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Brief Report

Population Structure of *Aetobatus narinari* (Myliobatiformes) Caught by the Artisanal Fishery in Northeast Brazil

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Abstract: In Brazil, the Whitespotted Eagle Ray *Aetobatus narinari* is considered data-deficient due to the scarcity of basic information on its biology, ecology, and vulnerability to capture. Despite this, the species is caught by artisanal fishing along most of the coast, especially in the northeast of Brazil. This study analyzed mitochondrial DNA data in specimens of *A. narinari* caught by artisanal fishing in the northeast coast of Brazil to understand their population structure. For this, 42 individuals were sequenced at three mitochondrial genes: cytochrome oxidase 1 (COI), cytochrome b (Cytb), and NADH dehydrogenase subunit 4 (ND4). Concatenated COI-Cytb-ND4 sequences yielded 14 haplotypes, with moderate haplotype diversity (h = 0.646), low nucleotide diversity (π = 0.00087), and low fixation index ΦST values, indicating no population structure. Our results suggest that there is only one population of *A. narinari* in the study area. Genetic studies can contribute to improving management plans in these areas, avoiding the overexploitation of this and other species.

Keywords: Myliobatiformes; Aetobatidae; genetic diversity; artisanal fishing; conservation

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1. Introduction

The Whitespotted Eagle Ray *Aetobatus narinari* (Euphrasen, 1790) [1] was previously considered a circumglobal species [2]. However, phylogenetic studies—based on variations in color pattern, parasitology, and genetic evidence—have shown that this species only occurs in the Atlantic Ocean [3,4]. Due to this taxonomic redefinition, the conservation status of the species throughout its range is currently classified as endangered (EN) by the International Union for Conservation of Nature (IUCN) [5], while in Brazil, *A. narinari* is classified as data-deficient (DD) [6].

Studies of *A. narinari* in the Atlantic have encompassed topics such as reproduction [7–11], population genetic connectivity, and phylogeography based on mitochondrial (cytochrome oxidase 1 (COI) [4] and cytochrome b (Cytb) [4,12]) and nuclear markers [4,12–14], feeding ecology [15–17], age and growth [18], and variation in catch rate in artisanal fisheries [7,19]. Nevertheless, basic information on the biology of *A. narinari* is still scarce, and further studies are needed, especially in areas where this species is targeted or incidentally captured in fisheries. The fishing and commercialization of the *A. narinari* occurs

in several countries of the Atlantic (Mexico, Puerto Rico, Venezuela, Cuba, Brazil) [7,9,19–21]; as such, data on population connectivity are critical in managing these populations.

Describing the molecular variation and genetic structure of populations is fundamental to understanding species health and long-term viability, as well as to better informing conservation directives [12–14]. This information is even more important in areas where populations are exploited by fisheries. Overexploitation of marine stocks can drastically reduce effective population size, resulting in a decrease in genetic diversity and possibly a concurrent increase in deleterious alleles and in inbreeding depression [22], preventing the replenishment of a species' stocks and having unforeseen ecosystem consequences through trophic cascades [23]. This is especially true for elasmobranchs, since fishing pressure can deplete and possibly isolate populations of the Silky Shark *Carcharhinus falciformis* [24] and the Spotted Eagle Ray *Aetobatus ocellatus* [25].

Aetobatus narinari is caught by artisanal fisheries [20], which exploit individuals from nursery areas in the northeast coast of Brazil [26]. Despite the species' vulnerability, there are still scarce studies on its biology in this region [11,26], hampering the assessment of its local conservation status. More information about *A. narinari*, such as its population structure, can direct more appropriate management measures for its conservation in the region. In this study, we aimed to (1) assess the genetic diversity of *A. narinari* specimens caught by artisanal fishing in different locations in northeastern Brazil and (2) test if mtDNA analysis was performed for a single Brazilian population or for other independent populations of this species in order to understand their population structure.

2. Materials and Methods

2.1. Study Area

The study was carried out in three artisanal fishing landing areas: on the southern coast of Paraiba in Acaú (07°30′51″ S; 34°49′11″ W) in the municipality of Pitimbu; on the northern coast of Pernambuco, in the localities of Barra de Catuama (07°40′31″ S; 34°49′48″ W); and in the municipality of Goiana, Jaguaribe (07°47′7″ S; 34°51′22″ W), in the municipality of Itamaracá (Figure 1). The distance from the capture and landing sites was approximately 10 km. Samples from landed individuals were collected monthly from April 2016 to August 2018.

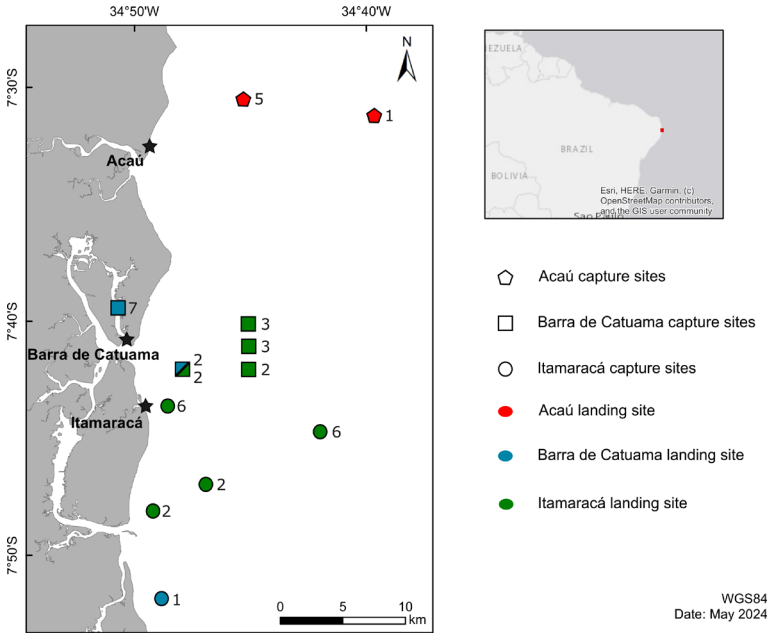

Figure 1. Map of the study area indicating the capture sites (pentagone: Acaú; square: Barra de Catuama; circle: Itamaracá) and landing sites (red: Acaú; blue: Barra de Catuama; green: Itamaracá) of *Aetobatus narinari* samples on the coast of Paraíba and Pernambuco, Brazil. Number of individuals

are indicated next to capture sites. For capture sites with several landing locations, the numbers of samples per landing sites are indicated vertically in alphabetical order. Map was generated with QGIS v 3.36.2 (https://www.qgis.org/ (accessed on 17 May 2024)) using polygons extracted from Google Earth (https://earth.google.com/web/ (accessed on 15 April 2024)).

2.2. Sampling

Tissue samples of 42 *Aetobatus narinari* [females: N = 24, 97–155 cm disc width (DW); males: N= 17, 49.6–141 cm DW; and one unidentified sex without DW information] were collected. Samples were taken at landing sites, and the catch locations were reported by fishermen. Individuals were caught in different fishing grounds throughout the study area, which were grouped into three larger areas and named according to their proximity to the landing sites (Figure 1). Since not all the individuals were caught and landed in the same area, we analyzed the data in two independent forms, considering both the capture sites and the landing sites (Table S1). For genetic analysis, a small piece of pelvic fin was collected from each specimen and stored in 95% ethanol in a freezer at −20 °C.

2.3. DNA Extraction, PCR, and Sequencing

Genomic DNA was extracted using the DNeasy Blood and Tissue Kit (Qiagen, Valencia, CA). Three partial mitochondrial genes were amplified and sequenced: cytochrome oxidase 1 (COI), cytochrome b (Cytb), and NADH dehydrogenase subunit 4 (ND4). PCR amplifications were carried out in 25 µL reactions containing 12.5 µL of GoTaq®Green Master Mix (Promega, Madison), 7.9 µL sterile water, 1.5 µL each primer (concentration: 10 µM), and 1.6 µL total gDNA (concentration: 10 ng/µL). COI fragment was amplified using the primers developed by Meyer (2003) [27]: dgLCO-1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′—forward) and dgHCO-2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′—reverse). Cycling conditions for COI were 2 min at 95 °C, followed by 5 cycles of 30 s at 95 °C, 30 s at 46 °C, and 45 s at 72 °C and 30 cycles of 30 s at 95 °C, 30 s at 51 °C, 30 s at 72 °C, and 5 min at 72 °C for final extension. Cytb fragment was amplified and sequenced using the primers AnarCBF1 (5′-GAGGGGCAACTGTCATCACTAACC-3′—forward) and AnarCBR1 (5′-CGATTGGGAAAAGGAGGAGGAA-3′—reverse) from Richards et al. (2009) [28]. Cycling conditions were the same as in Sellas et al. (2015) [12]: 3 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 53 °C, and 45 s at 72 °C. ND4 fragment was amplified using the primers ND4 (5′-CACCTATGACTACCAAAAGCTCATGTAGAAGC-3′—forward) [29] and H12293-LEU (5′-TTGCACCAAGAGTTTTTGGTTCCTAAGACC-3′—reverse) [30]. ND4 cycling conditions were as follows: 2 min at 95 °C, followed by 35 cycles of 15 s at 94 °C, 30 s at 55 °C, 1 min at 72 °C, and 5 min at 72 °C. PCR products were purified with ExoSAP-IT (Thermo Fisher Scientific, Pittsburgh, PA, USA) and sequenced in both directions on an ABI 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences were edited and aligned in BioEdit [31].

2.4. Analysis of Genetic Diversity and Structure

For each individual, the three mitochondrial fragments were concatenated and trimmed to the same length, creating a single sequence per individual of COI-Cytb-ND4 of 1976 base pairs (the length of each gene was 695 base pairs for COI; 639 for Cytb; and 864 for ND4). Haplotype diversity (h), nucleotide diversity (π), and divergences of concatenated COI-Cytb-ND4 sequences within and between sites were estimated using average pairwise distances (p-distances) calculated in Arlequin 3.5 [32]. To examine the relationships between sampling sites, a haplotype network for concatenated sequences was constructed using the TCS algorithm [33] in PopART (http://popart.otago.ac.nz (accessed on 13 February 2024)). Additionally, to examine genetic differentiation, the fixation index (Φ_{ST}) and analyses of molecular variance (AMOVA) were calculated using Arlequin 3.5 [32]. AMOVAs were performed based on population pair differences divided into two geographical groups (1Itamaracá and Barra de Catuama, corresponding to the north coast

of Pernambuco, and 2Acaú, corresponding to the south coast of Paraíba) to determine the amount of partitioned variation within and between geographical groupings of landing and capture sites. To test whether the populations evolved under neutrality, Tajima's D was calculated in DnaSP v. 6 [34].

3. Results

The COI-Cytb-ND4 concatenated sequences yielded 14 haplotypes. The average value for haplotype diversity was moderate (h = 0.646) and low for nucleotide diversity (π = 0.00087). When considering capture sites as populations, the highest haplotype diversity was observed in Itamaracá (h = 0.745), and the lowest nucleotide diversity was in Acaú (π = 0.00044) (Table S1). When considering landing sites as populations, the highest haplotype diversity and lowest nucleotide diversity were observed in Acaú (h = 0.733/π = 0.00044) (Table S1). As a general pattern, there was not a large difference in haplotype and nucleotide diversity when comparing capture and landings sites. For the samples in general, Tajima's D was significantly negative (TD = −1.99287, $p < 0.05$).

Fixation index values (Φ_{ST}) were not significantly different, considering either landing sites or sampling sites as populations. The values ranged from −0.007 (Acaú–Itamaracá) to 0.096 (Acaú–Barra de Catuama—this value seemed to show moderate differentiation but was not statistically significant) for landing sites and ranged from −0.010 (Barra de Catuama–Itamaracá) to 0.017 (Acaú–Itamaracá) for capture sites (Table 1).

Table 1. Population analyses for *Aetobatus narinari* samples. Pairwise fixation index values (Φ_{ST}) of concatenated COI-Cytb-ND4 sequences between sampling regions.

Landing Sites			
	Itamaracá	**Barra de Catuama**	**Acaú**
Itamaracá	0.00000		
Barra de Catuama	0.01049	0.00000	
Acaú	−0.00713	0.09579	0.00000
Capture Sites			
	Itamaracá	**Barra de Catuama**	**Acaú**
Itamaracá	0.00000		
Barra de Catuama	−0.01053	0.00000	
Acaú	0.01698	−0.00429	0.00000

Hierarchical AMOVAs attributed 99.31% of the overall variation within populations for landing sites and 99.90% within populations for capture sites (Table 2). There was no significant difference between the geographical groups for either landing or capture sites.

Table 2. AMOVA measures of population subdivision in *Aetobatus narinari* for populations from Itamaracá, Barra de Catuama, and Acaú in northeast Brazil.

Landing Sites				
Source of Variation	**d.f.**	**Sum of Squares**	**Variance Components**	**Percentage of Variation**
Among groups	1	0.885	−0.00802 Va	−0.93
Among populations within groups	1	1.054	0.01396 Vb	1.63
Within populations	39	33.251	0.85260 Vc	99.31
Total	41	35.190	0.85854	
Fixation Index	Φ_{ST}: 0.00692			
Capture Sites				
Source of Variation	**d.f.**	**Sum of squares**	**Variance components**	**Percentage of variation**
Among groups	1	0.885	0.00698 Va	0.81

Among populations within groups	1	0.750	−0.00613 Vb	−0.71
Within populations	39	33.556	0.86040 Vc	99.90
Total	41	35.190	0.85854	
Fixation Index	Φ_{ST}: 0.00099			

Only one haplotype (Hap_1) was found in all the sampled sites (either landing or sampling) according to the TCS haplotype network (Figure 2) and was shared by 25 individuals. Since some of the rays caught in Barra de Catuama were landed in Itamaracá, the diversity of haplotypes and the number of individuals collected on the north coast of Pernambuco varied. Consequently, Itamaracá represented 61.9% of the individuals and 71.4% of the haplotypes for the landing sites and 42.8% of the specimens and 57.1% of the haplotypes for the capture sites (Figure 2).

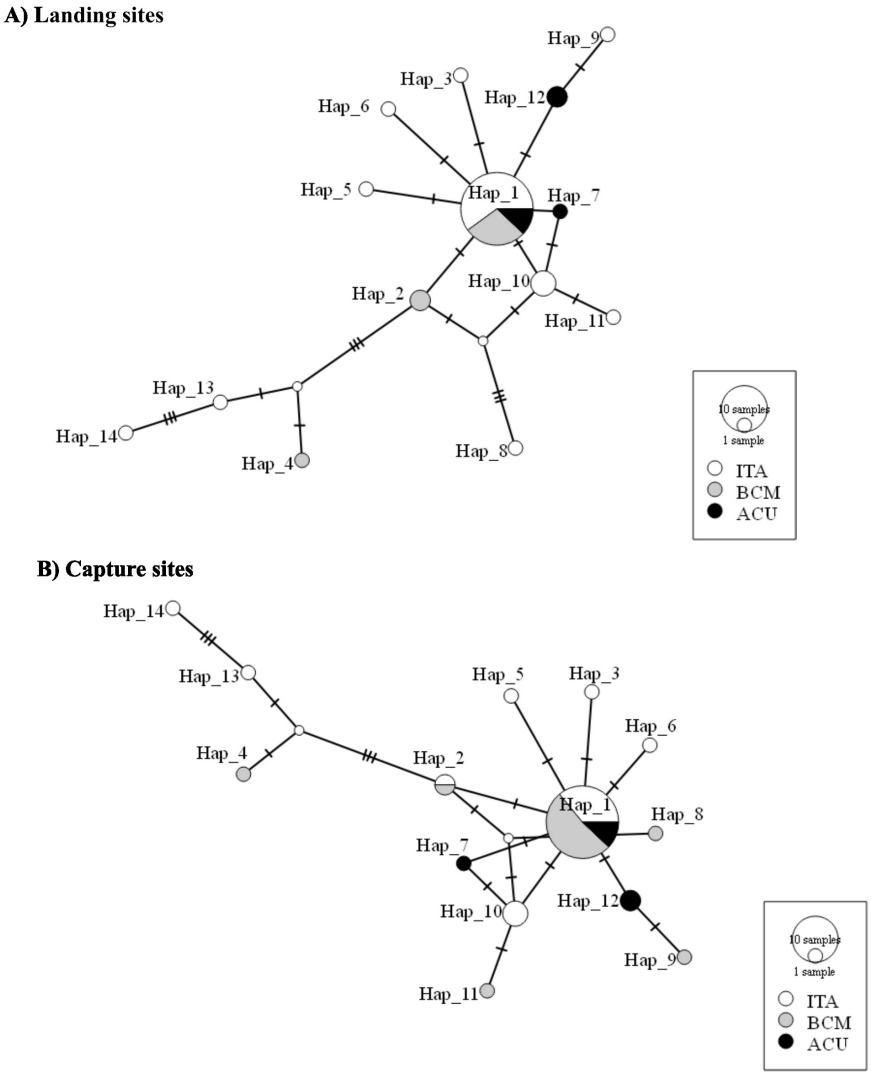

Figure 2. TCS haplotypes of *Aetobatus narinari* from northeast Brazil that were collected for this study. (**A**) Landing sites and (**B**) capture sites. Haplotype networks were constructed based on 1976 bp concatenated COI-Cytb-ND4 sequences. Each circle represents a different haplotype, with its size proportional to the number of individuals found with that haplotype; dashes on branches indicate base pair differences. The sampling sites are indicated by different colors referring to the region in which haplotypes were found. ITA—Itamaracá; BCM—Barra de Catuama; ACU—Acaú.

4. Discussion

We investigated the genetic structure and connectivity of *A. narinari* along part of the northeastern Brazilian coast using three fragments of the mitochondrial genome. Our results revealed that individuals sampled in this region originate from a single genetic stock, as neither capture nor landing sites could be differentiated genetically. Populations are not geographically structured throughout the study area. The absence of genetic differentiation, the moderate haplotype diversity, low nucleotide diversity, and significant negative Tajima's D values suggest that the haplotypes are closely related, and also that the population has a greater number of closely related haplotypes and may have expanded recently.

In this study, samples were analyzed according to landing sites and capture sites independently, since not all individuals were captured and landed in the same area. Indeed, accounting for the potential mixing of independent genetic stocks in fisheries landing is key for successful management measures and policies (e.g., [35]). Information on catch sites is therefore essential for studies of specimens caught by fishing activities whose samples are obtained in landing areas, as conducted here. Based on the information provided by fishermen, some rays caught in Barra de Catuama were landed in Itamaracá, underlining the importance of this information. Based on our results, however, there are no significant differences in mitochondrial haplotype frequencies between populations, either considering capture or landing sites, indicating that all individuals of this study likely originate from a single genetic stock. This is not unexpected, as high gene flow between populations, moderate to high levels of genetic diversity, and high estimates of effective population size of *A. narinari* have been documented in the northwestern Atlantic, Gulf of Mexico, and Caribbean Sea based on mitochondrial and microsatellite data [12,14]. The long-distance movement capacities of *A. narinari* (thousands of kilometers along continental coasts and the open ocean; [12,14,36]) and its migratory behavior [9,19,36] may explain part of this general lack of genetic structuring at the ocean basin scale, as geographic distances do not represent a strong barrier to gene flow [14]. However, low genetic differentiation patterns on a local scale have also been documented, notably between populations of Florida and Mexico [12], which may be linked to site fidelity of some individuals [9,36,37].

Our results are consistent with this general pattern of low to undetectable levels of genetic differentiation between populations, pointing towards fisheries management policies that could cover large geographic regions of the Brazilian Atlantic coast. However, wider geographic coverage would be beneficial to confirm our results. Additionally, this first study is based only on mitochondrial data and therefore provides a useful, but incomplete, picture of the genetic diversity of *A. narinari* populations in the southwestern Atlantic. Additional genetic studies based on nuclear data, using microsatellite loci [38] or a genotyping-by-sequencing approach, would refine our results and provide estimates of extant effective population sizes and their historical variations [39]. These analyses will be performed in a future paper.

Despite the genetic parameters observed here for *A. narinari* populations (i.e., no genetic structuring and high genetic diversity), anthropogenic disturbance, such as targeted and incidental fishing, can threaten the species' ability to persist at a resilient and stable genetic level [14]. Indeed, moderate and high levels of haplotype and nucleotide diversity have been found in other elasmobranch species undergoing fishing exploitation. Specimens of *Aetobatus ocellatus* (Vulnerable—VU) from the Indo-Pacific showed high haplotype diversity and nucleotide diversity statistically similar for Cytb ($h = 0.80$; $\pi = 0.0126$) and ND4 ($h = 0.81$; $\pi = 0.0085$) [40]. According to Cruz et al. (2021) [41], two species of Rhinobatidae on the southwest Atlantic coast showed considerable levels of haplotypic and nucleotide diversity in the mitochondrial control region, despite their conservation status, $h = 0.6518$, and $\pi = 0.0053$ for Brazilian Guitarfish *Pseudobatos horkelii* (critically endangered—CR); $h = 0.5185$ and $\pi = 0.0014$ for Chola Guitarfish *Pseudobatos percellens* (endangered—EN). Specimens of Caribbean Sharpnose Shark *Rhizoprionodon porosus*

(vulnerable—VU) from the Caribbean Sea and the Brazilian coast showed high haplotype diversity (h = 0.881), and their nucleotide diversity was π = 0.00278 [42], while on the northeast Pacific coast, Pacific Cownose Ray *Rhinoptera steindachneri* (near threatened—NE) showed low levels of genetic diversity (h = 0.077; π = 0.255%) for the mitochondrial NADH2 gene [43].

Since most genetic studies of *A. narinari* are based almost exclusively on areas where fishing for the species is prohibited, little is known about the genetic diversity of populations that occur in areas where the species is a fishing target and traded. According to Sellas et al. (2015) [12], in areas where *A. narinari* is fished, management units for the conservation of the species should be identified because unsustainable fishing practices can have significant effects on local populations. Thus, reliable management plans must consider avoiding fishing of pregnant females, newborns, and young, in addition to avoiding periods and areas of reproduction and parturition to mitigate the impacts of overexploitation on local populations. Therefore, in areas where *A. narinari* is caught by fishing, such as in northeastern Brazil, appropriate management measures must be taken to avoid depletion of this population.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d16070377/s1, Table S1: Specimen collection location, number of samples (N), sex, disc width (DW), number of haplotypes, haplotype diversity (h), and nucleotide diversity (π) of *Aetobatus narinari* in northeast Brazil.

Author Contributions: P.R.V.A. conceived and designed the study and carried out field work; P.R.V.A., B.D.P., and K.A.F. performed genetic analyses and interpretation of results; K.A.F. sequenced the PCR products at the Pritzker Laboratory for Molecular Systematics and Evolution (Fields Museum, Chicago); P.R.V.A., B.D.P., K.A.F., K.B.-H., R.L., and M.J.A. wrote, reviewed, and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The samples were collected under approval from the Chico Mendes Institute for Biodiversity Conservation (ICMBIO) authorization under Permanent License nº 49663-1.

Data Availability Statement: The 126 sequences (42 individuals, 3 loci) were submitted to NCBI on the 18 June 2024. The mitochondrial sequences produced for this study are available on GEnBank under the accession numbers BankIt2843312: PP951253 - PP951378.

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Conflicts of Interest: The authors declare no conflicts of interest.

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