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Investigating an Unknown Biodiversity: Evidence of Distinct Lineages of the Endemic Chola Guitarfish *Pseudobatos percellens* Walbaum, 1792 in the Western Atlantic Ocean

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Abstract: Anthropogenic actions have affected marine species for a long time, through overexploitation of natural stocks and habitat degradation, influencing the life strategies of several taxa, especially rays and sharks, which have suffered significant population declines in recent years. Therefore, conservation actions and stock management have become paramount. In this regard, chola guitarfish, *Pseudobatos percellens*, distributed throughout the Brazilian coast, is often commercially fished by local artisanal fleets or as by-catch in shrimp trawl fisheries. Therefore, this study aimed to understand the genetic diversity of *P. percellens* throughout the Brazilian coast, using single nucleotide polymorphisms (SNPs). Genetic analyses employing 3329 SNPs revealed a hidden biodiversity within *P. percellens*, with at least one lineage occurring in the Northern and Northeastern regions and another distributed in the Southeastern/Southern Brazilian coast, with high genetic differentiation between them. However, the Discriminant Analysis of Principal Components (DAPC) indicated the presence of in fact three lineages distributed in these regions that must still be better investigated. Therefore, to ensure adequate conservation of chola guitarfish biodiversity, populations must be managed separately along the Brazilian coast. Furthermore, the need for a taxonomic review for this group is noted.

Keywords: genomic; SNPs; threatened species; conservation; elasmobranch

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1. Introduction

Dramatic and persistent population declines have been documented for many fish species worldwide in recent decades [1] due to rapid environmental changes caused by anthropogenic impacts, such as habitat loss and pollution, in addition to overfishing [1–6], which has resulted in an unprecedented crisis. According to Díaz et al. [7], about one million species are estimated to be threatened with extinction due to the acceleration of this global biodiversity crisis [7–11], with an increasing number of threatened species requiring management. Because of this, methods to assess these species and prioritize conservation actions are now paramount [6,12,13].

Management objectives often focus on distinct populations or independent lineages, aspects that are difficult to determine in some species. In this regard, population genetic methods provide a means of delineating geographic distribution patterns and molecular variations, inferring the processes that generate and maintain these standards and allowing for hypothesis postulations [14–18].

Population genetics plays an important role in the management and conservation of endangered species [19,20], revealing population peculiarities, such as inbreeding and loss of genetic diversity, both of which compromise the viability of wild populations [21]. Maintaining genetic diversity is one of the foundations of conservation genetics, mainly because genetic diversity maintenance provides species plasticity in the face of a series of environmental variables and supports their evolutionary potential [22–24].

Sharks and rays (Elasmobranchii) are one of the most vulnerable groups concerning anthropogenic effects, mainly due to their K-strategist life history characteristics, such as low fecundity rates, slow growth, longevity, large size at hatching, and late age at maturity [25–27]. These animals play an important role in controlling and regulating the populations of other species, in addition to influencing the structure and function of coastal and oceanic ecosystems and comprising good marine ecosystem health indicators [28–31].

Currently, about 1485 elasmobranchs species have been identified worldwide (665 sharks and 820 rays or stingrays) [32]. Despite the greater number of rays species, most studies focus on sharks rather than on rays [33–35], especially abundant species and/or those affected by industrial fisheries, such as the Tiger Shark [36–40] and Blue Shark [41–44].

Guitarfish belonging to the Rhinobatidae family that is comprised of three genera, *Acroteriobatus*, *Pseudobatos*, and *Rhinobatos* [45,46]. These genera can be identified based on their external nasal flap morphology and molecular data [45], with *Pseudobatos* presenting a more restricted distribution compared to the others, occurring only in amphi-American regions.

The chola guitarfish, *Pseudobatos percellens* Walbaum, 1792, is distributed along the continental shelf of the western Atlantic Ocean, from Panama to Brazil [46–49]. Marine environments presenting different biological characteristics due to oceanographic, glacial-eustatic, biogeographic, and ecological factors are noted along its almost 9000 km distribution stretch [50–52]. Among the different types of barriers, the Amazon River discharge plume [53–55] and water temperature [56–59] are the most noteworthy, as they both alter the physical and chemical components of the North Brazil Shelf Region and can act as barriers for sister species, leading to speciation in different marine species [60–63].

Among the few available studies on *P. percellens*, Yokota and Lessa's study [64] on shark and ray nursery regions in northeastern Brazil is highlighted, indicating separate ecological niches for sharks and spatial overlap with respect to the assessed species. In another study, Caltabellotta et al. [65] conducted an age and growth study, evidencing that parameters were markedly different between *P. percellens* males and females. Recently, Cruz et al. [66] identified that the *P. percellens* population in southern Brazil (São Paulo, Paraná, and Santa Catarina) displays low genetic diversity among specimens by employing a mitochondrial marker (Dloop).

Long-term population viability is associated with genetic variability levels [67], that is, if a certain population cannot evolve in response to environmental variable changes, it may face a greater risk of extinction. In addition, anthropic actions and coastal pollution are responsible for degrading habitats in certain distribution areas [68]. This has contributed to the current "Endangered" (EN) assessment of *P. percellens* as established by the International Union for Conservation of Nature (IUCN) Red List of Threatened Species [69] and "Vulnerable" (VU) according to the Brazilian National List of Threatened Species [70].

Despite its threatened status and presenting metal concentrations in their meat many times above safe limits for human consumption [71], *P. percellens* is fished commercially in Brazil by artisanal fleets or as non-targeted catch in the shrimp-trawling fishery [72–74], and its meat is marketed in several areas of the Brazilian coast [75–80].

In this context, this study aimed to understand the genetic diversity and population structure of *P. percellens* along the Brazilian coast using single nucleotide polymorphisms (SNPs) by double-digest restriction site associated DNA (ddRAD) sequencing as genetic markers. We first questioned what are the genetic variation levels among specimens from the northern, northeastern, and southeastern/southern coast of Brazil? Second, how is genetic variation structured across populations on a local and regional scale? Third, do northern, northeastern and southeastern/southern populations still maintain gene flow?

Finally, can biogeographic Brazilian coast barriers influence the distribution of this species? Genetic diversity and biological factors which may influence *P. percellens* distribution are discussed, testing the hypothesis of the existence of a single lineage throughout the species distribution range along the Brazilian coast.

2. Material and Methods

2.1. Sample Collection and DNA Barcoding

Total DNA from 52 *Pseudobatos percellens* samples were obtained from five locations in northern and northeastern Brazil, namely in Amapa (AP, N = 03), Pará (PA, N = 05), and Pernambuco (PE, N = 14), and from the southeastern/southern regions, in São Paulo (SP, N = 15) and Paraná (PR, N = 15) (Supplementary Table S1). Sampling was permitted by capture permit no. 13843-1 issued by SISBIO/ICMBio, Brazil. Tissues of all individuals were deposited at the Laboratory of Fish Biology and Genetics fish collection, UNESP in Botucatu, São Paulo, Brazil. The DNA samples were extracted from muscle tissue fragments preserved in 95% ethanol using the Wizard ® Genomic DNA Purification kit (Promega, Madison, WI, USA), following the manufacturer's instructions.

The COI barcode region was amplified according to Hebert et al. [81] for DNA barcode analyses of each sample. PCR amplicons were visualized on a 1% agarose gel E-Gels (Invitrogen) and bi-directionally sequenced using the BigDye Terminator v.3.1 Cycle Sequencing Kit (Applied Biosystems, Inc.) on an ABI 3130 capillary sequencer, following the manufacturer's instructions. Sequences were aligned in Geneious 4.8.5 [82]. Aligned consensus sequences were compared with those deposited in the National Center for Biotechnology Information (NCBI) database (http://www.ncbi.nlm.nih.gov/, accessed on 27 August 2022) using the Basic Local Alignment Search Tool—Nucleotide (BLASTn).

Genetic distances were obtained by the Kimura-2-Parameter Model (K2P) method [83]. The final matrix contained 126 sequences, comprising 40 obtained in the present study and 86 extracted from GenBank (ncbi.nlm.nih.gov/genbank, accessed on 3 January 2021). A maximum likelihood phylogenetic reconstruction was applied to construct a tree from the pairwise distances, estimated using the Tamura and Nei [84] substitution model, as implemented in MEGA X [85]. The phylogenetic tree was tested by the bootstrap method with 1000 pseudoreplicates [86] and all analyzed sequences were submitted to GenBank (Accession Nos. OQ255948–OQ255987).

2.2. ddRAD Library Preparation

The SNP dataset was generated using a double digest restriction site associated DNA sequencing (ddRADseq) method following Peterson et al. [87], with modifications as reported by Campos et al. [88]. Briefly, we used 34 µL of a 200 ng/µL DNA solution and 1 µL of each of the restriction enzymes EcoRI (20 U/µL) and MspI (10 U/µL) (New England Biolabs, NEB) and 4 µL of the TANGO buffer, in a total volume of 40 µL at this stage, for sample genomic DNA digestion. A pair of customized adapters for each restriction enzyme was then applied, and the adapter P1 (3 nM—EcoRI) and adapter P2 (6 nM—MSPI) were bound to 31.5 µL of the digestion product. The ligation reaction of the adapters was developed by using 2 µL of the adapter for each of the enzymes, 4 µL of T4 Ligase Buffer 1X (Promega), and 0.5 µL of the enzyme T4 Ligase (Promega), with a final volume of 40 µL. The samples were finally incubated at 23 °C for 30 min, 65 °C for 10 min, and 63°C for 90 s, and then the temperature was reduced by 2 °C every 90 s until 23 °C. The ligation reaction samples were then purified.

The indexing reaction was performed with the insertion of the complement sequence of Nextera® Index Primers (Illumina, San Diego, CA, USA) S500 and N700 (Nextera DNA CD Indexes—96 indices, 96 samples) in each sample, to perform indexing with the Nextera® DNA Sample Preparation Kit (Illumina). The indexing reaction contained 15 µL of the ligation product and 5 µL of the S500 and N700 indices, and each sample contained a unique index combination with 25 µL of Phusion High-Fidelity PCR Master Mix (Thermo Scientific) for a final volume of 50 µL. The indexing reaction was followed by consecutive

thermocycler steps, with an initial step at 72 °C for 3 min followed by two denaturation steps at 95 °C for 30 s, followed by 16 cycles at 95 °C for 30 s, an annealing step 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, remaining at 4 °C to infinity.

Final sample concentration was standardized to a final volume of 10 ng/µL per sample and were then pooled and purified. After pool quantification, size selection was performed to select the size of the fragments of interest using the Wizard® SV Gel and PCR Clean-Up System kit (Promega, USA) in 1% agarose gels. The pool was applied to the gel, with the selection of the fragment of interest between 300 and 500 base pairs (bp), and the fragment was then purified. The library was quantified by real-time PCR (qPCR) to determine concentrations and sequenced with single-end reads of 150 bp in a NGS Illumina Nextseq500 platform.

After sequencing, the programs FastQC [89] and MultiQC [90] were used to assess the quality of the readings and adapter detection of the raw reads. Low quality base adapters and reads (quality score < 20) were removed using the TRIMMOMATIC v.o.32 Program [91]. In addition, in silico digestion was performed according to Driller et al. [92] and all reads were trimmed to 140 bp again using the TRIMMOMATIC v.o.32. The samples were then processed using the de novo pipeline from STACKS v2.55 [93] employing parameter values m = 3, M = 2, and n = 1. At the end, the STACKS population pipeline was used with the application of three SNP filters, the first to select SNPs that occurred in at least 80% of analyzed individuals (r = 0.80) per population, the second to exclude SNPs that presented a minor allele frequency (MAF) value <0.05, and the third, to exclude very high levels of heterozygosity, where SNPs with maximum observed heterozygosity greater than 0.65 were filtered. The plink v1.9 software [94] was used to filter *loci* that significantly ($p < 0.05$) deviated from the Hardy–Weinberg Equilibrium and were in linkage disequilibrium ($R^2 > 0.4$).

2.3. Population Genomics Analysis

Private alleles were calculated using the Stacks population program. The ARLEQUIN v3.5.2.2 software [95] was used to estimate the observed heterozygosity (Ho), expected heterozygosity (He), inbreeding coefficient (F_{IS}), and probability of deviation from the Hardy–Weinberg Equilibrium (HWE p-value), calculated by using an exact test. Deviations from the Hardy–Weinberg Equilibrium (HWE) in the datasets were assessed with the Global Bartlett tests, performed between observed and expected heterozygosity available in the ADEGENET v package. 2.1.1 of the R Program [96].

Genetic differences were analyzed using pairwise F_{ST} values calculated in the Arlequin v.3.5.2.2 software [95]. The number of existing clusters among *P. percellens* samples was investigated applying a Bayesian Analysis using the STRUCTURE Software [97], and the k-value was estimated considering k = 1 to k = 7, with 500,000 MCMC, burn-in of 10%, 10 independent runs *per* K and was performed using an admixture model, with the assumption of correlated frequencies. Isolation by distance (IBD) by a Mantel test, was tested by computing the regression of *F*ST/1-*F*ST on geographic distances and the level of significance determined by performing a test with ISOLDE in GENEPOP 4.2 [98] based on 1000 randomization.

Identification of the number of genetic clusters (k) was performed by the Puechmaille Method [99], using the Structure Selector [100]. Based on the number of detected structure clusters, the *F*st pairwise was analyzed and a Discriminant Analysis of Principal Components (DAPC) was conducted using the Adegenet v.2.2.1 R package [96]. Clusters were generated using the *find.clusters* function, which uses the k-means algorithm to generate clusters for various k values, and the Bayesian Information Criterion (BIC) was then considered to determine the most likely k value.

For a better understanding of the *P. percellens* group results, dendrograms were generated applying two different approaches, the Maximum Likelihood (ML) and the Bayesian Inference (BI). The ML approach was performed using the RAxML 8.2.8 software [101]

applying the rapid bootstrapping option with 1000 replicates. Since the general time reversible model and its variants are the only available options in RAxML, we replaced the best-fit models observed in the jModelTest by the GTR+Γ. In the same way, the BI analyses were performed in MrBayes 3.2 [102] using four simultaneous runs, each with four Markov chains (T = 0.2). The analyses began with randomly chosen trees, being extended through 50 million generations, with sampling every 5000 generations and using a burn-in fraction of 25 % of the trees. An explorative network analysis using the NeighborNet Algorithm implemented in Splitstree v.4.14 [103] was also performed.

3. Results

The forty specimens used in this study were molecularly identified by DNA barcoding to confirm their previous morphological identification. The obtained barcode sequences ranged in length from 510 to 644 bases pairs, revealing high similarity (>99%) with the *P. percellens* sequences deposited in Genbank. The interspecific genetic distances values ranged from 1.3% between *P. percellens* and *Pseudobatos lentiginosus*, to 21.4% in *Pseudobatos horkelii* and *Rhinobatus rhinobatus* (Table S2).

The Maximum Likelihood (ML) tree based on the DNA barcoding identified two main groups, one for *Pseudobatos* and the other for *Rhinobatos*. A first clade with *P. percellens* from the Brazilian coast was observed in the *Pseudobatos* group for the specimens used in this study, followed by a second clade containing *P. lentiginosus* individuals from Mexico, and finally, a third clade consisting of *P. horkelii* from Brazil (Figure S1).

After confirming specimen identification, a ddRAD library was developed for the 52 *P. percellens* samples, resulting in 86,319,471 raw data reads and ranging from 207,416 to 3,044,397 reads per sample (Table S1). After quality filtering, 70,757,837 reads were maintained, ranging from 175,933 to 2,578,145. All reads were standardized at 140 bp, and a total of 3329 high-quality SNPs were used for subsequent analyses after quality filtering, with only 36% of data missing. These SNPs were selected by *loci* genotyped in >75 % of the individuals and showing an MAF > 0.05, among which 1779 transitions and 1550 transversions were detected (Figure S2).

Observed heterozygosity (Ho) ranged from 0.281 (SP) to 0.577 (AP) among the five analyzed populations (Table 1), while expected heterozygosity (He) ranged from 0.239 (SP) to 0.5487 (AP). The differences between observed and expected heterozygosity variations in all sampling locations were statistically significant (Bartlett's K-square = 573.47, df = 1, p-value < 2.2×10^{-16}), where Ho (General Ho = 0.260) was statistically different from He (general He = 0.348), suggesting that the global population deviated from the HWE.

Table 1. Genetic diversity statistics for *Pseudobatos percellens* using 3.329 SNPs. ID: sampling localities, N: number of individuals per location, Ap: number of private alleles, Np: number of polymorphic loci, Ho: observed heterozygosity, He: expected heterozygosity, F_{IS}: inbreeding coefficient, (π): nucleotide diversity.

Localities	ID	N	Ap	Np	Ho	He	F_{IS}	(π)
Amapá	AP	03	83	216	0.577	0.487	−0.542	0.115 ± 0.067
Pará	PA	05	191	448	0.471	0.415	−0.216	0.108 ± 0.057
Pernambuco	PE	14	389	331	0.315	0.298	−0.088	0.102 ± 0.050
São Paulo	SP	15	140	115	0.281	0.239	−0.213	0.031 ± 0.015
Paraná	PR	15	192	158	0.333	0.273	−0.233	0.038 ± 0.019

The average F_{IS} values were negative in all the populations, ranging from −0.542 (AP) to −0.088 (PE) (Table 1). The average nucleotide diversity (π) value across all populations ranged from 0.031 (SP) to 0.115 (AP) (Table 1).

High levels of population genetic differentiation among geographical *P. percellens* sample regions were also detected. The highest mean F_{ST} values were found between Amapá and São Paulo (F_{ST} = 0.764), and the lowest between São Paulo and Paraná (F_{ST} = 0.043),

indicating that *P. percellens* populations in the Brazilian coast are genetically different, with two extant lineages (Figure S3a, Table S3). The Mantel test demonstrated a correlation between geographic distance and fixation index values (R^2 = 0.5904) with P = 0.001.

The STRUCTURE software analyses including all 52 samples best supported a value of k = 2 genetic clusters, based on the Evanno Method ((ln (P) D) 3886 ± 43,141) (Figure 1), which correspond to Amapá, Pará, and Pernambuco for Cluster 1 and São Paulo and Paraná for Cluster 2. The performed DAPC involving the analyzed geographical regions indicated a clear separation between Amapá, Pará, and Pernambuco, whereas a clear overlap between São Paulo and Paraná was observed (Figure 2).

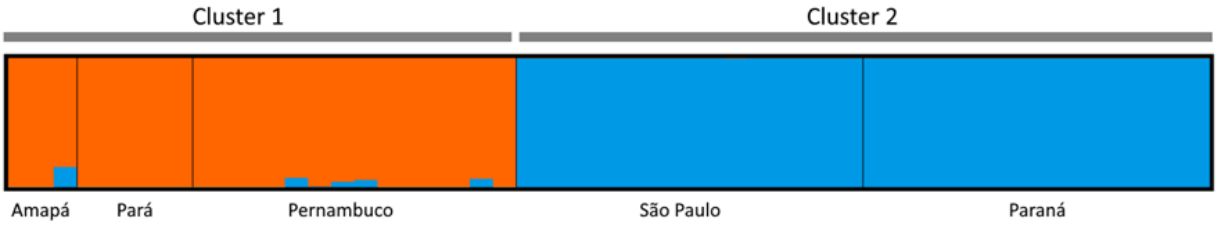

Figure 1. Bayesian Analysis of Genetic Similarity among geographical *Pseudobatos percellens* populations performed on STRUCTURE results (k = 2) for 52 individuals from 3329 SNPs.

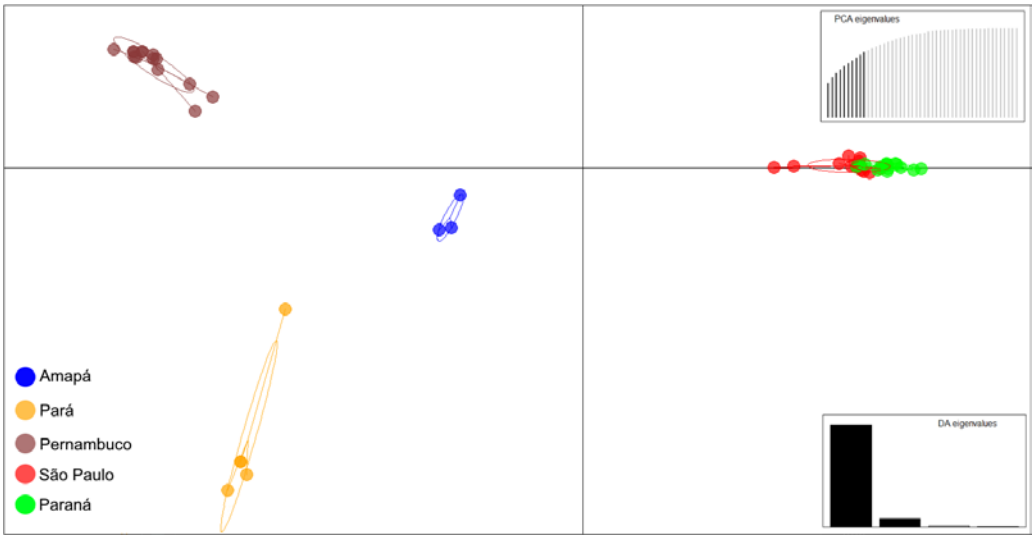

Figure 2. Scatterplots of the Discriminant Analysis of Principal Components (DAPC) om 3.329 SNPs from *Pseudobatos percellens*. Dots represent individuals and colored ellipses correspond to geographical populations. The Principal Component Analysis (PCA) and DA (Significance tests) eigenvalue representatives are highlighted in the inset.

The analyzed DAPC identified an optimum of k = 3 genetically distinct clusters (Figure 3a). All individuals displayed a 100 % membership probability to the group to which they were assigned. All clusters contained between 7 and 30 individuals, and some clusters spanned two geographical populations, with Cluster 1 including samples from Amapá and Pará, Cluster 2 with all samples from Pernambuco and one sample of Amapá, and Cluster 3 exclusively composed of all samples from São Paulo and Paraná, thus revealing a high genetic relatedness among those accessions (Figure 3b). The clustering was supported by results of the pairwise F_{ST} Analysis between pairs of clusters (Figure S3b). The highest F_{ST} values were detected between Cluster 1 and Cluster 3 (F_{ST} = 0.694) and between Cluster 2 and Cluster 3 (F_{ST} = 0.601), indicating a high genetic differentiation among clusters of accessions. The lowest F_{ST} was obtained between Clusters 1 and 2 (F_{ST} = 0.106).

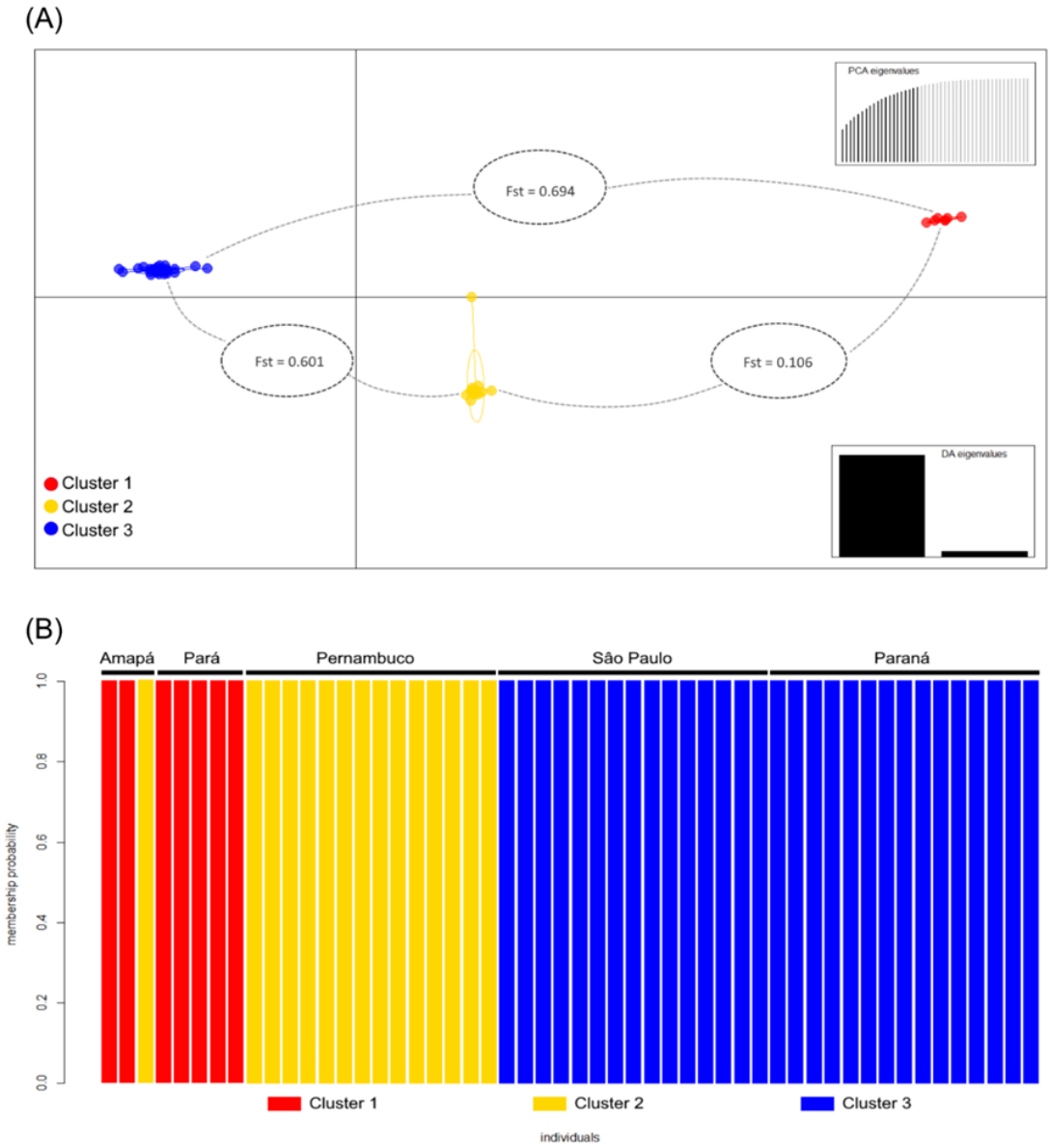

Figure 3. Inference of the number of clusters obtained in the Discriminant Analysis of Principal Components (DAPC) performed on the 3.329 SNPs dataset from *Pseudobatos percellens*. (**A**) DAPC scatterplot distinguishing between the three genetic clusters and F_{ST} pairwise values detected between them. The Principal Component Analysis (PCA) and DA (Significance tests) eigenvalue representatives are highlighted in the inset. (**B**) Subdivision of the individuals based on the DAPC membership probabilities into three genetic clusters (k = 3). The same color for individuals indicates that they belong to the same cluster.

The divMigrate Analysis unveiled a high gene flow in the southeastern region, between São Paulo and Paraná, and a weak gene flow in the northern and northeastern regions, between Amapá, Pará, and Pernambuco (Figure S4). In addition, a very limited and unexpected gene flow (<0.08) was found between the southeast and northern and northeastern regions.

For a more accurate investigation of the existence of two possible *P. percellens* lineages, the selection of SNPs that genotyped 100% of all individuals of the two lineages was performed, thus maintaining 1.448 SNPs. Both Bayesian Inference (BI) and Maximum Likelihood (ML) analyses supported the division of *P. percellens* into two groups representing the northern and northeastern (Amapá, Pará, and Pernambuco) and southeastern/southern

regions (São Paulo and Paraná) (Figure 4A,B). The group I lineage included populations from Amapá, Pará, and Pernambuco, totaling 22 samples, while the group II lineage, with 30 samples, included populations from São Paulo and Paraná.

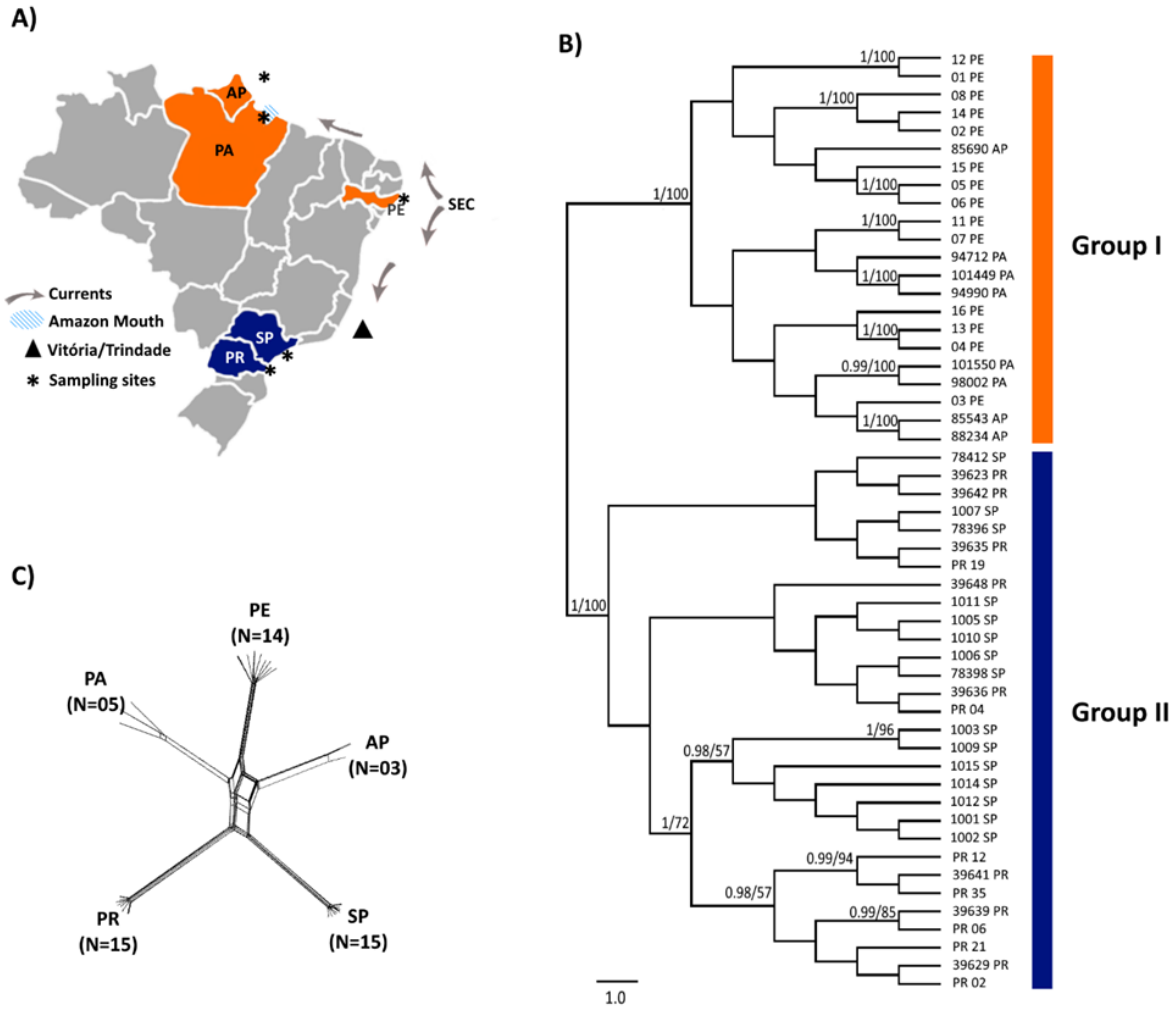

Figure 4. (**A**) Map of the analyzed *Pseudobatos percellens* Brazilian coast sampling sites, highlighted along the Southern Equatorial Current (SEC), region of the mouth of the Amazon River, and Victoria-Trindade Seamount Chain. (**B**) Phylogenetic tree based on Bayesian Inference with 1.448 SNPS identified in *P. percellens*, and posterior probabilities (≥0.98) and Bootstrap values (≥57) obtained from the Maximum Likelihood Analysis employing the same matrix. (**C**) NeighborNet splits based on the uncorrected p-distance with the groups identified in the samples AP, Amapá; PA, Pará; PE, Pernambuco; SP, São Paulo; PR, Paraná.

4. Discussion

This study was developed with SNP markers through ddRAD sequencing in individuals of five local chola guitarfish *P. percellens* populations, providing a new perspective into genetic differentiation patterns along the Brazilian coast for the first time. The analyses revealed at least one lineage occurring in the northern and northeastern region and another distributed along the southeastern/southern Brazilian coast, with high genetic differentiation between them. The DAPC analysis, however, pointed out the presence of three lineages distributed in these regions.

Pseudobatos percellens is an endemic species in the western Atlantic Ocean, occurring from Panamá to Brazil, and is generally found in sympatry with *P. horkelli* on the southeastern continental shelf from Rio de Janeiro to Mar del Plata (Argentina) [58,66,104,105]. Another species belonging to the same genus, *P. lentiginosus* Garman 1880, occurs in the

western north Atlantic region, although reports of occurrence in the northernmost region of Brazil are noted [106], although they still requiring confirmation [58,107].

Due to the occurrence of sympatric species belonging to the same genus in the region, the specimens used in this study were previously analyzed by DNA barcoding and compared to *P. lentiginosus* and *P. horkelli* sequences and to species belonging to the *Rhinobatos* genus deposited in Genbank. The results confirmed that all the analyzed specimens corresponded to *P. percellens*, without any taxonomic conflict with other species described for the *Pseudobatos* genus. It is also important to note that the DNA barcode in fishes does not often reveal genetic peculiarities or lineages within groups, more specifically in elasmobranchs. For example, shark species belonging to the *Squalus* genus [108] or freshwater *Potamotrygon* stingrays [109] present a high taxonomic complexity that is not well elucidated by solely employing the COI gene, and requires the use of other complementary genetic markers.

The genome-wide SNPs data obtained herein when analyzing *P. percellens* indicated a high degree of genetic differentiation between specimens from the northern and northeastern regions in comparison to specimens from southeastern/southern Brazil. These results may partially reflect the biological behavior of *P. percellens*, as this species is known to perform seasonal migrations or short-distance movements [50], supported by the Mantel test and isolation by distance model of population structure. These movements over time could be reflected in the complex speciation nature of this group, which ranges from the presence of continuous intragroup variations without reproductive isolation, to complete and irreversible reproductive isolation between groups [110]. Nevertheless, the extent of the effects of these seasonal movements on a long-term evolutionary time scale remain uncertain [110].

Factors Influencing Dispersal: Biogeography Barriers along the Brazilian Coast

The pairwise FST estimations and DAPC plots implied that the São Paulo and Paraná groups exhibit higher genetic similarity than evidenced between Amapá, Pará, and Pernambuco samples, revealing genome differences in these regions. These results were somehow expected, as the sampled regions in São Paulo (Santos/SP) and Paraná (Pontal do Paraná/PR) are geographically close (<270 km). In addition, these regions share similar environmental conditions, such as salinity and sediments and are located in or close to the Cananéia–Iguape–Paranaguá Estuarine Lagoon Complex [111].

Fish are well known for their phenotypic plasticity related to specific environmental changes, which results in thermal performance curve shifts with chronic changes in temperature, potentially leading to a compensation for negative temperature effects [112]. Temperature differences have been identified as an important biogeographic barrier (thermal insulation) for several marine fish species, even more so as the north coast region of Brazil is home to more temperate waters when compared to the south region [56,57,113–118].

Temperature is directly associated with Elasmobranchii biodiversity in the American continent, with the greatest richness observed in the Atlantic Tropical Zone, between the tropics of Cancer and Capricorn [119]. Considering latitudinal and vertical temperature water column variations, species with wide spatial distributions present greater physiological tolerance and, thus, manage to overcome thermal barriers, while restricted species generally display low adaptability to variations [52,120]. This particularity has been observed for different shark and ray species in the southwestern Atlantic [56].

Environmental temperature variation effects may be considered with regards to the distribution patterns of *P. horkelii*, an endemic ray species that occurs in the Argentine Zoogeographic Province, although it has also been recorded in northeastern Brazil [56–58,121]. Therefore, temperature differences may play an important role in separating the two genetic lineages identified herein in *P. percellens*. The results indicate that the lineages belonging to group I may be more adapted to the warmer tropical waters found in the north and northeast coastal Brazilian regions, while individuals from group II seem to be found in cooler water temperatures, in south and southeast Brazil.

The sampled Pará and Amapá regions are relatively close and located in neighboring states, while Pernambuco is located at a greater geographical distance (over 1200 km). Regardless of the geographic distance between the sampled locations, the northern and northeastern Brazil regions are considered more heterogeneous environments, due to unique characteristics as a result of the Plume in the Amazon and Orinoco River (PAOR) [53,54]. This specific environment is considered an important haline barrier for many local coastal fishes [55,60,61,63,122,123].

The results evidencing the structuring of *P. percellens* populations on both sides of the Amazon Estuary, that is, between Amapá/Pará and Pernambuco, may suggest that this haline barrier is unlikely to be responsible for the differentiation observed herein. In addition, the simple presence of the population located in northern Brazil under the direct influence of the Amazon River discharge and partially within the PAOR demonstrated that this species can withstand salinity changes, which is also confirmed by *P. percellens* records in estuarine areas throughout its distribution range [68,124–127].

Ocean currents may also influence the distribution of these specimens throughout the Brazilian coast and contribute to the separation of the two lineages. In this regard, the southern Equatorial Current (SEC) can be strongly considered among the acting factors in the region, contributing to species dispersion. When the SEC reaches the South American continent, it splits into the North Brazilian Current (NBC—northwards) and the Brazilian Current (BC—southwards) [128]. The NBC is an intense western current that curves back on itself after passing the mouth of the Amazon River [129]. The BC is a weak western boundary current carrying warm subtropical water, flowing south along the Brazilian coast, and plays an important role in determining water circulation in the southern Bay of Brazil (SBB). This biogeographical region comprises the area between Cabo Frio (Rio de Janeiro/RJ) and Cabo de Santa Marta (Santa Catarina/SC), where the BC meets the Falklands/Malvinas Cold Current (FMCC), a branch of the Antarctic Circumpolar Current at the Atlantic Subtropical Convergence (ASC), which limits the Argentine Zoogeographic Province (AZP) [130–132].

At the Vitória-Trindade Seamount Chain region, the BC is also influenced by the Central Water of the South Atlantic (CWSA), penetrating over the shelf, reaching coastal areas with a thermocline between 10 and 15 m, retracting during the winter, and consequently being replaced by typically tropical waters. This region thus represents an important faunal transition area, comprising the southern limit of occurrence of several tropical fish species and the northern limit of species from temperate regions, thus leading to population or lineage differentiation, as reported herein and also detected for *Sardinella brasiliensis* [54,113,114,130,133].

5. Conclusions

Pseudobatos is a Rhinobatidae family genus of rays comprising only nine species [134], together presenting an amphi-American distribution. *Pseudobatos percellens* presents the widest distribution area in the Atlantic Ocean, including individuals inhabiting the maximum recorded depth of 162 m, almost twice the area of the congeneric species *P. horkelii* and *P. lentiginosus* in this ocean [58]. This wide *P. percellens* distribution differs from the patterns observed in other species belonging to this family, which generally present a more restricted distribution pattern. This may be considered an indication of the existence of cryptic undescribed species in this group [107], corroborating the results reported in the present study.

Our findings emphasize the need for studies on the regional biodiversity of this group of fishes, in order to understand different distribution patterns and their limiting factors, especially in species presenting active dispersion processes, as noted for Chondrichthyes, carried out mainly during adult life stages [46,52,116,135–137]. In this context, the knowledge of biogeographic species limits and the distribution of their respective populations, as well as their genetic diversity patterns and connectivity, provide data to define management and conservation priority areas. This is especially important when species are distributed

in areas comprising various management authorities and threat levels of connectivity, as in the case of *P. percellens, P. horkelii*, and *P. lentiginosus* [52,56,136].

Moreover, the low gene flow between components belonging to group I found in north and northeast Brazil, and to group II, which inhabit the southeastern/southern regions, is noteworthy, due to several aforementioned factors, and may prevent the occurrence of shared haplotypes. In this case, the genetic drift could lead to genetic differences between individuals from these regions, leading to reciprocal lineages and ultimately, speciation, as pointed by Avise [138].

Finally, the findings reported herein revealed a hidden biodiversity within *P. percellens* and support the need for a taxonomic revision of this group, with the recommendation of including areas not sampled in the present study. In addition, the identification of a hidden diversity within *P. percellens* should be used to improve the management of the Neotropical ichthyofauna that inhabits modified and degraded regions impacted by pollution and habitat fragmentation and are subject to fisheries pressure. Therefore, considering our results employing genome-wide SNP data, two distinct lineages were identified for *P. percellens* on the Brazilian coast that should, therefore, be managed separately to ensure adequate biodiversity conservation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d15030344/s1, Figure S1: The Maximum Likelihood tree of the *Pseudobatos* and *Rhinobatos* specimens, based on mitochondrial cytochrome c oxidase subunit I gene sequences under the K2P Model. The asterisks* represent the 40 samples sequenced in this study; Figure S2: Identification of 3.329 SNPS (in blue) in transitions (in grey) and transversions (in red) characterized in *Pseudobatos percellens;* Figure S3: Heatmap depicting the pairwise Fst Distance matrices using the 52 *Pseudobatos percellens* sample dataset with 3.329 SNPs, between geographical populations (a) and based on clustering (b). Non-significant *p*-value >0.05 represented by x. AP, Amapá; PA, Pará; PE, Pernambuco; SP, São Paulo; PR, Paraná; Figure S4: Contemporary gene flow based on number of migrants (Nm), illustrating relationships based on geographic distribution for the P. *percellens* samples. Arrows indicate the migration direction, while stronger gene flows are represented by larger numbers, as well as thicker and darker colored lines. Fine and clear lines represent limited gene flow with a coefficient < 0.3. AP, Amapá; PA, Pará; PE, Pernambuco; SP, São Paulo; PR, Paraná. Table S1: Summary of the sequencing and processing of 52 *Pseudobatos percellens* samples. Asterisks* represent samples not sequenced in the DNA barcoding analysis; Table S2: Genetic distances (Kimura-2-Parameter—K2P) based on COI sequences among *Pseudobatos* and *Rhinobatos* species (below the diagonal) and standard errors (above the diagonal). Numbers in bold represent the intraspecific K2P genetic distances; Table S3: Matrix of Pairwise F_{ST} (below the diagonal) and *p-values* (above the diagonal divide) among geographical state of *Pseudobatos percellens* populations based on data from 3.329 SNPs.

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Data Availability Statement: The data presented in the present study was deposited and made openly available through the GenBank genetic sequence database, accession Nos. OQ255948–OQ255987).

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