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Genetic Population Structure and Diversity of the Whitetail Dogfish *Squalus albicaudus* (Chondrichthyes, Squaliformes) along the Brazilian Coast as Identified by SNP Markers

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Abstract: The shark *Squalus albicaudus*, categorized by the International Union for Conservation of Nature red list as Data Deficient due to lack of minimal information for classification, is distributed throughout the Brazilian coast. High pressures such as overfishing and anthropic activities, as well as certain biological characteristics, including k strategists, comprise influential shark stocks reduction agents. However, genetic diversity, population structure, connectivity, and effective population size data are still limited for *S. albicaudus*, indicating the need for further studies. In this context, the genetic variability and population structure of *S. albicaudus* were investigated herein to test for panmixia. Samples were obtained from coasts of the Brazilian states of Pernambuco, Rio de Janeiro, and São Paulo along the species distribution range, and single nucleotide polymorphisms (SNPs) were assessed by the ddRADseq method. The findings revealed a panmitic *S. albicaudus* population, explained by certain life strategies, such as polyandry and migratory behavior. Based on the genomic findings reported herein, a single *S. albicaudus* population should be considered in the study area, indicating the need for specific management and conservation plans at the regional scale.

Keywords: whitetail dogfish; sharks; genetic structure; ddRADseq; conservation

Key Contribution: This study is the first to assess *Squalus albicaudus* shark genetic diversity and population structure.

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1. Introduction

Marine biodiversity requires urgent support due to extensive human activities throughout different oceans, including, but not limited to, overfishing, environmental degradation, pollution, and climate changes consequences [1–3]. Elasmobranchs, a group that includes sharks and rays, are especially vulnerable to human activities, as they exhibit certain life history and biological characteristics, such as k strategies, leading to low reproductive, high longevity, and slow growth rates [4,5]. These particularities make this taxonomic group highly susceptible to environmental alterations and anthropogenic factors that determine physical habitat changes, as well as the introduction of new biological elements that can lead to competition dynamics changes between species and within population structures [2,6,7].

Most elasmobranchs have suffered worldwide population declines during the last decades, indicating the need for further studies and better protective actions [1,8–10]. In this regard, the extensive exploitation of marine species can lead to genetic diversity imbalances, requiring further fish stock and population assessments in order to establish suitable management and effective conservation actions [11–13].

On the Brazilian coast, there are approximately 163 species of elasmobranchs, being considered a biodiversity hotspot [7]; among these species, many are threatened and are endemic species, such as the daggernose shark *Isogomphodon oxyrhynchus* Müller and Henle 1839, listed as Critically Endangered by the International Union for Conservation of Nature (IUCN) [14], which exhibits a restricted distribution area, from the south of the state of Maranhão, in Brazil to Venezuela [15], and has suffered significant population collapses since the 1990s [16], which have not recovered even after three generations [14]. Another example comprises the guitarfish *Pseudobatos horkelii* (Müller & Henle, 1841). This species is found between Rio de Janeiro in Brazil and Mar del Plata in Argentina [17] and is also categorized as Critically Endangered [14], suffering severe population declines of over 80% due to overfishing [18]. Both species experience severe fishing pressures, mainly due to bycatch, and are marketed for their meat in different Brazilian regions [19–25].

A taxonomic revision of the *Squalus* genus for Southwest Atlantic components has led to the redescription of *Squalus cubensis* (Howell-Rivero, 1936) or *Squalus cubensis/megalops* (Gadig, 2001), which has recently been renamed *Squalus albicaudus*, Carvalho & Gomes 2016 [26]. *Squalus albicaudus* is a demersal shark that can inhabit depths between 50 and 400 m, geographically distributed throughout the Brazilian coast from Southern Bahia to São Paulo [14], although it has also been described in Pernambuco [27] and Rio Grande do Sul, Uruguay and Argentina [28]. It is categorized by the IUCN as Data Deficient [14], mostly due to the lack of biological and distribution information. The species is popularly known as the white-tailed dogfish and is a mesopredator, feeding on benthic invertebrates and small bony fishes [26].

Although not an important commercial fisheries component, *Squalus* species are commonly caught by industrial fishing as bycatch [29] and reported in artisanal fishery landing ports, and their meat is sold in local markets in Brazil [19,20,30]. However, mercury contamination has been recently reported for *S. albicaudus* in southeastern Brazil, indicating potential human health risks [31].

In the last decade, genetic methodologies have been applied as a powerful tool for conservation, with the genomics revolution positively affecting species identification and population structure investigations [32–35]. New molecular approaches in the next-generation sequencing (NGS) era have led to further knowledge of non-model species, thereby providing information on the genetic structure of wild stocks and populations [36]. One promising approach in this regard is the reduced representation sequencing (RRS) method, which allows for the sequencing of thousands of single nucleotide polymorphisms (SNPs) in population studies without the need for a reference genome [34]. Population structure and genetic diversity investigations, therefore, provide effective data on genetic diversity sustainability, useful in the development of management and conservation plans [13,37–39].

Multiple paternity, when a single brood of offspring is fertilized by multiple males [40], has been documented for *S. albicaudus* [41], *S. acanthias*, Linnaeus 1758 [42–44]), and *S. mitsukurii*, Jordan & Snyder 1903 [40]. This mating system can influence elasmobranch populations, leading to increased genetic variability and decreasing inbreeding [41,45]. In this context, this study aimed to assess the genomic population structure of *S. albicaudus* from samples obtained from three locations within the species distribution area along the northeast and southeast Brazilian coasts. Population structuring analyses were performed, employing SNP markers to assess genomic diversity and differentiation and infer gene flow patterns, thus testing the panmixia hypothesis. The data generated herein on *S. albicaudus* population structure and diversity along the Brazilian coast can contribute to further understanding this species distribution and behavior and prove useful in conservation programs and in the suitable management of the species.

2. Material and Methods

2.1. Sample Collection and DNA Extraction

Total DNA was extracted from 31 *S. albicaudus* samples obtained from individuals sampled from 3 different northeast and southeast Brazilian coast locations, namely Recife, in the state of Pernambuco (n = 14), Angra dos Reis, in the state of Rio de Janeiro (n = 4), and Santos, in the state of São Paulo (n = 13) (see Supplementary Figure S1). Fin and muscle samples preserved in 96% ethanol deposited at the Laboratory of Fish Biology and Genetics (LBP), UNESP, fish collection in Botucatu, SP, Brazil, were identified by the DNA barcode technique [46]. Due to the species demersal habits, captures are not frequent and most samples were obtained from local fishers and research collaborators. All sample collections were organized in accordance with the Brazilian government (SISBIO protocol 13843-1) and Animal Ethical Committee rules. The DNA was extracted from preserved tissue fragments using the Wizard® Genomic DNA Purification Kit (Promega, Madison, WI, USA) following the manufacturer's instructions.

2.2. ddRAD Library Preparation

Library construction was performed using a double digest restriction site-associated DNA (ddRAD) method following Peterson et al. [47], with modifications proposed by Campos et al. [48]. For a final volume of 34 µL of DNA (200 ng/ul), 1 µL of the restriction enzymes EcoRI (20 U/µL) and MspI (10 U/µL) (New England Biolabs (NEB) and 4 µL of TANGO buffer, in a total volume of 40 µL at this stage, were used to digest the genomic DNA of each sample. The reaction products were then purified with Agencourt AMPure XP beads following the manufacturer's protocol. A pair of customized adapters P1 (3 nM—EcoRI) and P2 (6 nM—MSPI) were used for each restriction enzyme, which were ligated in the 31.5 µL of the digestion product. The adapter ligation reactions were performed with 2.0 µL of the adapter for each of the enzymes, 4 µL of T4 Ligase Buffer 1X (Promega), and 0.5 µL of the T4 Ligase enzyme (Promega), with a final volume of 40 µL. Samples were incubated at 23 °C for 30 min, 65 °C for 10 min, and 63 °C for 90 s, followed by a temperature decrease of 2 °C every 90 s, up to 23 °C, followed by sample purification.

An indexing reaction was performed during the adapter ligation steps, with the insertion of the complement sequences of Nextera® Index Primers (Illumina, San Diego, CA, USA) S500 and N700 (Nextera DNA CD Indexes—96 indexes, 96 samples) in each sample, using the Nextera® DNA Sample Preparation Kit (Illumina) on the inserts attached to the adapters. The indexing reaction contained 15 µL of the ligation product, 5 µL of each S500 and N700 index, with each sample presenting a unique index combination, and 25 µL of Phusion High-Fidelity PCR Master Mix (Thermo Scientific), to a final volume of 50 µL. The reactions were performed in consecutive steps using a thermocycler and initiated with an incubation step at 72 °C for 3 min, followed by 2 denaturation steps at 95 °C for 30 s, 16 cycles at 95 °C for 30 s, an annealing reaction at 55 °C for 30 s, an extension step at 72 °C for 30 s, and a final extension step at 72 °C for 5 min, with the final product resting at 4 °C to infinity. Following a new purification step, samples were standardized to 10 ng/µL each, followed by pooling and purification. Fragments between 300 and 500 bp were selected using the Wizard® SV Gel and PCR Clean-Up System Kit (Promega, USA) by 1% agarose gel electrophoresis and purified. The obtained library was quantified by real-time PCR (qPCR) to determine adequate concentrations for sequencing single-end 150 pb reads on an NGS Illumina Nextseq550 platform.

2.3. SNP Analysis Filtering

The quality of the reads and adapter detection of the raw reads were accessed prior to the data analysis by the FastQC [49] and MultiQC [50] programs. Subsequently, low quality adapters and reads (quality score < 20) were removed using the Trimmomatic vo32 software [51]. In silico digestion was then performed and reads with enzyme digestion sites were removed. Finally, the filter-retained reads were trimmed to 140 bp using the Trimmomatic vo32 program [51]. After processing, downstream SNPs discovery was

conducted using the Stacks v2.0 pipeline package [52] with a de novo and reference map approach to assemble the marker catalog and the SNPs, considering that a reference genome is not available for *S. albicaudus*.

The SNP analyses were performed considering the m = 2 parameter, which controls the number of incompatibilities between two alleles of a given *loci*, the m = 3 parameter, which controls the number of minimum identical readings necessary to initiate a possible allele and the n = 1 parameter, related to the maximum mismatch between *loci* for catalog building. The reference parameter was established by the Bowtie2 software (v2.2.4), and the Stacks population module was used, applying three SNPs filters; the first to select SNPs that occur at least in 70% of individuals (r = 0.70) per population, the second to exclude SNPs in minor allele frequency (MAF) values < 0.05, and the third to eliminate the SNPs with a maximum observed heterozygosity value greater than 0.80.

2.4. Genetic Diversity and Population Structure

Private alleles were initially calculated using the Stacks population program. The ARLEQUIN v3.5.2.2 software [53] was used to calculate observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficients (FIS), and the probability of Hardy–Weinberg equilibrium (HWE *p*-value) deviations by the exact test. These parameters were calculated using the GENEPOP v4.1.0 program [54]. Global Bartlett tests were performed to assess Hardy–Weinberg Equilibrium (HWE) deviations in the datasets between observed and expected heterozygosity by the adegenet v package. 2.1.1 available in the R program [55].

Pairwise FST values were calculated using the Arlequin v.3.5.2.2 [53] for the population structure analyses of the investigated sampling site. A Bayesian analysis was performed using the STRUCTURE software [56] to test sample partitioning into genetic clusters. K-value estimations considered K = 1 to K = 5, with 1,000,000 MC and a burn-in of 10% with 20 independent runs per k, assuming the correlated admixture model and frequencies. Genetic cluster numbers (K) were determined by the Puechmaille method [57] based on the Structure Selector [58]. Results were then averaged and displayed using the main CLUMPAK software pipeline [59]. A Discriminant Analysis of Principal Components (DAPC) was conducted to identify genetic clusters using the adegenet R package [55].

Gene flow patterns and relative migration rate estimations based on the number of migrants (Nm) among locations were obtained with the divMigrate function employing the R program diveRsity package [60,61] with 1000 bootstraps.

3. Results

The ddRAD library reduction method application resulted in 41.401.332 raw reads of 150 bp single-end sequencings obtained by the Illumina NextSeq platform. A total of 29,612,986 reads were retained after the filtering process, resulting in 455 SNPs (see Supplementary Figure S2).

Genetic Diversity and Structure

The number of private alleles obtained for each sample locations revealed 30 alleles for Pernambuco, 2 for Rio de Janeiro, and 17 for São Paulo. Observed heterozygosity (Ho) values were higher than expected heterozygosity (He) values for all locations, (Ho = 0.386 and He = 0.266 for Pernambuco, Ho = 0.423 and He = 0.377 for Rio de Janeiro, and Ho = 0.230 and He = 0.212 for São Paulo). Statistically significant differences between observed and expected heterozygosity were noted for all locations (Bartlett's K-square = 158.81, df = 1; P = 2.2×10^{16}), where the overall Ho = 0.348 was statistically different from the overall He = 0.288, suggesting that the investigated population moves away from the Hardy–Weinberg Equilibrium (HWE). Inbreeding coefficient (FIS) values were negative for all sampled locations −0.233 for Pernambuco, −0.245 for Rio de Janeiro, and −0.227 for São Paulo), indicating absence of inbreeding.

Genetic differentiation was not identified by the pairwise FST analysis between locations (0.028 (*p*-value 0.015 ± 0.033) between Pernambuco and Rio de Janeiro, 0.015 (*p*-value 0.009 ± 0.009) between Pernambuco and São Paulo, and 0.036 (*p*-value 0.441 ± 0.043) between Rio de Janeiro and São Paulo), indicating lack of population structure in the sampled locations. The cluster number characterization carried out by the Puchmaille method revealed that the highest probability of clusters was K = 2 (see Supplementary Figure S3) and that the three analyzed populations did not display any distinct population genetic structure based on the datasets (see Supplementary Figure S4). The DAPC, also performed to estimate the appropriate genetic cluster number, was determined by the Bayesian Information Criterion (BIC), where K was equal to 1 (see Supplementary Figure S5). All localities are close to the graph axis, indicating genetic closeness (Figure 1).

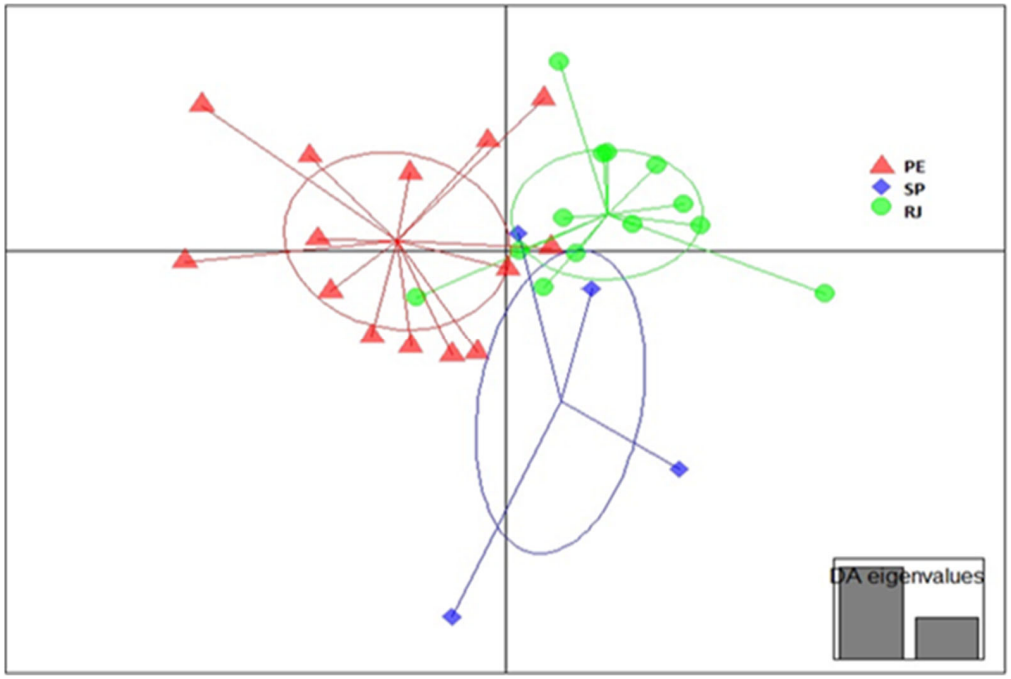

Figure 1. Discriminative Analysis of Principal Components graph for the three *Squalus albicaudus* groups indicated according to legend symbols and colors. Sampling sites: PE—Pernambuco (Recife); SP—São Paulo (Santos); and RJ—Rio de Janeiro (Rio de Janeiro). The DA eigenvalues representation is highlighted in the box.

The migration rates based on number of migrants (Nm) estimates for the sampling sites revealed a gene flow between the three localities, ranging from 0.33 to 1.0. The gene flow value between Pernambuco and Rio de Janeiro was 0.33, and the reverse, 0.77. The gene flow between Rio de Janeiro and São Paulo was 0.44 and between São Paulo and Rio de Janeiro, 0.30. Values as high as 0.83 were observed between Pernambuco and São Paulo, reaching 1.0 from São Paulo to Pernambuco (Figure 2). In general, the migration network indicates the main gene flow process directed to Pernambuco and genetic connectivity among the other regions.

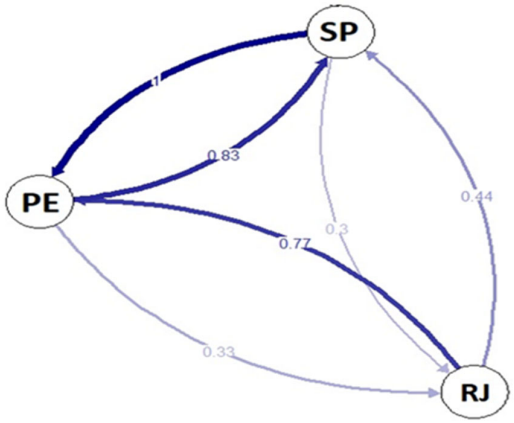

Figure 2. Relative migration network between the *Squalus albicaudus* sampling sites along the Brazilian coast analyzed herein. Analyses were processed using the divMigrate program based on the effective number of migrants (Nm method). Lines indicate migration directions and intensity values and arrows indicate gene flow directions. Numbers indicate migration intensity. Sampling sites: PE—Pernambuco (Recife); RJ—Rio de Janeiro (Rio de Janeiro); SP—São Paulo (Santos).

4. Discussion

The genetic diversity and structure for the *S. albicaudus* samples obtained along the Brazilian coast were investigated using 455 SNPs markers. The findings indicate panmixia for this species in the study area, covering coastal locations in the states of Pernambuco, Rio de Janeiro, and São Paulo. A high connectivity pattern was also identified between the investigated samples through gene flow, reinforcing a possible mix of individuals within the stock and explaining the low differentiation detected herein. These data may contribute to further understanding *S. albicaudus* distribution and population structure patterns, which are still unknown, leading to a Data Deficient IUCN categorization [14].

A moderate genetic diversity and heterozygosity excess for *S. albicaudus* was noted, similarly to *Carcharhinus* sharks identified by SNP markers for two species developed by Junge et al. [62], revealing Ho values ranging between 0.21 and 0.37 and He values from 0.23 to 0.29 for *Carcharhinus brachyurus* (Günther 1870) and *Carcharhinus obscurus* (Lesueur 1818), with both species commercially targeted in many parts of their distribution area.

In addition, the STRUCTURE analysis revealed k = 2, that is, the individuals corresponding to the analyzed samples are part of two groups, but this can be explained according to Janes et al. [63], as there is a tendency of the Structure k = 2, when using the program only by default and not following the necessary recommendations, and, in addition, ΔK does not allow an evaluation of k = 1, so it may be that k = 2 is reported as a default. The authors Pritchard and Wen [64] and Evanno et al. [65] recommend using more than one method for selecting the best k'.

The findings reported herein indicate *S. albicaudus* is still resilient at the genetic level. However, environmental changes and species exploitation may result in genetic alterations, such as a decreased genetic diversity and population structure changes, as noted for other marine species [66].

Bathymetric habit variations between sexes have been described for the two *Squalus* genus species, *S. acanthias* and *S. megalops*, in which males and females are distributed at different depths, where males are often captured in deeper waters than mature females, leading to susceptibility to further population declines due to fishing [67–69]. In this context, best practices should be applied to *S. albicaudus* stock management, mainly considering all locations investigated herein as a single conservation unit. Knowledge concerning genetic stocks is paramount for fishery management actions [70], aiding in understanding if populations can evolve even when suffering environmental changes, overfishing, and loss of habitat, which usually lead to genetic diversity losses and affect long-term population survival [71].

The *S. albicaudus* samples from Pernambuco may be considered as part of a geographic distribution expansion for the species, confirming previous reports [27] by a deep-water scientific survey carried out as part of the "Brazilian Program for the Assessment of Living Resources in the Exclusive Economic Zone" (REVIZEE), wherein a probable *Squalus* cf. *albicaudus* specimen was captured on the Northeast Continental Slope above the coast of Bahia. Another study using the distribution modeling method identified *S. albicaudus* in Rio Grande do Sul (Brazil), Uruguay, and Argentina [28]. The available references on the occurrence and distribution of this species in the Southern Atlantic Ocean thus indicate and emphasize the need for robust analyses and species descriptions.

Shark life strategies can increase connectivity between populations within distribution ranges. Some shark species carry out extensive oceanic movements and generally exhibit low genetic structures within oceans or only at small scales between regions, such as certain pelagic sharks, i.e., spiny dog sharks, *Squalus acanthias,* and the tope sharks, *Galeorhinus galeus*, as well as *S. albicaudus*, which prefer temperate and cold water [43,72,73]

The high gene flow involving all analyzed locations may be due to the polyandrous life reproduction strategy of *S. albicaudus* [41], defined as the participation of multiple males fertilizing a single female and generating mixed litters [74]. The hypothetical benefits of this behavior include maintaining or increasing genetic diversity of certain populations [75], which can determine and increase the number of viable offspring in a single litter [76]. This may, in turn, interfere with the probability and risk of fertilization by genetically incompatible sperm and decrease the chance of inbreeding depression [77]. In addition, the *S. albicaudus* distribution range is associated with cold waters that rise from the south to the northern regions of the Brazilian coast, with an optimum depth of around 100 m. However, although both features can aid gene flow and maintain genetic diversity over time, stochastic events or overfishing could cause population declines on a smaller time scale [78].

Due to restricted distributions, many species are directly or indirectly threatened by human activities, including climate change [79,80], fishing, pollution, and habitat destruction [81,82]. These stressors threaten marine biodiversity sustainability and existence [83,84] and the suite of benefits these ecosystems provide [85,86].

5. Conclusions

The results reported herein provide the first genetic evaluation concerning the real population distribution status and structure for *S. albicaudus* sampled from different Brazilian coast areas. A panmitic population was characterized, covering different distribution areas, confirmed by gene flow between regions. The findings indicate that the sampled individuals can be considered a single genetic stock. In this context, when combining elasmobranchs as K strategists, this indicates limited long-term evolutionary potential. This, therefore, indicates the need for effective conservation efforts, such as effective fishing legislations, mainly due to lack of accurate information about natural *S. albicaudus* stocks and populations.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/fishes8070373/s1, Figure S1: Map of the Brazilian coast indicating the collection sites of the shark *S. albicaudus* samples analyzed; Figure S2: Analysis after sequencing of the *Squalus albicaudus* and the steps performed to obtain the SNP markers used for analysis; Figure S3: Puchmaille method estimation, demonstrating the highest probability of clusters of k = 2; Figure S4: Result of STRUCTURE visualized by the CLUMPACK program applied to samples of the shark *Squalus albicaudus*; Figure S5: Number of the best cluster (k = 1) of the shark species *Squalus albicaudus*.

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Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in the present study will be deposited and made available openly through the GenBank genetic sequence database.

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