. mokarran*[5]	-			Only head
325	29-Mar-21	Nouadhibou	*G. altavela*	*G. altavela*			Y
326	29-Mar-21	Nouadhibou	*R. rhinobatos*	New barcode			Y
327	29-Mar-21	Nouadhibou	*R. acutus*	*R. acutus*			Y
328	29-Mar-21	Nouadhibou	*Carcharhinus sp*	*C. obscurus* *C. brachyurus*[1] *C. brevipinna*[1] *C. longimanus*[1] *C. amboinensis*[1]	*C. obscurus* *C. galapagensis*		Y
329	29-Mar-21	Nouadhibou	*Carcharhinus sp*	*C. obscurus* *C. brachyurus*[1] *C. brevipinna*[1] *C. longimanus*[1] *C. amboinensis*[1]	*C. obscurus* *C. galapagensis*	*C. obscurus*	Y
330	29-Mar-21	Nouadhibou	*S. mokarran*[5]	-			N
331	29-Mar-21	Nouadhibou	*D. pastinaca*	*D. pastinaca*			Y
332	29-Mar-21	Nouadhibou	*Dasyatis sp*	New barcode	*D. marmorata* *D. pastinaca*[3]		Y
333	30-Mar-21	Iwik	*L. smithii*	New barcode	-		Y
334	30-Mar-21	Iwik	*L. smithii*	New barcode			Y
335	30-Mar-21	Iwik	*R. rhinobatos or R. irvinei*	New barcode			Y
400	2-Apr-21	Nair	*F. margarita / margaritella*	New barcode			Y
401	2-Apr-21	Nair	*F. margarita / margaritella*	New barcode	New barcode		N
402	2-Apr-21	Nair	*F. margarita / margaritella*	New barcode	New barcode		N
403	2-Apr-21	Nair	*F. margarita / margaritella*	New barcode	New barcode		N
404	7-Apr-21	Belawakh	*Rhinobatos sp*	New barcode			Y
405	7-Apr-21	Belawakh	*G. cuvier*	*G. cuvier*			Y
406	7-Apr-21	Belawakh	*Gymnura sp*	New barcode	-		Y
407	7-Apr-21	Belawakh	*G. altavela*	*G. altavela*			Y
408	7-Apr-21	Belawakh	*G. altavela*	*G. altavela*	*G. altavela* *G. natalensis*		Y
409	7-Apr-21	Belawakh	*T. grabata*	New barcode			Y

Similarity percentage of all species > 99%, except:
- Sequence not included due to poor quality
[1] NCBI percentage of similarity between 98-99%
[2] NCBI percentage of similarity < 98%
[3] Species possibly mislabeled
[4] *Paragaleus pectoralis*. Species identified through sequence similarity with other sample sequences
[5] Species not confirmed due to insufficient morphological and genetic evidence

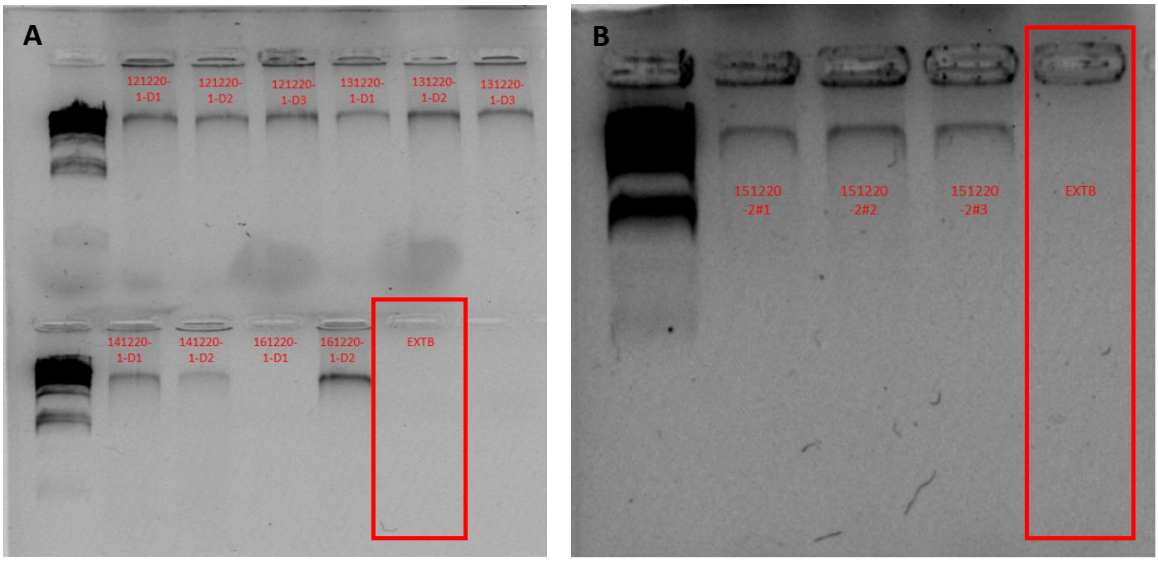

Figure S1. Extraction blanks visualized on 0.8% agarose gel. **A** Red rectangle shows ExtB260121 **B** red rectangle shows ExtB030221

Table S2. P-distance matrix for 12S barcodes of sampled elasmobranch species

	Fmar	Dmar	Dpas	Pvio	Tgra	Hrud	Gymn	Galta	Rmar	Abov1	Abov2	Rund	Rpar	Torp	Gcem	Rrhi	Zsch	Ioxy	Asup	Gcir	Racu	Cobs	Slew	Szyg	Mpun	Gcuv	Lsmi	Ppec	Hper
Fmar																													
Dmar	0.1605																												
Dpas	0.1572	0.0316																											
Pvio	0.1575	0.0364	0.0330																										
Tgra	0.1466	0.0590	0.0557	0.0560																									
Hrud	0.1450	0.0837	0.0803	0.0807	0.0698																								
Gymn	0.1719	0.1253	0.1220	0.1223	0.1115	0.1098																							
Galta	0.1791	0.1325	0.1292	0.1295	0.1186	0.1170	0.0566																						
Rmar	0.1566	0.1100	0.1067	0.1070	0.0962	0.0946	0.1150	0.1222																					
Abov1	0.1576	0.1110	0.1077	0.1080	0.0971	0.0955	0.1080	0.1152	0.1007																				
Abov2	0.1601	0.1135	0.1102	0.1105	0.0996	0.0980	0.1105	0.1177	0.1032	0.0125																			
Rund	0.2585	0.2516	0.2483	0.2486	0.2377	0.2361	0.2630	0.2702	0.2477	0.2487	0.2512																		
Rpar	0.2631	0.2561	0.2528	0.2531	0.2422	0.2406	0.2676	0.2747	0.2523	0.2532	0.2557	0.0500																	
Torp	0.2734	0.2665	0.2631	0.2635	0.2526	0.2510	0.2779	0.2851	0.2626	0.2636	0.2661	0.2662	0.2707																
Gcem	0.2289	0.2219	0.2186	0.2189	0.2081	0.2064	0.2334	0.2406	0.2181	0.2191	0.2216	0.2287	0.2333	0.2436															
Rrhi	0.2048	0.1978	0.1945	0.1948	0.1840	0.1823	0.2093	0.2165	0.1940	0.1950	0.1975	0.2046	0.2092	0.2195	0.1321														
Zsch	0.2343	0.2274	0.2240	0.2243	0.2135	0.2119	0.2388	0.2460	0.2235	0.2245	0.2270	0.2342	0.2387	0.2490	0.1881	0.1641													
Ioxy	0.3056	0.2986	0.2953	0.2956	0.2848	0.2832	0.3101	0.3173	0.2948	0.2958	0.2983	0.2862	0.2907	0.3133	0.2758	0.2517	0.2812												
Asup	0.2864	0.2795	0.2761	0.2765	0.2656	0.2640	0.2909	0.2981	0.2756	0.2766	0.2791	0.2670	0.2715	0.2941	0.2566	0.2325	0.2620	0.0875											
Gcir	0.3164	0.3094	0.3061	0.3064	0.2955	0.2939	0.3208	0.3280	0.3055	0.3065	0.3090	0.2970	0.3015	0.3240	0.2865	0.2624	0.2920	0.2384	0.2192										
Racu	0.3133	0.3063	0.3030	0.3033	0.2925	0.2908	0.3178	0.3250	0.3025	0.3035	0.3060	0.2939	0.2984	0.3210	0.2835	0.2594	0.2889	0.2211	0.2019	0.2461									
Cobs	0.3098	0.3029	0.2995	0.2999	0.2890	0.2874	0.3143	0.3215	0.2990	0.3000	0.3025	0.2904	0.2950	0.3175	0.2800	0.2559	0.2854	0.2177	0.1985	0.2427	0.0625								
Slew	0.2940	0.2871	0.2837	0.2841	0.2732	0.2716	0.2985	0.3057	0.2832	0.2842	0.2867	0.2746	0.2791	0.3017	0.2642	0.2401	0.2696	0.2018	0.1827	0.2268	0.0892	0.0858							
Szyg	0.3095	0.3025	0.2992	0.2995	0.2886	0.2870	0.3140	0.3211	0.2987	0.2996	0.3021	0.2901	0.2946	0.3171	0.2797	0.2556	0.2851	0.2173	0.1981	0.2423	0.1162	0.1128	0.0969						
Mpun	0.3291	0.3222	0.3188	0.3192	0.3083	0.3067	0.3336	0.3408	0.3183	0.3193	0.3218	0.3097	0.3143	0.3368	0.2993	0.2752	0.3047	0.2370	0.2178	0.2620	0.1540	0.1506	0.1348	0.1502					
Gcuv	0.3030	0.2961	0.2927	0.2931	0.2822	0.2806	0.3075	0.3147	0.2922	0.2932	0.2957	0.2836	0.2882	0.3107	0.2732	0.2491	0.2786	0.2109	0.1917	0.2358	0.1527	0.1492	0.1334	0.1489	0.1685				
Lsmi	0.3386	0.3316	0.3283	0.3286	0.3178	0.3161	0.3431	0.3503	0.3278	0.3288	0.3313	0.3192	0.3237	0.3462	0.3088	0.2847	0.3142	0.2722	0.2530	0.2829	0.2799	0.2764	0.2606	0.2761	0.2957	0.2696			
Ppec	0.2583	0.2514	0.2480	0.2484	0.2375	0.2359	0.2628	0.2700	0.2475	0.2485	0.2510	0.2389	0.2435	0.2660	0.2285	0.2044	0.2339	0.2158	0.1966	0.2265	0.2235	0.2200	0.2042	0.2197	0.2393	0.2132	0.2488		
Hper	0.2706	0.2636	0.2603	0.2606	0.2497	0.2481	0.2751	0.2822	0.2598	0.2607	0.2632	0.2512	0.2557	0.2782	0.2408	0.2167	0.2462	0.2585	0.2393	0.2692	0.2662	0.2627	0.2469	0.2623	0.2820	0.2559	0.2915	0.2112	

Table S3. Metadata from eDNA samples.

Sample name	Replicates	Extraction blank	Sampling Date	Filtration Date	Site	In/outside PNBA	Habitat	Longitude	Latitude
Ile des Pelicans	3	ExtB161220	14/02/2020	14/02/2020	Mid Banc d'Arguin	Inside PNBA	no information	20.58314	-16.64489
Akadir	3	ExtB161220	15/02/2020	15/02/2020	Ile d'Arguin	Inside PNBA	no information	20.60795	-16.44831
Selac_Puit 1	1	ExtB161221	01/11/2020	01/11/2020	Nouadhibou Processing Site	Outside PNBA	NA	21.071735	-16.993395
Selac_Puit 2	1	ExtB161222	01/11/2020	01/11/2020	Nouadhibou Processing Site	Outside PNBA	NA	21.071735	-16.993395
Iwik	3	ExtB161223	04/11/2020	04/11/2020	Iwik	Inside PNBA	NA	19.877914	-16.304943
Mamghar	3	ExtB161224	07/11/2020	07/11/2020	Mamghar	Inside PNBA	NA	19.355537	-16.516715
Idis	3	ExtB161225	07/11/2020	07/11/2020	Idis	Inside PNBA	no information	19.525811	-16.495327
Baye St Jean	3	ExtB260121	12/12/2020	12/12/2020	Baye St Jean	Inside PNBA	Seagrass	19.37591	-16.46436
L'oeil	3	ExtB260121	13/12/2020	13/12/2020	L'oeil	Inside PNBA	No vegetation	19.37359	-16.53738
Belkeiznaya	2	ExtB260121	14/12/2020	14/12/2020	Belkeiznaya	Inside PNBA	Seagrass matte?	19.902757	-16.3080
West Tidra 1	3	ExtB030221	15/12/2020	16/12/2020	West off Tidra/Kiji	Inside PNBA	Seagrass	19.84629	-16.6180
West Tidra 2	3	ExtB030221	15/12/2020	16/12/2020	West off Tidra/Kiji	Inside PNBA	Seagrass	19.78945	-16.59858
Kiji	2	ExtB260121	16/12/2020	16/12/2020	Kiji	Inside PNBA	no information	19.73805	-16.49369
Mouzan	3	ExtBNair	31/3/2021	01/04/2021	Mouzan	Inside PNBA	Seagrass	19.893577	-16.518452
Nair	2	ExtBNair	01/04/2021	03/04/2021	Nair	Inside PNBA	Sand	19.859644	-16.407293

Table S4. eDNA reads from samples taken inside the PNBA. Cells marked in yellow represent reads removed from the final dataset due to contamination in extraction blanks.

Sample location	*Aetomylaeus bovinus*	*Alopias vulpinus*	*Carcharhinus obscurus*	*Dasyatis pastinaca*	*Dasyatis marmorata*	*Fontitrygon margarita/margaritella*	*Ginglymostoma cirratum*
Ile des Pelicans	0	0	59	0	1	15	0
Akadir	1	1	1	0	3	22	0
West Tidra 1	31	0	240	23	100	668	0
West Tidra 2	388	0	3	1	75	4	176
Kiji	55	0	1	0	0	7	1
Idis	0	0	0	3	0	1	0
Mouzan	2673	0	0	0	3	252	0
Nair	1	0	565	0	1	352	0
Belkeiznaya	0	0	1	1	0	3	0
Iwik	3	0	1	0	1115	26	1
Mamghar	0	1	1	0	0	3	1
Baye St jean	18	0	0	1	0	35	3
L'oeil	7	0	1	0	2	9	0
TOTAL	***3177***	***2***	***873***	***29***	***1300***	***1397***	***182***

Sample location	*Glaucostegus cemiculus*	*Gymnura altavela*	*Gymnura sp*	*Heptranchias perlo*	*Hypanus rudis*	*Isurus oxyrinchus*	*Leptocharias smithii*	*Mustelus punctulatus*
Ile des Pelicans	8	19	10	0	1	0	1	27
Akadir	0	307	1	0	2	0	676	2
West Tidra 1	732	95	33	0	14	0	673	77
West Tidra 2	312	44	10	0	71	1	1	2
Kiji	4	17	4	0	56	0	0	1
Idis	65	510	156	0	68	0	0	0

Mouzan	51	55	3	0	412	0	59	18
Nair	0	3	0	0	0	0	0	1
Belkeiznaya	9372	1	0	1	0	2	0	0
Iwik	0	295	5	246	417	0	5	0
Mamghar	36	1	1	1	2	3	0	744
Baye St jean	98	7	0	0	0	0	1	1
L'oeil	95	90	108	0	1	2	4	4
TOTAL	***10773***	***1444***	***331***	***248***	***1044***	***8***	***1420***	***877***

Sample location	***Myliobatis sp***	***Paragaleus pectoralis***	***Prionace glauca***	***Pteroplatytrygon violacea***	***Raja parva***	***Raja undulata***	***Rhinobatos rhinobatos***
Ile des Pelicans	1	2	0	0	0	0	16
Akadir	0	0	0	0	0	0	26
West Tidra 1	0	2	4	36	61	1	4990
West Tidra 2	0	0	5	0	0	0	31
Kiji	0	0	0	0	0	0	0
Idis	0	0	0	0	0	0	783
Mouzan	0	0	2	0	0	0	3212
Nair	0	0	0	0	0	0	4
Belkeiznaya	0	0	2	0	0	0	15
Iwik	0	0	2	0	0	0	6
Mamghar	0	0	0	0	0	0	12
Baye St jean	3	1	0	1	0	4	20
L'oeil	1	2	1	121	0	0	17138
TOTAL	***5***	***7***	***16***	***158***	***61***	***5***	***26253***

Sample location	***Rhinoptera marginata***	***Rhizoprionodon acutus***	***Scyliorhinus canicula***	***Squalus acanthias***	***Taeniura grabata***	***Torpedo sp***	***Zanobatus schoenleinii***
Ile des Pelicans	3	21	2	18	2	0	1
Akadir	982	847	0	5365	1	1	2015
West Tidra 1	3701	1364	0	3	72	0	823
West Tidra 2	6805	147	38	50	218	0	4
Kiji	4	27	0	0	429	62	26
Idis	0	691	1	1	0	1	3
Mouzan	1	116	2	90	2	1	5
Nair	2	76	0	3	0	0	1
Belkeiznaya	1	5	4	11	0	0	1
Iwik	14	29	0	7	0	758	426
Mamghar	9	50	0	10	0	1	264
Baye St jean	11	5	0	14	0	0	7
L'oeil	5	5673	0	36	0	2	501
TOTAL	***11538***	***9051***	***47***	***5608***	***724***	***826***	***4077***

Table S5. eDNA reads from samples taken outside the PNBA (Cap Blanc).

Species	Total
Carcharhinus obscurus	1
Rhizoprionodon acutus	718
Paragaleus pectoralis	412
Leptocharias smithii	241
Mustelus punctulatus	3
Dasyatis pastinaca	1244
Fontitrygon margarita/margaritella	63
Hypanus rudis	1
Gymnura altavela	3
Rhinoptera marginata	14
Ginglymostoma cirratum	1776
Raja undulata	1
Rhinobatos rhinobatos	2354
Zanobatus schoenleinii	3110
Squalus acanthias	2

Table S6. eDNA reads from samples taken at salting wells at the Nouadhibou processing site.

Species	SELAC PUIT 1	SELAC PUIT 2	TOTAL
Aetomylaeus bovinus	602	1	603
Dasyatis pastinaca	2	1	3
Dasyatis marmorata	158	55	213
Fontitrygon margarita/margaritella	3	2	5
Galeocerdo cuvier	38	1	39
Ginglymostoma cirratum	0	123	123
Glaucostegus cemiculus	940	87	1027
Gymnura altavela	22790	24356	47146
Gymnura sp	7163	28	7191
Hypanus rudis	9	181	190
Leptocharias smithii	177	17	194

Mustelus punctulatus	2464	621	3085
Paragaleus pectoralis	525	247	772
Prionace glauca	2	0	2
Pseudotriakis microdon	1	1	2
Raja undulata	96	101	197
Rhinobatos rhinobatos	39	929	968
Rhinoptera marginata	291	0	291
Rhizoprionodon acutus	30211	25901	56112
Sphyrna lewini	6	0	6
Sphyrna mokarran	1	1	2
Sphyrna zygaena	440	75	515
Squalus acanthias	7	11	18
Taeniura grabata	520	82	602
Torpedo marmorata	0	2	2
Zanobatus schoenleinii	1	6	7

Table S7. Read count in control samples (extraction blanks and PCR blanks). Values marked in red represent species that were excluded from eDNA samples extracted with the respective extraction blank.

Species	PCRBlank_020621	PCRBlank_240521	ExtB_Nair	ExtB_030221	ExtB_161220	ExtB_260121	ExtB_91220
Aetomylaeus bovinus	1	0	1	0	1	1	0
Alopias vulpinus	0	0	0	0	0	0	0
Carcharhinus obscurus	0	0	0	1	0	1	0
Dasyatis pastinaca	0	0	0	0	1	4	4
Dasyatis marmorata	0	0	0	1	0	1	0
Fontitrygon margarita/margaritella	0	0	2	28757	11	10	11
Galeocerdo cuvier	0	0	0	0	0	0	0
Ginglymostoma cirratum	0	0	1	6	3	0	3
Glaucostegus cemiculus	0	0	1	4	1	0	1
Gymnura altavela	0	0	19	1	4	0	8
Gymnura sp	0	0	6	0	0	0	1

Heptranchias perlo	0	0	0	0	0	0	0
Hypanus rudis	0	0	0	0	0	0	0
Isurus oxyrinchus	0	0	0	0	0	0	0
Leptocharias smithii	0	0	0	2	0	0	0
Mustelus punctulatus	0	0	0	0	2	1	0
Paragaleus pectoralis	0	0	0	0	0	1	2
Prionace glauca	0	0	1	354	0	1	0
Pseudotriakis microdon	0	0	0	0	0	0	0
Pteroplatytrygon violacea	0	0	0	0	0	0	0
Raja parva	0	0	0	0	0	0	0
Raja undulata	0	0	1	0	1	0	0
Rhinobatos rhinobatos	0	1	10	7	14	6035	7
Rhinoptera marginata	0	0	5	2	1	5	2
Rhizoprionodon acutus	0	0	28	1	16	1	14
Scyliorhinus canicula	0	0	0	1	0	0	0
Sphyrna lewini	0	0	0	0	0	0	0
Sphyrna mokarran	0	0	0	0	0	0	0
Sphyrna zygaena	0	0	0	0	0	0	0
Squalus acanthias	0	0	20	5	8	7070	10
Taeniura grabata	0	0	0	0	0	0	1
Torpedo marmorata	0	0	0	0	0	0	0
Torpedo sp	0	0	0	0	0	0	0
Zanobatus schoenleinii	0	0	1	1	5	0	1