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Multispecies genetic approach reveals divergent connectivity patterns in marine fish from Western Atlantic

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Abstract

Traditionally, the apparent paucity of biogeographic barriers in marine environments when compared to terrestrial and freshwater habitats has been associated with high gene flow rates among geographically distant populations. However, physical traits such as tide currents, temperature, and salinity levels may serve as ecological boundaries thus leading to restricted-range phylogeographic patterns (e.g., the outflow plume from the Amazonas-Orinoco rivers between the Caribbean and the Brazilian Province) according to adaptive features of coastal organisms. To assess the degree of cohesiveness among populations and species of marine and estuarine fishes along a latitudinal gradient from Western South Atlantic, we carried out comparative phylogenetic and species delimitation analyses based on Cytochrome C Oxidase I (COI) sequences of 34 fish taxa from the Caribbean and Brazilian coasts. Distinct values of genetic diversity were revealed for both Provinces, ranging from moderate (1 to 2%) to high (≥ 2%) in 11.76% and 20.59% of the analyzed taxa, respectively. Furthermore, a significant genetic differentiation was observed within the nominal taxa *Diapterus auratus*, *Citharichthys spilopterus*, and *Scorpaena plumieri* from the Caribbean, as well as for *Haemulon plumierii* between the Caribbean and Brazilian Provinces. Such divergence is likely to result from temporal isolation among local populations during sea-level fluctuations during the Pliocene-Pleistocene period. The present findings demonstrate that similar biogeographic boundaries may result in species-specific patterns of genetic connectivity, possibly associated with ecological constraints. Since molecular operational taxonomic units (MOTUs) were identified in certain formal taxa from both Provinces, a systematic revision of these groups is highly recommended. At last, multispecies COI data proved to be helpful to phylogeographic inferences and to support appropriate policies for the conservation of natural resources.

Keywords Amazonas-Orinoco plume · COI · Coastal fish · Genetic divergence · Marine biogeography · Species delimitation

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Introduction

The apparent lack of conspicuous barriers in marine environments has often been used as a proxy for intensified gene flow among geographically distant populations of marine species (Palumbi 1994). However, geomorphological, hydrographic, or historical features can act as ecological or biogeographic boundaries for evolutionary-related fauna populations (Toonen et al. 2016). These regions are referred to as marine provinces, being characterized by their unique biota resulting from long-term or recent evolutionary histories (Spalding et al. 2007; Toonen et al. 2016). Based on the distribution of marine fish species, Briggs and Bowen (2012) redefined the original biogeographic marine provinces proposed by Spalding et al. (2007) by distinguishing four provinces in the Western Atlantic: (i) Caroline (divided into Northern Mexico Gulf and Atlantic Coast), (ii) the Caribbean (from Bermudas to the mouth of Amazonas River), (iii) Brazil (from the mouth of Amazonas River to Santa Catarina, Southern Brazil), and (iv) Argentina (from Santa Catarina to the Valdez Peninsula in Argentina).

Several studies have reported that the Amazonas-Orinoco Plume (AOP) between the Caribbean and Brazilian provinces act as a subtle but significant barrier influencing the current distribution of fish species in Western Atlantic (Rocha et al. 2007; Luiz et al. 2012; Cowman and Bellwood 2013; Luiz et al. 2013). This coastal barrier was formed approximately 11 million years ago (mya) and consists of a massive freshwater and sediment outflow that extends up to 2300 km along the northern/northeastern coast of South America. As a result, the AOP induces significant physicochemical changes in coastal areas, acting as a barrier to the dispersal of several marine species, particularly reef-associated fishes (Rocha 2003; Luiz et al. 2012). Accordingly, previous comparative genetic studies have identified constraints in gene flow among populations of coastal species, resulting in unique lineages northwards and southwards the AOP barrier (Rocha et al. 2002; Rocha 2003; Robertson et al. 2004; Rocha 2004; Rocha et al. 2005; Ball et al. 2007; Argolo et al. 2018). However, additional reports have shown high gene flow rates among populations separated by the AOP in both pelagic and coastal species, mostly likely favored by long-term larval dispersal or migratory abilities at specific life stages (Rocha et al. 2002; Rocha 2003; Ross Robertson et al. 2006; Rocha and Bowen 2008; Luiz et al. 2012).

Understanding the influence of historical and ecological processes on the establishment of biogeographic patterns is a key issue in marine biogeography (Mora et al. 2012). Therefore, population, phylogenetic, and phylogeographic models based on genetic data provide an effective strategy for inferring biogeographic boundaries and pattern of species distribution under a spatiotemporal context (Schlötterer 2004; Sunnucks 2000). For instance, climatic changes during the Pliocene-Pleistocene have determined sea-level fluctuations and eventually the extinction or isolation of marine populations along the Atlantic coast (Bellwood and Wainwright 2002; Gysels et al. 2004; Hoarau et al. 2007).

Over recent years, the analyses of Cytochrome C oxidase subunit I (COI) sequences from the mitochondrial genome (or mtDNA) have been successfully employed to infer the diversity levels in animal kingdom (Hebert et al. 2003a). This approach, known as DNA barcoding, is a method of molecular identification at species level based on amplification and sequencing of a COI fragment (Hebert et al. 2003a; Hebert et al. 2003b). In fishes, DNA barcoding has evidenced several cases of cryptic species and evolutionarily significant units within a nominal taxon. Furthermore, COI data has proved to be informative to assess vicariant events in a biogeographic perspective (Argolo et al. 2018) or to infer phylogenetic and phylogeographic relationships (Jacobina et al. 2018; Jacobina et al. 2020).

Even though the main focus of DNA barcoding is the identification of specific taxa, the availability of georeferenced sequences from various species/populations across different ecoregions in public databases is useful to analyze the patterns of genetic variation over wide geographic ranges, such as the Western Atlantic. In fact, Briggs and Bowen (2012) stressed out that further biogeographic studies in fish species from this region was needed. Fortunately, the number of COI sequences from marine fish species collected along a latitudinal gradient in Western Atlantic in open databases (e.g., BOLD Systems) has increased. Most of these sequences refer to samples from the Caribbean and Brazilian ecoregions, representing a plentiful source of potential insights about how the partitioning of genetic variation in fish populations from each province might have been driven by ecological or historical constraints. Nevertheless, there is a gap in the COI information from fish populations along most of northeastern Brazilian coast, regarded as a putative transition zone between both provinces.

Therefore, the goal of this study was to perform a multispecies genetic analysis to investigate the connectivity of coastal and estuarine fish species along a latitudinal gradient in the Western Atlantic, including additional COI sequences from fish populations collected in Northeastern Brazil. Based on COI data and distinct methods of phylogenetic, phylogeographic, and species delimitation inferences, we attempted to address the following questions: (i) are the populations of marine-estuarine fishes from distinct biogeographic regions in Brazil and the Caribbean significantly divergent?; (ii) what is the influence of the AOP on fish populations and their diversity patterns?; and (iii) what was the role of climate changes during the Pliocene-Pleistocene period in the evolutionary diversification of fish lineages in Western Atlantic?

Material and methods

Sampling

A total of 1230 COI sequences from 34 taxa of marine fish were collected from the Caribbean (CA) and Brazilian (BR) provinces (Table S1). These sequences comprised two datasets: (i) 57 specimens from 12 species collected along Northeastern coast of Brazil (6° 18′ 26.97″ S/35° 01′ 55.45″ W), identified using specific keys (Carpenter and De Angelis 2002; Menezes et al. 2003), and stored in the fish collection at the Oceanography Department of the Federal University of Rio Grande do Norte; and (ii) public sequences of 1162 specimens from 34 species from both provinces downloaded from the BOLD Systems platform (http://www.boldsystems.org/index.php/). The selection of COI sequences from the public database was based on the presence of scientific names and information about the geographic coordinates of collection sites.

Isolation, amplification, and sequencing of DNA

Total DNA was isolated from fragments of muscle tissue stored in 96% ethanol using the DNeasy Tissue Kit (Qiagen) following the manufacturer's instructions. Subsequently, a 659-bp (base pair) COI fragment was amplified using the universal forward FishR1 (5′TCAACCAACCACAAAGACATTGGCAC3′) and reverse FishR2 (5′ACTTCAGGGTGACCGAAGAATCAGAA3′) primers (Ward et al. 2005). The polymerase chain reaction (PCR) was performed using 12.5 µl of 2× Taq master mix (Vivantis), 0.5 µl of each primer (10 mM), 0.5 µl $MgCl_2$ (50 mM), 2 µl of template DNA at 40 ng, and purified water to a final volume of 25 µl. The PCR conditions encompassed an initial denaturation step at 95 °C for 2 min, followed by 35 cycles of 30 s at 94 °C, 30 s at 57 °C, and 2 min at 72 °C, with a final extension step at 72 °C for 10 min. The PCR products were purified using the ExoSap-IT® Kit (Affymetrix) according to the manufacturer's instructions. Both forward and reverse COI sequences were obtained using the Big DyeTM Terminator v3.1 Cycle Sequencing Ready Reaction (Applied Biosystems) in an automatic sequencer (ABI 3130 – Applied Biosystems) using the M-13 primer.

Table 1 Genetic distances of marine and estuarine fish species that presented no significant genetic divergence between and within the Caribbean and Brazilian biogeographic provinces in Western Atlantic

Order	Family	Species	Genetic divergence between provinces	Genetic divergence within provinces	
			Caribbean-Brazil	Caribbean	Brazil
Clupeiformes	Clupeidae	*Opisthonema oglinum*	0.006	0.005	0.005
Acanthuriformes	Acanthuridae	*Acanthurus chirurgus*	0.002	0.001	0.001
Acanthuriformes	Acanthuridae	*Acanthurus coeruleus*	0.005	0.003	0.002
Carangiformes	Carangidae	*Caranx hippos*	0.004	0.001	0.006
Carangiformes	Carangidae	*Caranx latus*	0.003	0.002	0.003
Carangiformes	Carangidae	*Selene vomer*	0.001	0.001	0.000
Carangiformes	Carangidae	*Trachinotus carolinus*	0.001	0.001	0.001
Carangiformes	Carangidae	*Trachinotus falcatus*	0.001	0.000	0.000
Acanthuriformes	Chaetodontidae	*Chaetodon striatus*	0.001	0.001	0.001
Acanthuriformes	Ephippidae	*Chaetodipterus faber*	0.001	0.001	0.000
Perciformes	Haemulidae	*Anisotremus virginicus*	0.002	0.002	0.002
Perciformes	Haemulidae	*Conodon nobilis*	0.007	0.002	0.000
Perciformes	Haemulidae	*Haemulon parra*	0.001	0.001	0.001
Labriformes	Labridae	*Doratonotus megalepis*	0.006	0.004	0.006
Labriformes	Labridae	*Halichoeres radiatus*	0.002	0.003	0.000
Blenniiformes	Labrisomidae	*Labrisomus nuchipinnis*	0.006	0.004	0.006
Perciformes	Lutjanidae	*Lutjanus synagris*	0.003	0.005	0.000
Perciformes	Lutjanidae	*Ocyurus chrysurus*	0.003	0.005	0.000
Perciformes	Lutjanidae	*Rhomboplites aurorubens*	0.001	0.001	0.001
Mulliformes	Mullidae	*Pseudupeneus maculatus*	0.001	0.001	0.001
Perciformes	Scaridae	*Sparisoma radians*	0.003	0.004	0.003
Dactylopteriformes	Dactylopteridae	*Dactylopterus volitans*	0.002	0.002	0.003
Gerreiformes	Gerreidae	*Diapterus rhombeus*	0.003	0.003	0.002

Sequence analyses and DNA barcoding

Both forward and reverse COI sequences of fish samples from Northeastern Brazil (Table 1) were edited using the Geneious software (Kearse et al. 2012). All sequences were aligned using the ClustalW method (Thompson et al. 1994) implemented in BioEdit 7.0.5.3 (Hall 1999), followed by visual inspection for final adjustments. Each species was aligned separately, and the sequences were classified based on the biogeographic province of their collection sites. No stop codons, insertions, or deletions were observed in the DNA sequences, which were deposited in GenBank (Supplementary material 1).

Genetic distances were calculated for each species within and between the two provinces in Western Atlantic using the Kimura-2-parameter (K2P) model available in MEGAX (Tamura et al. 2013), following the conventional protocol established in DNA barcoding (www.boldsystems.org). In marine fish, the COI distances of up to 2% typically represents intraspecific divergence values, being thus referred to as a threshold for delimiting distinct taxa (Ward et al. 2009). The software PopArt (Leigh and Bryant 2015) was employed to generate a haplotype network based on the Median-Joining algorithm for those species exhibiting deep genetic divergence (above 2%).

Species delimitation

Three methods of single-locus species delimitation based on trees were included in the present analysis: the Bayesian Poisson Tree Process (bPTP) (Zhang et al. 2013), the Generalized Mixed Yule-Coalescent (GMYC) (Pons et al. 2006), and the Automatic Barcode Gap Discovery (ABGD) (Puillandre et al. 2012). The bPTP analysis was conducted using the web server http://species.h-its.org/ptp/, using a non-ultrametric tree obtained through maximum likelihood (ML) inference as input. This analysis comprised 500,000 generations with sampling every 500 generations assuming a burn-in of 10%. On the other hand, the GMYC analysis was performed using the GMYC web server (http://species.h-its.org/gmyc/), based on a single threshold for a non-calibrated tree generated in BEAST (Drummond et al. 2012). Finally, the ABGD analysis was carried out in the web server http://wwwabi.snv.jussieu.fr/public/abgd/, with a gap width of 1.0 for all available distances (p, Kimura-2, and Jukes-Cantor) (Berbel-Filho et al. 2018). The concordance among the delimited Molecular Operational Taxonomic Units (MOTUs) was evaluated by comparing the clusters from each species delimitation algorithm.

Estimation of divergence times

The time of divergence was estimated for each species characterized by moderate to high genetic distance values. We employed a Bayesian approach, and the support values for each branch were calculated based on posterior probabilities (PP) using the software BEAST v2.4.8 (Bouckaert et al. 2014). For estimating the divergence time, we considered two calibration points: (1) the uprising of the Panama Isthmus (3.5–5.5 mya) as a dating point, including congeneric species occurring in the Pacific Ocean as an outgroup, and (2) a general substitution rate of COI sequences estimated at 0.01 mutations/sites/million years (Thomaz et al. 2015). We employed the Yule prior and a log-normal relaxed clock (Drummond et al. 2012), considering that all species are part of the same macroevolutionary process to generate a reliable estimate of diversification times (Heath et al. 2014). Two independent runs using Bayesian inference were performed, composed of 20 million generations each with sampling every 2000 generations. Stationarity and effective sample size values (ESS > 200) were assessed using Tracer v1.7 (Rambaut et al., 2018). Log values and the trees were combined using software LogCombiner v2.4.5, following a burn-in of 10%. The most reliable tree was generated using TreeAnnotator v2.4.5 (Bouckaert et al. 2014) and visualized using the software FigTree (http://tree.bio.ed.ac.uk/software/figtree/). Only nodes with *P* values above 0.95 were considered highly supported, while the nodes representing the same lineage were removed for better visualization.

Results

Distinct patterns of genetic variation were observed in marine fish from both biogeographic provinces (CA and BR) in Western Atlantic. Most taxa (23 species; 67.65%) showed slight genetic distance values, reaching up to 0.006 within or between provinces (Table 1). However, moderate levels of genetic divergence (≥ 1 to 2%) were detected in 11.76% of taxa, including *Acanthurus bahianus*, *Elops saurus*, *Chloroscombrus chrysurus*, and *Xyrichtys novacula* (Table 2). Furthermore, deep genetic differences (≥ 2%) were found in 20.59% of nominal species, such as *Polydactylus virginicus*, *Achirus lineatus*, *Haemulon plumierii*, *Stegastes variabilis*, *Scorpaena plumieri*, *Citharichthys spilopterus*, and *Diapterus auratus* (Table 2). In addition, high genetic differentiation was observed for *Diapterus auratus* (2.2%), *Haemulon plumierii* (2.8%), *Citharichthys spilopterus* (5.7%), and *Scorpaena plumieri* (4.0%) within the CA province, as well as for the samples of *Haemulon plumierii* (2.2%) within the BR province (Table 2).

Genealogical inferences were carried out for those taxa characterized by moderate to high intraspecific genetic divergence, revealing a combination of either exclusive and shared haplotypes within and between both provinces (Fig. 1). Overall, this approach revealed a remarkable genetic structure among samples, with unique haplotypes for samples from each province. Nevertheless, shared haplotypes were observed among samples from the CA and BR, suggesting gene flow

Table 2 Marine and estuarine fish species showing moderate to high values of genetic divergences between the Caribbean and Brazilian biogeographic provinces

Order	Family	Species	Genetic divergence between provinces	Genetic divergence within provinces	
			Caribbean-Brazil	Caribbean	Brazil
Elopiformes	Elopidae	*Elops saurus/smith*	0.015	0.009	0.003
Acanthuriformes	Acanthuridae	*Acanthurus bahianus*	0.010	0.004	0.007
Carangiformes	Carangidae	*Chloroscombrus chrysurus*	0.017	0.007	0.005
Perciformes	Haemulidae	*Haemulon plumierii*	0.031	0.028	0.022
Labriformes	Labridae	*Xyrichtys novacula*	0.018	0.005	0.001
Perciformes	Polynemidae	*Polydactylus virginicus*	0.020	0.001	0.001
Perciformes	Pomacentridae	*Stegastes variabilis*	0.032	0.007	0.016
Gerreiformes	Gerreidae	*Diapterus auratus*	0.032	0.022	0.001
Pleuronectiformes	Achiridae	*Achirus lineatus*	0.022	0.011	0.003
Pleuronectiformes	Paralichthyidae	*Citharichthys spilopterus*	0.039	0.057	0.002
Scorpaeniformes	Scorpaenidae	*Scorpaena plumieri*	0.097	0.040	0.000

between populations from these provinces (Fig. 1). The species delimitation methods (ABGD, bPTP, and GMYC) identified distinct lineages within 32.3% of taxa (Fig. 1). From this total, 8.8% (*A. lineatus*, *P. virginicus*, and *S. variabilis*) were characterized by the genetic structure associated with differences between the CA and BR provinces.

A comparative analysis of those species showing deep genetic divergence (Fig. 1) revealed distinct divergence times for such putative cryptic lineages. The divergence time estimated for the whole set of analyzed species ranged from 9 million years ago (Mya) to 200,000 years ago, with an average time of 4 Mya. Most divergence events have

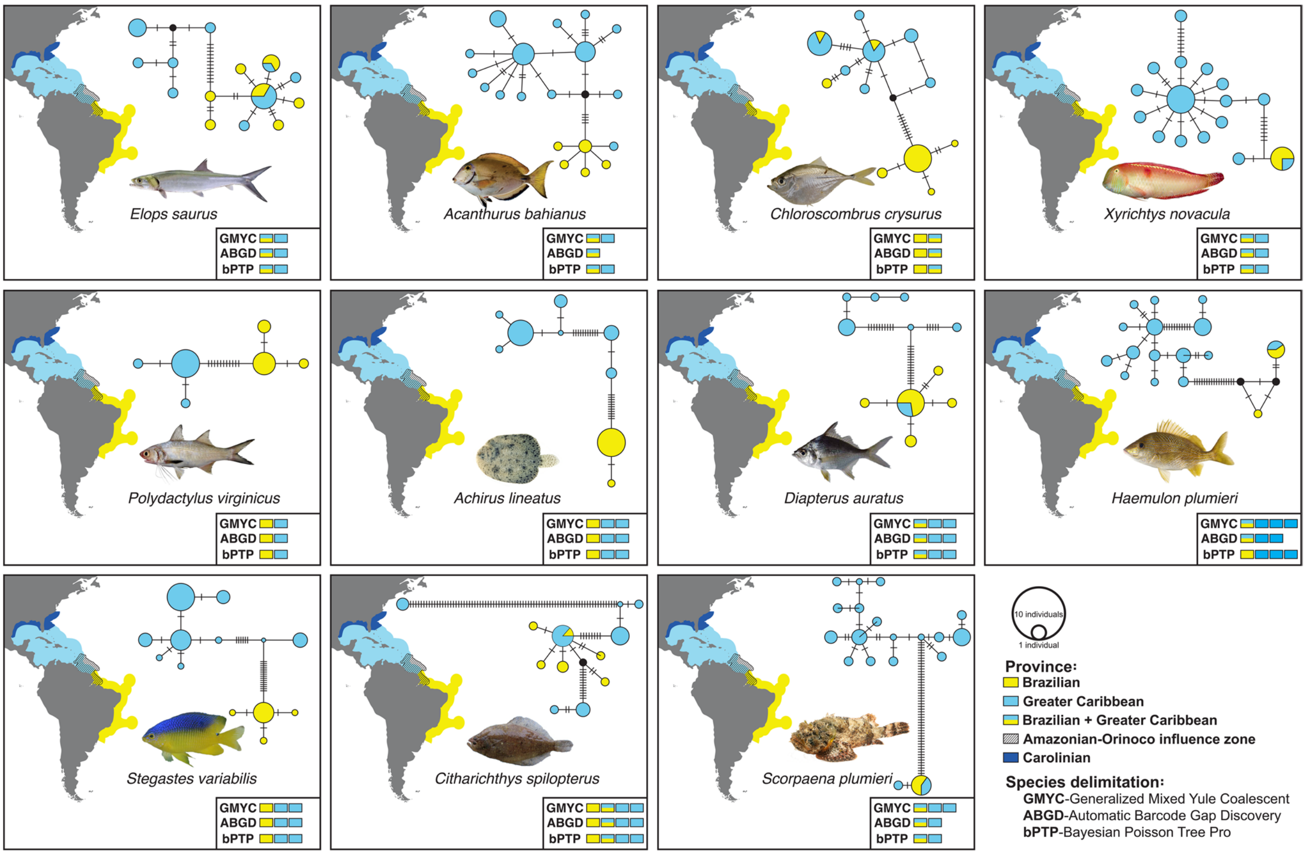

Fig. 1 Species delimitation methods (bPTP, GMYC, and ABGD) and haplotype network in 11 species showing moderate to high genetic divergence between and within the Caribbean and Brazilian biogeographic provinces

taken place between the Late Pliocene (5 Mya) and the Pleistocene (2 Mya). Interestingly, the divergence times in the three species exhibiting genetic structure between the CA and BR provinces differed among each other. Accordingly, *P. virginicus* (500,000 years ago) and *S. variabilis* (200,000 years ago) have undergone recent split events in the Pleistocene, while *A. lineatus* showed a more ancient diversification process (2.5 Mya) in the Late Pliocene (Fig. 2).

Discussion

The Amazon-Orinoco plume impact on fish populations diversity

Our data revealed contrasting patterns of genetic divergence in marine fish with distinct habitat preferences along Western Atlantic. Moderate to high genetic distance values (ranging from 1 to 9.7%) were detected in 32.3% of sampled species. Some lineages were unique to each province, while others were shared between the CA and BR provinces. The former was characterized by the highest number of exclusive lineages ($n = 17$), supporting the assumption that the Caribbean played a key role as the center of origin and diversity of marine organisms in Tropical Western Atlantic (Rocha et al. 2008). Rather than being homogeneous, the CA province displays high environmental heterogeneity, including variations in sea temperature and habitats, as well as differences between insular and continental environments. The peculiar environmental features along this region, encompassing coral reefs, mud bottom coastlines, mangroves, and large river outflows, accounted for most of the genetic divergence among fish populations in the Caribbean (Robertson and Cramer 2014). Nevertheless, unique genetic lineages from the BR province were also identified ($n = 5$), suggesting that peripheral areas can also act as a potential source of regional biodiversity driven by parapatric or ecological speciation (Pinheiro et al. 2018; Peluso et al. 2018).

In addition to the divergent diversity patterns between the CA and Br provinces related to the particular features of each province, the outflow of Amazon/Orinoco rivers is likely to have molded the range of marine species along this region (Rocha 2003; Rocha et al. 2005; Luiz et al. 2012; Jacobina et al. 2020). Even though the massive discharge of freshwater and sediments from Amazon/Orinoco river mouth has been referred to as a permeable barrier, vicariant effects related to the AOP have been reported in reef fish (Araujo et al. 2022), sponges, and scleractinian corals, particularly over shallow areas (Miloslavich et al. 2011). The effects of AOP have also been associated with ecological speciation processes (Rocha et al. 2005). Therefore, the areas northwards the river mouth (i.e., the Caribbean) are mostly composed of islands and relatively stable conditions, including clear waters, low sedimentation rates, and high concentration of calcium carbonate on the seafloor. On the other hand, the southern regions (e.g., the Brazilian shore) are highly influenced by continental environments, particularly related to riverine discharges and high turbidity (Rocha et al. 2002; Rocha 2003; Rocha et al. 2005). Such

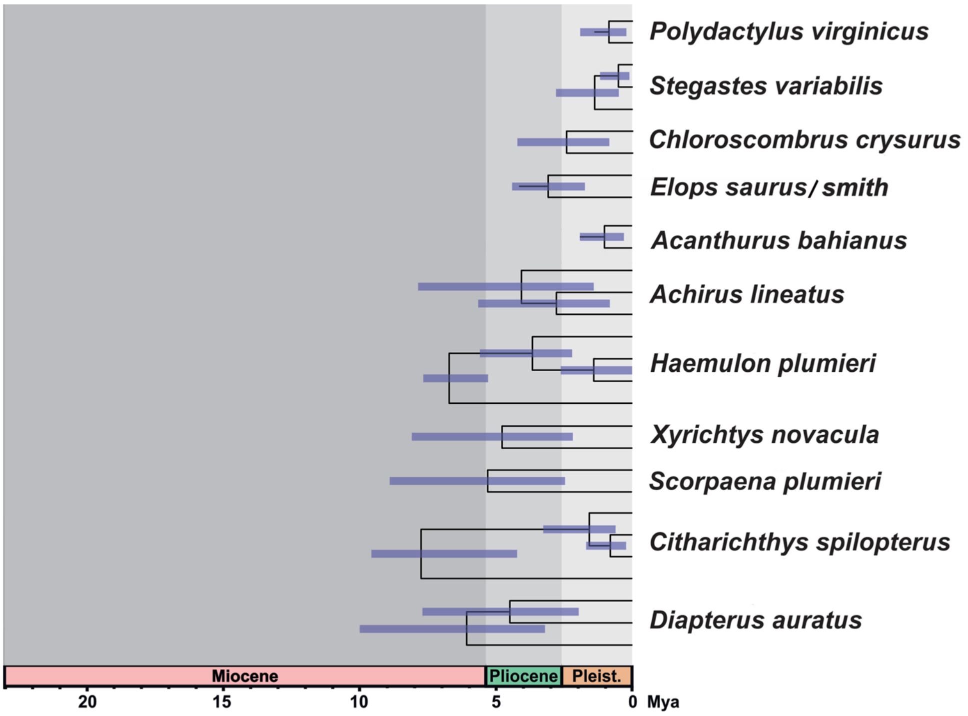

Fig. 2 Multispecies divergence time highlighting the cladogenetic events among lineages

contrasting habitats between the coastline of Brazil and the insular Caribbean should impose divergent selection pressures that underlie the speciation events in Western Atlantic (Rocha et al. 2005). In fact, Rocha et al. (2002) observed that the AOP represented an efficient barrier to the dispersal of *A. bahianus*. However, the AOP acted as a moderate barrier for *A. coeruleus* and had no significant effects on the gene flow among populations of *A. chirurgus* from both provinces as a result of distinct habitat preferences in this group of surgeonfish. The present results reinforce the role of ecological particularities of each species in their response to the effectiveness of AOP as dispersal barrier. Accordingly, the species characterized by high (*A. lineatus*, *S. variabilis*, *H. plumierii*, *D. auratus*, *C. spilopterus*, and *S. plumieri*) to moderate (*A. bahianus, E. saurus*, *C. chrysurus*, *X. novacula*, *P. virginicus*) genetic divergence between populations northwards and southwards the AOP are closely associated with shallow coral reefs and estuarine regions.

On the other hand, the high gene flow in 67.6% of the analyzed species across the AOP may be attributed to long-term larval dispersal or adult migration rates (Rocha 2003; Luiz et al. 2012). Furthermore, the abilities of species in inhabiting deep-water environments would facilitate their dispersal, eventually leading to interpopulation homogeneity between both provinces. For instance, Moura et al. (2016) documented an extensive system of carbonate reefs (9300 km^2) below the AOP, serving as a corridor for fish and other organisms between the CA and BR provinces. Moreover, several studies have reported low genetic distances between populations across these regions, primarily influenced by the multidirectional coastal currents that enable larval migration and increase the permeability between these putative biogeographic barriers (Freitas et al. 2014; Liedke et al. 2020). Moreover, a recent study on reef fish reported a strong correlation between total body length and barrier permeability, in as much as small-sized species usually present increased rates of diversification (Araujo et al. 2022).

We also point out the first evidence of remarkable genetic divergence in *Chloroscombrus chrysurus*, *Stegastes variabilis*, *Diapterus auratus*, *Achirus lineatus*, *Citharichthys spilopterus*, *Polydactylus virginicus*, and *Scorpaena plumieri*, as revealed by the species delimitation methods. In the case of *Elops saurus*, both molecular and morphological data identified two morphotypes corresponding to a temperature gradient, where one morphotype is restricted to cool waters while the other morphotype (*Elops* sp.) is found in warm waters from the Caribbean Province, being later named as *Elops smithi* (Obermiller and Pfeiler 2003; McBride and Horodysky 2004; Horodysky et al. 2010).

The present results also revealed a deep genetic differentiation in *E. saurus* between the regions separated by the AOP, indicating the presence of two MOTUs according to species delimitation algorithms. Interestingly, both lineages coexist in sympatry and remain as unique entities due to differences in their breeding periods. Most likely, the records of *E. saurus* along the BR province should actually correspond to *E. smithi* (McBride and Horodysky 2004). Therefore, integrative analyses that incorporate additional genetic data and morphological traits can provide useful insights to the recognition of the species status in this fish group and their respective geographical distribution in Western Atlantic.

Likewise, genetic differences have also been reported in *H. plumieri* within the CA province (O'Donnell et al. 2019) based on analyses of 10 microsatellite loci. This report identified four distinct populations across North and South Carolina, southeastern Florida, eastern Gulf of Mexico, and the Caribbean to the USA. Nonetheless, further investigations are required to refine the extension of genetic divergences between the populations of *H. plumieri* between and within each province since our data suggest the presence of a shared lineage occurring in both the CA and BR provinces.

In turn, a similar pattern of divergence in COI sequences and chromosomal variation was observed among populations of *Xyrichtys novacula* from the Mediterranean, the Caribbean, and Brazil, with no differences in samples from each province (Almeida et al. 2017; Nirchio et al. 2019). Therefore, a comprehensive phylogeographic study is encouraged to be performed in this taxon to elucidate the phylogenetic relationships of these genetically divergent groups according to each region. Such studies could contribute to our understanding of the patterns and processes shaping the fish diversity in Western Atlantic, being particularly helpful to direct effective strategies for the management of fisheries stocks and conservation policies.

Pliocene-Pleistocene effects in the Western Atlantic

The closure of the Panama Isthmus during the Plio-Pleistocene, approximately 5.5 to 3.6 million years ago, was one of the most significant cladogenetic events responsible for the speciation processes on both sides of Atlantic and Pacific Oceans. In addition, the dynamics of the current tides in Atlantic and the outflow of freshwater and sediments from the Amazon River have molded the patterns of biodiversity distribution between South Atlantic and the Caribbean. The estimated time of sediment deposition from Amazonian basins in Atlantic coast is still a topic of debate, since some authors suggest it has taken place 5 to 6 million years ago (Joyeux et al. 2001) while others assume an earlier origin (around 11 million years ago), with the maximum sediment deposition during the Pleistocene, about 1.6 million years ago (Floeter and Gasparini 2001).

Sea-level fluctuations have also played a key role in shaping the seascape worldwide, being a potential driver of speciation in marine biota, particularly for species from shallow coastal waters and estuaries (Hewitt 1996; Lambeck and

Chappell 2001; Miller et al. 2005; Grant and Bowen 2006; Jacobina et al. 2020). In Western Atlantic, sea-level fluctuations have also affected the dynamics of the AOP, resulting in significant differences in the sediment distribution during the glacial maxima when sea level was approximately 130 m lower than that presently observed (Rocha et al. 2005). In general, the uprising and retreats of sea level would have determined the disappearance, contraction, or isolation of certain populations and species (Beheregaray et al. 2002; Grant and Bowen 2006). Therefore, these combined effects would result in periods of population expansion or contraction (Pereyra et al. 2010; Fernandez-Iriarte et al. 2011).

Our results highlight that distinct historical processes that shaped the seascape of Western Atlantic have direct effects on the times of divergence of studied fish species, being particularly modulated by the cladogenetic events from the Pliocene and Pleistocene. Moreover, the distinctive species-specific patterns observed in this study may be rather associated with unique adaptive responses to stochastic abiotic factors such as temperature, depth, and marine current dynamics (Fernandez-Iriarte et al. 2011). In fact, the continental shelf along the Western Atlantic is quite heterogeneous, being wider in Southern and Southeastern Brazil and narrower along the Northeastern and Northern coast. This differential pattern could have favored more pronounced vicariant effects related to habitat changes in shallow coastal regions characterized by a narrow continental shelf (Ludt and Rocha 2015). As expected, the multispecies comparison of taxa from the CA and BR provinces usually revealed high values of genetic distance and increased speciation events in small-sized fish from shallow waters.

Conclusions

The present study revealed significant genetic divergence among samples of 11 marine fish taxa from coastal coral reefs and estuarine regions between two biogeographic provinces in Western Atlantic. These findings confirm the efficiency of DNA barcoding in identifying genetic discontinuities and cryptic diversity in marine populations according to their geographic range, as previously reported by other authors (Serra-Pereira et al. 2011; Landi et al. 2014; Neves et al. 2016). Overall, these reports place the COI markers as a suitable and primary tool to identify significant evolutionary units and to indicate further taxonomic revisions. Accordingly, the present results provide a baseline for refined multilocus phylogeographic approaches and the development of appropriate strategies for both the management of fisheries and conservation of marine biodiversity.

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Declarations

Conflict of interest The authors declare no competing interests.

Ethical approval The collection license was obtained by the government agency SISBIO (27027-5). No approval of research ethics committees was required to accomplish the goals of this study because experimental work was conducted with unregulated fish species.

Sampling and field studies All necessary permits for sampling and observational field studies have been obtained by the authors from the competent authorities.

Data availability All faunistic data generated or analyzed during this study are included in this published article, whereas COI sequences are deposited in GenBank (see electronic supplementary Table S1).

Author contribution DGM and UPJ conducted the literature search and wrote the first draft of the manuscript. LLC, JAB, PRAMA, RAT, and GS performed data analysis and revised/edited the final versions of the manuscript. All authors read and approved the final manuscript.

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