Received: 16 November 2021 | Revised: 8 April 2022 | Accepted: 19 April 2022

DOI: 10.1111/jbi.14398

Journal of Biogeography

WILEY

RESEARCH ARTICLE

The Amazon-Orinoco Barrier as a driver of reef-fish speciation in the Western Atlantic through time

Gabriel S. Araujo[1] | **Luiz A. Rocha**[2] | **Naomi S. Lastrucci**[3] | **Osmar J. Luiz**[4] | **Fabio Di Dario**[5] | **Sergio R. Floeter**[3]

[1]Programa de Pós-Graduação em Ciências Ambientais e Conservação, Universidade Federal do Rio de Janeiro, Instituto de Biodiversidade e Sustentabilidade - NUPEM/UFRJ, Macaé, Rio de Janeiro, Brazil

[2]Section of Ichthyology, California Academy of Sciences, San Francisco, California, USA

[3]Marine Macroecology and Biogeography Lab., Departamento de Ecologia e Zoologia, Universidade Federal de Santa Catarina, Florianópolis, Santa Catarina, Brazil

[4]Research Institute for the Environment and Livelihoods, Charles Darwin University, Darwin, Northern Territory, Australia

[5]Universidade Federal do Rio de Janeiro, Instituto de Biodiversidade e Sustentabilidade – NUPEM/UFRJ, Macaé, Rio de Janeiro, Brazil

Correspondence

Gabriel S. Araujo, Universidade Federal do Rio de Janeiro, Instituto de Biodiversidade e Sustentabilidade – NUPEM/UFRJ, Av. São José Barreto, Macaé, RJ 27910–970, Brazil.

Email: gabrielsoaraujo@gmail.com

Funding information

California Academy of Sciences Hope For Reefs Initiative; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 443302/2020 and 307340/2019-8; Coordenação de Aperfeiçoamento de Pessoal de Nível Superior, Grant/Award Number: 88882.461696/2019-01, 88881.624424/2021-01 and 4551-13-3; Lakeside Foundation, Grant/Award Number: 4-22-0314

Editor: Cynthia Riginos

Abstract

Aim: The Amazon-Orinoco plume is the major biogeographical barrier between the Great Caribbean and the Brazilian Province. No study has so far addressed the influence of this barrier in a broad chrono-phylogenetic context. Here, we evaluate the effects of the Amazon-Orinoco plume barrier on the patterns of diversification of Western Atlantic reef fishes through time.

Location: Tropical Western Atlantic.

Taxon: Reef Fishes (Percomorpha).

Methods: We compiled DNA sequence data of 21 molecular markers from sister species/population pairs across 33 reef-fish families in the Greater Caribbean biogeographical region and the Brazilian Province. The data included estimated divergence times between sister-pairs based on previously published phylogenies and 94 newly proposed time-trees. Divergence dates were compared with variations in the sedimentation discharge rate of the Amazon River and sea-level fluctuations. To address the hypothesis that fishes with different adult sizes are affected differently by the Amazon-Orinoco plume, we used a phylogenetic least squares regression to test the relationship between maximum total body length and divergence times between the Caribbean and Brazilian sister-pairs.

Results: We identified an increase in the rates of lineage diversification between sister-pairs of the Greater Caribbean and Brazilian Province reef fishes in the past 2.4 million years, in accordance with the period of higher sedimentation rates of the Amazon River and the more frequent sea-level fluctuations in the Pleistocene. A strong relationship between total body length and effectiveness of the barrier through time was confirmed, with smaller species typically diverging earlier in periods when sedimentation rates of the Amazon River were significantly lower.

Conclusions: The strong biogeographical signal detected in this study across different lineages of reef fishes clearly indicates that the Amazon-Orinoco plume played a significant role in the diversification of the Atlantic reef-fish fauna. The temporal pattern of diversifications indicates a correlation between the permeability of the plume, sea-level fluctuations, variability in the volume of the discharge and fish body size, with the barrier's influence being weaker on larger fishes.

KEYWORDS
Amazon River, body size, Brazil, Caribbean, Coral Reefs, divergence time, marine biogeography

1 | INTRODUCTION

Biogeographical barriers are some of the most relevant determinants of species distributions. In the sea, biogeographical barriers can be either hard, such as landmasses, or soft, such as sediment and temperature barriers, with the latter being more permeable than the former (Bowen et al., 2013; Cowman & Bellwood, 2013). Depending on the nature of the barrier and the region where it is located, the barrier may be traversed by taxa with specific biological attributes, or at certain times, but not by others. Biogeographical provinces, which are largely separated and maintained distinct by biogeographical barriers, are geographical subdivisions defined by the proportion of endemic taxa and/or the dissimilarity of their biotas (Briggs, 1974; Briggs & Bowen, 2012; Floeter et al., 2008; Vermeij, 2004). The influence of a barrier on speciation can be estimated and evaluated through analyses and comparisons among patterns of species distribution or genetically distinct populations inhabiting the distinct provinces (e.g., Boehm et al., 2013; Rocha, 2003).

The Brazilian Province is defined as the marine region extending from the Amazon River mouth to southern Brazil (Santa Catarina State), including the oceanic islands of St. Peter and St. Paul Archipelago, Rocas Atoll, Fernando de Noronha Archipelago and the Trindade–Martin Vaz complex (Floeter et al., 2008; Pinheiro et al., 2018; Rocha, 2003). The Greater Caribbean biogeographical region, in turn, extends from Bermuda and Florida to the coastal region off northeastern South America, and from the Gulf of Mexico to the islands of Dominica and Barbados in the Northwestern Atlantic (Figure 1; Robertson & Cramer, 2014; Chollett & Robertson, 2020).

The massive outflow of freshwater and sediments into the ocean from the combined Amazon and Orinoco rivers discharges in northern South America is considered the major biogeographical barrier responsible for the faunal discontinuity between the Caribbean and Brazilian Provinces (Rocha, 2003; Robertson et al., 2006; Figure 1). This outflow forms the Amazon-Orinoco plume, which extends for about 2300 km along the northeast coast of South America (Floeter et al., 2008; Rocha, 2003). The plume started to form about 9.4 million years ago (Mya), but the degree of sediment deposition and freshwater discharge in the region fluctuated greatly through time (Figueiredo et al., 2009, 2010; Gorini et al., 2013; Hoorn et al., 1995).

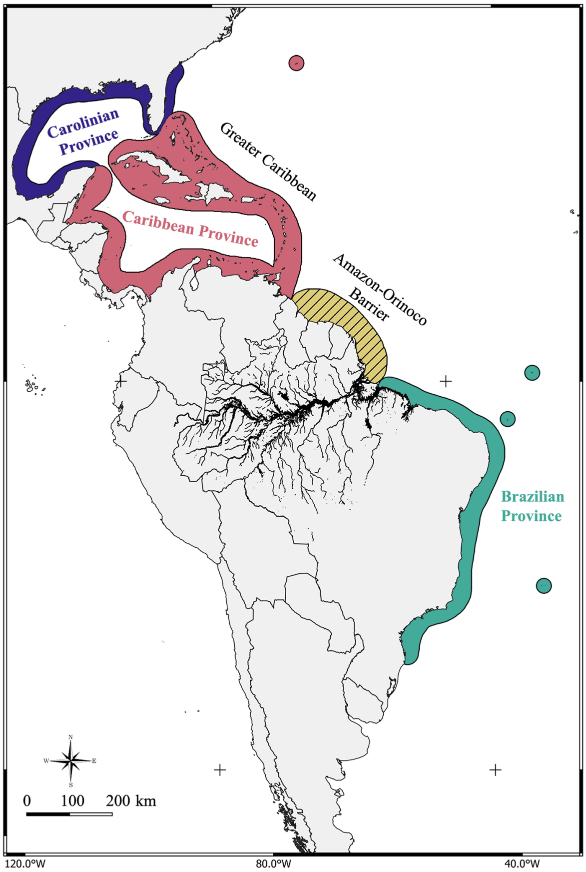

FIGURE 1 Map showing the two tropical biogeographical regions of the Western Atlantic and the area of influence of the Amazon-Orinoco Barrier. Carolinian Province and Caribbean Province correspond to Northern Province and Central+Southern Provinces of Robertson and Cramer (2014), respectively. The map was drawn in an equal-area projection (Mollweide)

The Amazon River has by far the largest freshwater discharge in the world, with up to 300,000 m^3/s, which is roughly an order of magnitude higher than the average discharge of the Orinoco River (Moura et al., 2016; Ward et al., 2015). Consequently, the Amazon River is largely responsible for the structuration of the Amazon-Orinoco plume. However, three stages of development of average sedimentation rates of the Amazon River have been identified in the geological time (Figueiredo et al., 2009, 2010; Gorini et al., 2013; Hoorn et al., 2017). The Amazon River was not yet fully entrenched during the middle to late Miocene (9.4–5.6 Mya). Consequently, sediments were partially trapped in continental basins, resulting in low sedimentation rates at the plume (mean value of ~0.05 m/ka). From the late Miocene to early Pleistocene (5.6–2.4 Mya), the Amazon River was more fully entrenched, and a sixfold increase in sedimentation rates has been estimated during that period (mean value of ~0.3 m/ka). The last stage of development of the sedimentation rate of the Amazon River started during the Pleistocene, at about 2.4 Mya, and is coincident with the formation of the modern Amazon River. During this period, it is presumed that sediment deposition rates were 24 times higher than during the first phase and four times higher than the second one, with a mean value of ~1.2 m/ka. Therefore, the Amazon-Orinoco barrier was possibly more permeable in the early periods of development of the Amazon River due to relatively modest sedimentation rates. As the river matured and sedimentation rates increased, the barrier gradually became less permeable to shallow reef organisms.

In addition to the massive amount of freshwater discharge and sediment deposition primarily resulting from the flow of the Amazon River into the Atlantic, the northeastern coast of South America is a region influenced by highly energetic physical conditions due to the fast-flowing North Brazil Current, strong winds and great variation in tides (Moura et al., 2016). All those factors combined hamper the formation of shallow coral reefs (Briggs, 1974; Veron, 1995), impairing the passage of shallow marine organisms dependent on those environments (Tosetto et al., 2022), despite the existence of an outstanding mesophotic reef system (>70 m deep) underneath the Amazon River plume (Collette & Rützler, 1977; Francini-Filho et al., 2018; Moura et al., 2016; Rocha, 2003). The effectiveness of this barrier fluctuated over time due to sea-level fluctuations. During high sea-level stands, the freshwater and sediment outflow of the Amazon River is mainly carried to the north over shallow waters by the North Brazil Current, which flows mostly in a westerly direction (Peterson & Stramma, 1991). This allows the colonization of the deep outer shelf of the region by sponges and other reef organisms (Collette & Rützler, 1977; Moura et al., 2016; Rocha, 2003; Rocha et al., 2002), forming The Great Amazon Reef System (GARS) that supposedly reduces the effectiveness of the Amazon-Orinoco Barrier. During lower sea-level periods, in turn, sediment is directly deposited at the Amazon mouth on the Atlantic Ocean floor, contributing to the formation of the Amazon Fan (Nittrouer & DeMaster, 1986; Rocha, 2003). As a result, deeper reef environments in the sponge corridor along the outer shelf of the region are under more turbid conditions, that more effectively isolate the Caribbean and Brazilian regions (Collette & Rützler, 1977; Rocha, 2003).

The comparison of the distributions of different taxa may shed light on the biogeographical history overshadowed by deep time (Delrieu-Trottin et al., 2019; Nelson & Platnick, 1981; Toonen et al., 2011). Despite the availability of molecular phylogenies and nucleotide sequences from multiple species of different families of reef fishes, no study has so far addressed the influence of the Amazon-Orinoco Barrier in a comparative and taxonomically diverse phylogenetic context. Superimposing divergence date times among species or populations of different taxa across the Amazon-Orinoco Barrier timeline is crucial to understanding this barrier's influence and effectiveness over time and to assessing the primary drivers involved in this process.

In this study, we analysed the impact of the Amazon-Orinoco Barrier on the diversification patterns of the reef-fish fauna of the Tropical Western Atlantic. We analysed the genetic divergence between Caribbean and Brazilian sister-pairs across 110 taxa of either sister species or populations of the same species living on opposite sides of the barrier to determine how the Amazon-Orinoco River plume influenced diversification events in the Atlantic. We also tested if body size, in terms of total length, was relevant to a species' ability to cross the Amazon-Orinoco Barrier in the past, as it seems to be today (Luiz et al., 2012). We tested the hypothesis of dynamic temporal permeability of the Amazon-Orinoco Barrier by seeking the significance of the relationship between the maximum total length of the sister-pairs in the Caribbean and Brazilian regions and their respective divergence times in relation to the three development stages of the Amazon River freshwater and sediment discharge. To do that, previously published and newly generated data for divergence times among sister-pairs in both regions were considered in a broad phylogenetic framework. This is, therefore, the first meta-analytic study including all available published data and public sequence records of reef fishes conducted to explore general patterns of temporal diversification among reef fishes in the Western Atlantic.

2 | MATERIALS AND METHODS

Our methodological approach is diagrammed in Figure S1 and detailed in the following sections. Table S4 contains the summarized dataset.

2.1 | Taxonomic selection and geographical distribution screening

The definition of 'reef-fish families' and the categories of size used here, Cryptobenthic Reef Fishes (CRFs) and Large Reef Fishes (LRFs), follow Brandl et al. (2018) and resulted in the selection of 58 families of the Teleostei. For the 44 families represented in the Western Atlantic, on both sides of the barrier, the following procedure was implemented: geographical distributions were scored for each species based on Fricke et al. (2022) in addition to the more detailed geographical information available at the IUCN website (https://www.iucnredlist.org/); when not available at the IUCN, distributional data were also compiled from Fishbase (https://www.fishbase.de/). Species were then categorized into either (1) species that are reported for the entire Tropical Western Atlantic or (2) pairs of sister species in which one of them occurs in the Caribbean and the other occurs off Brazil. A careful search in the published literature focusing on molecular phylogenetic studies that include those sister-pairs was then executed. Only phylogenies with divergence time estimates were considered. In addition, selected phylogenies include sequences based on samplings from both the Carolinian/Caribbean and the Brazilian Provinces in the case of species reported for the entire Tropical Western Atlantic (category 1 above), or include sister-pairs on each side of the Amazon-Orinoco Barrier in the case of category 2. A total of 16 phylogenies met all these criteria (Table S1). When the phylogeny did not include an estimate of the divergence time or when it did not fulfil all the criteria indicated above, the sequences and topology were used for subsequent analysis for this purpose, that is, we used the 'sibling species' relationships retrieved in the topologies to implement the analysis of divergence time. In the case of sister-pairs not represented in any molecular phylogenetic study, an extensive search for sequences was carried out in

GenBank (https://www.ncbi.nlm.nih.gov/genbank/) and Barcode of Life Data System (http://www.boldsystems.org/) to perform analyses of divergence time between clades occurring in the Caribbean and Brazilian regions. In the case of published phylogenies of *Clepticus*, *Coryphopterus* and *Myripristis* (Beldade et al., 2009; Bowen et al., 2006; Volk et al., 2021), either the sequences were not available at online repositories or only the haplotypes were available. We then contacted the authors of the original publications, and all requested sequences and information were kindly provided. GenBank and BOLD accession numbers for each species-pair are available in Table S3.

2.2 | Molecular phylogenies and haplotype distribution

Divergence date intervals were calculated for 94 sister-pairs of Caribbean and Brazilian regions (Figures S6–85) using BEAST 2.6.3 (Bouckaert et al., 2019), with a relaxed lognormal clock and the Calibrated Yule Model as priors, and divergence times for 16 pairs were obtained from the literature. Substitution models were estimated using jModelTest2 (Darriba et al., 2012). Secondary calibration points provided by Rabosky et al. (2018) or by time-tree phylogenies of the target group (Table S1) were also considered. In multi-locus analyses, the genes were individually partitioned. At least two independent Markov Chain Monte Carlo (MCMC) were implemented for each analysis. The MCMC chain length was refined to be sufficient once all convergence statistics of effective sample sizes were >200, sampling trees at every 0.01% generation of the chain length. The run parameters were accessed in Tracer 1.7 (Rambaut et al., 2018) and the log and tree files were combined in LogCombiner (Bouckaert et al., 2019). The target tree was summarized in TreeAnnotator (Bouckaert et al., 2019) after excluding 20% burning from each run. The substitution models and calibration points for each analysis are indicated in Figures S6–S85. In the analyses, divergence dates were considered only when a clear phylogenetic separation between Caribbean and Brazilian populations (i.e. clearly distinct clades) was observed. When distinct clades were not observed for those regions, indicating an absence of isolation between populations from the Caribbean and Brazilian regions, divergence date was computed as zero, regardless of the estimated crown age. Haplotype networks for pairs of populations were built with the TCS algorithm (Clement et al., 2000) present in PopArt (Leigh & Bryant, 2015).

2.3 | Effect of body size on barrier transposal across time

Previous studies have concluded that marine species with larger body sizes generally have a greater capacity to withstand environmental variability and also potentially have a stronger active dispersal capacity (Luiz et al., 2012; Roy et al., 2002; Vermeij, 2004; Vermeij et al., 2008). Large adult size was indeed proposed as a significant predictor for a given reef fish's ability to cross the Amazon-Orinoco Barrier in its current configuration (Luiz et al., 2012). The GARS, in particular, seems to function as an intermittent corridor for species dispersal depending on sea-level height (Rocha, 2003). However, since there has been a gradual increase in the sedimentation and freshwater discharge rates of the Amazon River into the Atlantic over the last few million years, it is likely that permeability of the Amazon-Orinoco Barrier has also varied more broadly across time. To estimate the relationship between barrier crossing and body size through time, the maximum total length (cm) of each species was obtained from FishBase. Then, each sister-pair was assigned to one of the three temporal categories: those that diverged in (1) the first sedimentation phase of the Amazon River (9.4–5.6 Mya); (2) the second (5.6–2.4 Mya); and (3) the third (2.4 Mya–present). A fourth category including species without divergence between the regions (presumably panmictic species) was also considered. Because animal traits, including body size, tend to be more similar in closely related species, consideration of phylogeny autocorrelation is critical for exploring the linkage of traits among species (Li & Ives, 2017). We performed a phylogenetic generalized least squares regression (PGLS; Lajeunesse & Fox, 2015), considering the phylogenetic relationships between species and assuming body size variation under Brownian motion. PGLS can incorporate potential bias in phylogenetic relatedness among target species, assuming that the similarity of model residuals between species depends on their phylogenetic relatedness. To feed on the PGLS, we constructed a single phylogeny of the species analysed in this study only, using the R package 'FishPhyloMaker' (Nakamura et al., 2021; Figure S2). Because data points in the analyses included both populations of a single species and pairs of sister species, we considered the species pair (instead of a single species) as the tip of the phylogenetic tree when necessary (Figure S2). The null hypothesis was that there is as much variation in the maximum total length of the species within the temporal windows as between the temporal windows. The maximum total length of the species was converted to log to satisfy assumptions of normality and homoscedasticity. The PGLS was fitted using the R package 'nlme' and the generation of plots were implemented using the R Software Package (R Core Team, 2020).

3 | RESULTS

A total of 110 estimates of divergence time based on 21 molecular markers (seven mitochondrial and 14 nuclear; Figures S3, S6–85; Table S4) were recovered in 33 reef-fish families. Of those, 34 correspond to pairs of sister species and 76 correspond to pairs of disjunct populations within a species. In all, 60 pairs showed some level of divergence between the Caribbean and Brazilian regions, whereas 50 seem to have genetically panmictic populations along the regions (Figure S3; Table S1). Of the 110 estimates of divergence time, 23 consisted of Cryptobenthic Reef Fish (CRF) sister-pairs in 10 families, whereas 87 are Large Reef Fish (LRF) pairs in 23 families. Among those with some level of divergence between regions, 20 (33.3%)

are CRF sister-pairs and 40 (66.6%) are LRF sister-pairs. In total, 20 of the 23 sister-pairs of CRFs showed some divergence between the regions (87.0%), whereas 40 of the 87 pairs of LRFs showed some level of divergence (45.9%).

Among the 60 pairs with some level of divergence between the Caribbean and Brazilian regions, 33 were pairs of sister species, and 27 represent disjunct populations labelled as single species names. Among those population pairs, 15 (*Coryphopterus glaucofraenum*, *C. thrix*, *Diplectrum bivittatum*, *Enneanectes altivelis*, *Epinephelus adscensionis*, *Gobiesox barbatulus*, *Gobioclinus kalisherae*, *Haemulon atlanticus*, *H. plumierii*, *Hippocampus erectus*, *Lagocephalus laevigatus*, *Scartella cristata*, *Scorpaena plumieri*, *Sphoeroides spengleri* and *Stephanolepis hispidus*) appear to represent species pairs not formally recognized as distinct taxa. In those 15 cases, we found a clear biogeographical compartmentalization, identified in the haplotype networks—which configures distinct Caribbean and Brazilian haplogroups—and the number of mutations between the haplogroups. Furthermore, haplotypes are not shared between the regions in 11 of those 15 population pairs, reinforcing the possible species-level category of those populations (Figure 2a,b). Notably, in all cases of shared haplotypes between the regions, haplotypes from Brazilian lineage were found in the Caribbean populations, but the opposite was never observed (Figure 2b). Also, haplotypes of a single pair of sister species, *Apogon maculatus*/*Apogon americanus*, are the same in both the Caribbean and Brazilian populations, but sequences from *A. americanus* are all from the Fernando de Noronha Archipelago, a location known to have direct links with the Caribbean (Rocha et al., 2005).

In the cases of *Hemicaranx amblyrhynchus*, *Hyporthodus flavolimbatus* and *Selene setapinnis*, sequences from the Caribbean were not available, but sequences of those species from the Carolinian Province and Brazil indicated a clear separation between the populations inhabiting those regions. Examined sequences of *Halichoeres bivittatus* and *Haemulon aurolineatum*, in turn, indicate the separation, followed by secondary contact, between populations of those species from the Carolinian Province and the Caribbean and Brazilian Provinces, which are grouped in a single panmictic unity (Figure 2c).

In Figure 3, we show the estimated divergence dates of the 60 sister-pairs with some degree of isolation between the Caribbean and Brazilian regions in relation to sea-level fluctuations of the last 12 Mya and the three stages of freshwater and sediment deposition of the Amazon River. The estimated divergence dates ranged from around 0.1 to 17.1 Mya (Table S1; Figure 3), with all but two presumably occurring after the onset of the transcontinental Amazon River and its Atlantic discharge (~9.0–9.4 Mya). Only two cladogenetic events—one between *Gobiosoma spilotum* and the clade formed by *Gobiosoma alfiei* and *G. hemigymnum*, and the other between *Lythrypnus nesiotes* and *L. brasiliensis* (both in the Gobiidae)—were estimated as having occurred in a period prior to the opening of the Amazon River in the Atlantic Ocean (13.2 and 17.1 Mya, respectively). The eight oldest dates of cladogenetic events estimated are all from small and cryptobenthic sister-pairs.

Only 10 cladogenetic events are estimated as having occurred between 9.4 and 5.6 Mya, that is, during the first phase of formation of the Amazon River, when the freshwater discharge and sedimentation rate was relatively low (~0.05 m/ka). Eight of those clades include cryptobenthic reef fishes. The average maximum TL of sister-pairs that diverged in/before this period is 12.3 cm, with 41.2 cm being the maximum TL recorded (Figures 3 and 4). Among the CRFs, *Scartella cristata* and *Gobiesox barbatulus* have isolated populations in the Caribbean and Brazilian regions that diverged at about 7.6 and 5.8 Mya, respectively, with the populations off Brazil (and also the eastern Atlantic in the case of *S. cristata*, which is more closely related to the Brazilian population than to the Caribbean one) likely representing distinctive unrecognized species. The three examined sister-pairs in the Opistognathidae also separated at this stage of formation of the Amazon River (Table S1; Figure S64). The only sister species in the Haemulidae and Labridae that diverged in this phase are formed by species with the lowest maximum TL of both families included in this study, *Haemulon parra* + *H. bonariense* (41.2 and 40 cm TL, respectively) vs. *H. squamipinna* (19.4 cm TL, Rocha, C. *personal communication*) and *Halichoeres maculipinna* (18.0 cm TL) vs. *Halichoeres penrosei* (11.9 cm TL).

During the second phase of formation of the Amazon River (5.8–2.4 Mya; sedimentation rate ~ 0.3 m/ka), 17 cladogenetic events resulting in Caribbean and Brazilian sister-pairs were detected, eight of them including cryptobenthic reef fishes and nine represented by large reef fishes. The average maximum TL of the sister-pairs that diverged in this phase is 24.2 cm, with a maximum TL of 79.1 cm (Figures 3 and 4). Disjunct populations of currently recognized single species in the regions were detected in the cases of *Coryphopterus glaucofraenum* (estimated divergence date = 5.3 Mya), *Diplectrum bivittatum* (4.2 Mya), *Scorpaena plumieri* (3.1 Mya), *Sphoeroides spengleri* (3.1 Mya), *Gobioclinus kalisherae* (2.7 Mya), *Enneanectes altivelis* (2.5 Mya), *Coryphopterus thrix* (2.5 Mya) and *Haemulon plumierii* (2.5 Mya). Lineages of those species in the Brazilian Province likely represent species not formally recognized. Results also indicate that the Caribbean population of *Scorpaena plumieri* is more closely related to *S. mystes* from the Tropical Eastern Pacific than to the Brazilian population of the genus, also currently recognized as *S. plumieri*.

In the last 2.4 Mya, which corresponds to the third and most intense period of mean sedimentation rates and freshwater discharge of the Amazon River (sedimentation rate ~ 1.2 m/ka), 31 sister-pairs have some level of divergence between the Caribbean and Brazilian regions. Only two of those sister-pairs include cryptobenthic reef fishes, with the vast majority of 29 lineages that diverged during this period represented by large reef fishes. The average maximum total length of specimens from lineages that originated in this phase is 50.6 cm, with the largest total length recorded at 150.0 cm (Figures 3 and 4). A total of seven possibly not yet formally recognized species were detected in *Serranus flaviventris* (estimated divergence between populations = 2.1 Mya), *Selene setapinnis* (1.8 Mya), *Hippocampus erectus* (1.2 Mya), *Epinephelus adscensionis* (1.1 Mya), *Stephanolepis hispidus* (1.1 Mya), *Lagocephalus laevigatus* (0.7 Mya) and *Haemulon atlanticus* (0.4 Mya).

Among the 50 sister-pairs with populations apparently not distinct in the Caribbean and Brazilian regions, only three correspond to CRFs. Therefore, the vast majority of species living in both

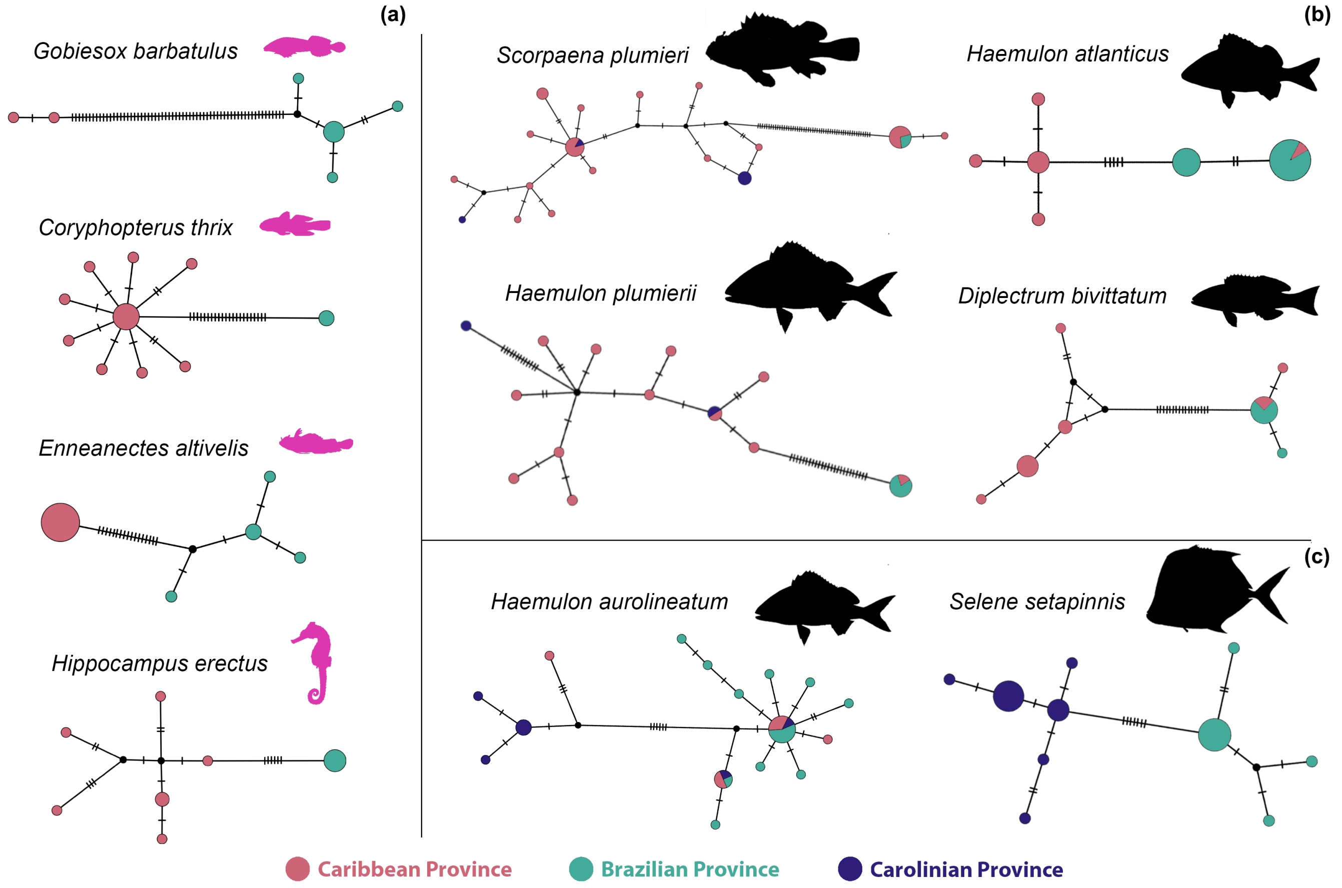

FIGURE 2 Haplotype networks of species reported for the entire Tropical Western Atlantic. In light red, haplotypes sampled in the Caribbean. In cyan, haplotypes sampled from off the Brazilian coast. Violet indicates haplotypes sampled in the Carolinian Province. Relative frequencies indicated by the size of partitions inside circles. (a) Species without shared haplotypes between the Caribbean and Brazilian regions. (b) Species that share haplotypes between the Caribbean and Brazilian regions. c) Species with genetic structure between the Carolinian Province and the Caribbean+Brazil, and between the Carolinian Province and Brazilian region. Pink and black silhouettes indicate cryptobenthic species and large reef-fish species, respectively

regions among the clades analysed is composed of LRFs, with an average maximum TL of 63.2 cm and the maximum TL record of 250.0 cm (Figures 4; Figure S3; Table S1). Our results indicate a statistically significant relationship between dates of cladogenetic events and values of maximum TL recorded in species or disjunct populations of reef fishes living in the Caribbean and the Brazilian regions: the average TL of sister-pairs tends to get smaller as older the cladogenetic event is (Table 1; Table S2; Figure 4; Figures S4 and S5). Indeed, this relationship sets up a size/sedimentation rate gradient. The average maximum TL of pairs that diverged in phase 3 of the formation of the Amazon River (50.6 cm) is more than twice the TL value of pairs that diverged in phase 2 (24.2 cm) and more than four times the average size of pairs that diverged in phase 1 (12.3 cm). That pattern is further corroborated by the maximum TL of the seemingly panmictic populations in the Caribbean and Brazilian regions: the average maximum TL in the panmictic populations (63.2 cm) is more than 2.5 times larger than the maximum TL of populations that diverged in phase 2 of the formation of the Amazon River and more than five times the TL of populations that diverged in the older phase 1.

4 | DISCUSSION

Here we presented the largest and most comprehensive attempt to understand the role of the Amazon-Orinoco Barrier in shaping the Western Atlantic reef-fish fauna within a temporal and phylogenetic context. Results of over a hundred dated molecular phylogenies based on different genetic markers reinforce and detail the role of the Amazon-Orinoco Barrier in the configuration of the modern and past reef-fish fauna of the Western Atlantic. Almost all cladogenetic events that resulted in distinct sister species or sister populations of reef fishes in the Caribbean and Brazilian Provinces addressed in our meta-analysis can be associated with the onset and development of the Amazon-Orinoco Barrier at different times.

The estimated dates of cladogenic events of most sister-pairs of reef fishes in the Caribbean and Brazilian regions included in our dataset seem to be strongly correlated to two main factors: changes in freshwater discharge and sedimentation rate of the Amazon River throughout its geologic history and sea-level fluctuations of the Pleistocene. Sister-pair lineage splitting of 31 out of 60 identified cladogenic events (~51.6%) occurred within the past 2.4 Mya, a

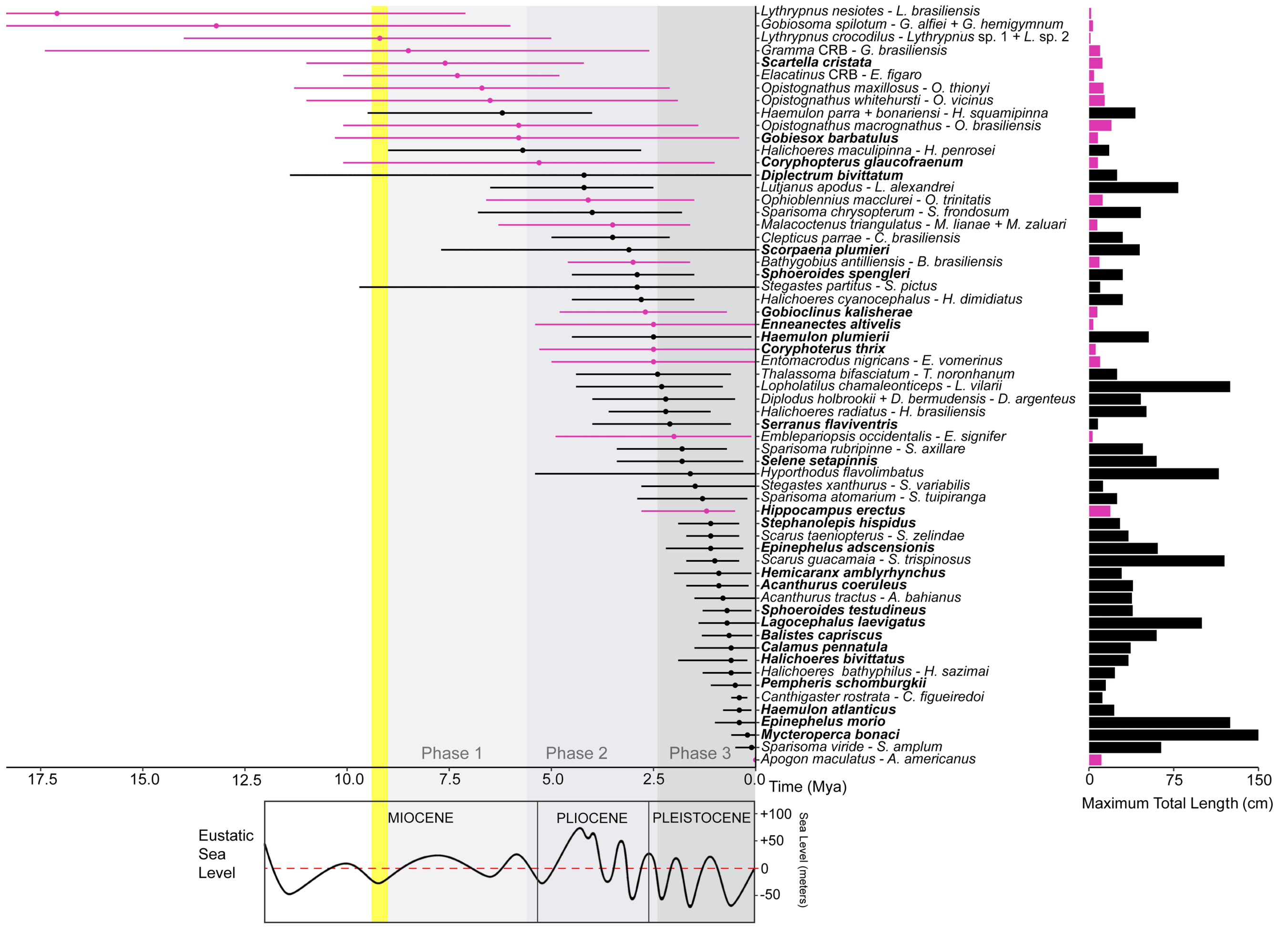

FIGURE 3 Estimates of divergence dates between Caribbean–Brazilian sister species (regular) or sister populations of the same species (bold) and fluctuations in sea level in the last 17.5 Mya, in relation with the three stages of increasing sediment and freshwater discharge of the Amazon River (in shades of grey: phase (1), 9.4–5.6 Mya; phase (2), 5.6–2.4 Mya; phase (3), 2.4 Mya–present). Red dotted line indicates current sea level; sea-level curve modified from Haq et al. (1987) and Johnson et al. (2006), according to Hilgen et al.'s (2012) timescale. Yellow stripe corresponds to the presumed time interval of the 'birth' of the Amazon River (9.0–9.4 Mya). Pink bars indicate species of cryptobenthic reef-fish families (CRF), and black bars indicate species of large reef-fish families (LRF). Mean and 95% High Posterior Probabilities are indicated by the dots and lines in each species/population pair, respectively (oldest Posterior Probability for the divergence between *Lythrypnus nesiotes* vs. *L. brasiliensis* and between *Gobiosoma spilotum* vs *G. alfiei* + *G. hemigymnum* estimated at 27.3 and 21.7 Mya, respectively). Timescale in millions of years. Maximum total length in cm

period characterized by the higher sedimentation rate and freshwater discharge of the Amazon River in the Atlantic (Figure 3; Floeter et al., 2008; Figueiredo et al., 2009; Hoorn et al., 2017; Rocha, 2003). This period is also significantly characterized by greater sea-level fluctuations, with the low sea-level periods likely contributing more to the isolation of populations. The lower frequency of cladogenic events between 2.5 and 9.4 Mya (29 of 60 pairs, about 48.3%), an interval of almost 7 My, was likely related to the comparatively lower rates of freshwater and sedimentation discharge of the Amazon River in the Atlantic in association with the more prolonged and constant high sea-level stands of the period. Lastly, only two cladogenic events older than 9.4 Mya, that is, before the formation of the Amazon River, were detected. However, the 95% highest posterior density interval of the estimated divergence dates of those cladogenic events also covers post-Amazon periods.

4.1 | Divergence time and body size

Another distinct result of our study is the clear correlation between divergence time of lineages and the size of the species, with smaller body sizes primarily associated with older cladogenic events (Figure 3). Luiz et al. (2012) showed that large-bodied reef-fish species are more able to cross soft biogeographical barriers, such as the Amazon-Orinoco Barrier in its current configuration. Body size is consistently considered a relevant predictor for long-distance migration capacity, as well as an indicator of a likely broader tolerance to environmental variability (Luiz et al., 2012; Roy et al., 2002; Vermeij, 2004). Our data tested those hypotheses on a broader geologic timescale, recognizing that the Amazon-Orinoco Barrier is a historically dynamic and complex system. The majority of events that originated cryptobenthic reef-fish sister species or populations in the Caribbean and

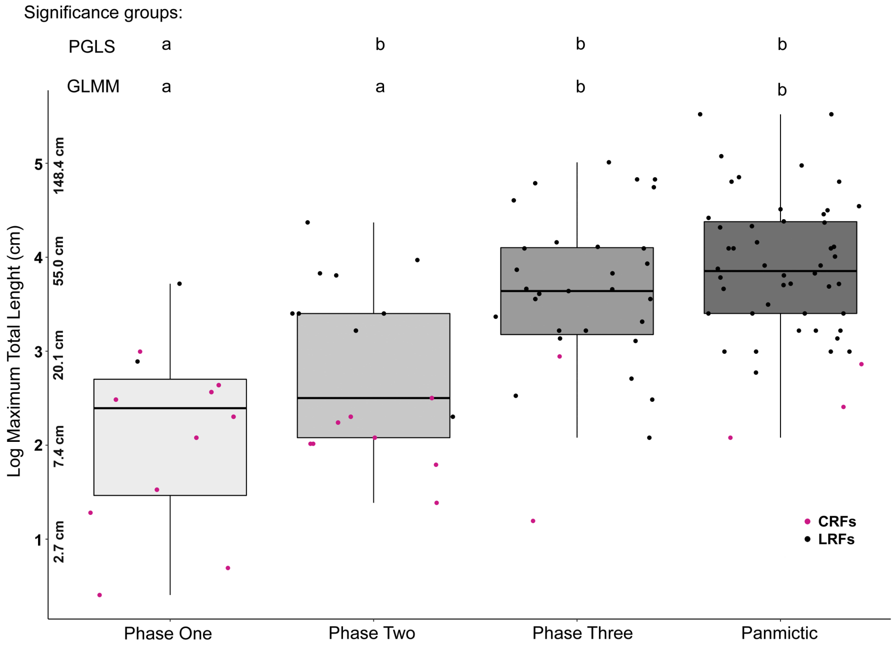

FIGURE 4 Boxplot comparing species maximum total length across four time periods: Phase One, species with populations isolated in the Caribbean and Brazilian regions in the first sedimentation phase of the Amazon River; Phase Two, species with populations isolated in the Caribbean and Brazilian regions in the second sedimentation phase of the Amazon River; Phase Three, species with populations isolated in the Caribbean and Brazilian regions in the third sedimentation phase of the Amazon River; Panmictic, species without isolated populations in the Caribbean and Brazilian regions. Maximum total length transformed to log. Vertical lines, grey bars, and black horizontal lines represent, respectively, data range, interquartile range and median. Letters indicate significance groups derived from the phylogenetic generalized least squares (PGLS) and the linear mixed-effect models (GLMM). Pink and black dots indicate cryptobenthic species and large reef-fish species, respectively

TABLE 1 Parameters of the phylogenetic generalized least squares model predicting phase of species or population divergence as a function of species maximum body size, including the parameter estimates (±standard error), degrees of freedom (df), test statistic (*t*-value) and probability (*p*-value). Reference levels for this model were set as 'Phase one' for the variable 'Phase'

Variable	Estimate ± SE	*t*-value	*p*-value
Intercept	2.372 ± 1.017	2.332	0.0216
Phase			
Phase two	0.538 ± 0.122	4.382	<0.001
Phase three	0.878 ± 0.208	4.212	<0.001
Panmictic	0.968 ± 0.165	5.857	<0.001

Brazilian Provinces (90.0%) are concentrated in phases 1 and 2 of the development of the Amazon River (including here the two events prior to 9.4 Mya). In contrast, only about one-third (27.5%) of all cladogenetic events resulting in distinct populations or species of large reef-fish families such as Carangidae, Epinephelidae, Haemulidae and Lutjanidae—mostly composed of medium- to large-bodied pelagic spawners and with a larger depth range than CRFs—occurred in that same time frame. In those families combined, a total of 10 cases of sister-pairs of species or populations with some degree of divergence were identified, contrasting with seven only in the Gobiidae, for instance. In addition, most (80%) divergences detected in those families resulting in pairs of species or populations in the Caribbean and Brazilian regions were concentrated in the last 2.5 Mya, the period with higher sedimentation rates and greater sea-level fluctuations. As expected, the only two cases of sister species detected among those families contain the species with the smallest size of their respective families: *Haemulon squamipinna* (19.4 cm TL; Brazil) and *Lutjanus alexandrei* (24.3 cm TL; Brazil). They are also the only two cases of divergence in these families in the early stages of the Amazon River.

Those results support and complement the findings of Luiz et al. (2012), demonstrating that the maximum total length of ancestral populations of reef fishes was also a decisive feature in the ability to overcome the Amazon-Orinoco Barrier in the past. Indeed, the general traits of cryptobenthic species—small, benthic, anatomically or behaviourally cryptic, with limited swimming and dispersal capacity and short life cycles—make them more sensitive to soft barriers, like the Amazon-Orinoco, and therefore more prone to allopatric speciation (Brandl et al., 2018).

4.2 | The Amazon-Orinoco Barrier and the patterns of diversification in the Western Atlantic

Our data also indicate that the Amazon-Orinoco Barrier acted in the past in a mixed way in the speciation processes of reef fishes in the Western Atlantic, either as a vicariant agent, when a new barrier separates previously existing populations of widely distributed species or by restricting the dispersion, when divergences dates are significantly younger than the barrier itself (Humphries et al., 1988; Robertson et al., 2006; Rocha, 2003). In other words, our results indicate that for some species, particularly of small fishes, the barrier was highly effective since the early stages of sedimentation rates and freshwater discharge of the Amazon River (Figure 3). For other species, the Amazon-Orinoco Barrier seemed to act as a dynamic semi-permeable barrier through time, restricting the dispersion of species but also becoming less permeable over time as the sedimentation and freshwater discharge increased.

Divergence dates of cladogenetic events close to the birth of the Amazon River, the similar dates of divergence observed among different sister-pairs of species of the Opistognathidae, and the presence of exclusive haplotypes in populations of the Caribbean and off Brazil in all identified cases of possible taxonomically cryptic CRFs reinforce the hypothesis that the Amazon-Orinoco Barrier contributed as a vicariant agent in those cases (Figures 2a and 3; Robertson et al., 2006). In addition, among the 79 species of fishes recorded below the Amazon plume, only two, *Hippocampus reidi* and *Apogon pseudomaculatus*, can be considered CRFs (Collette & Rützler, 1977; Moura et al., 2016). They are also components of the only two cryptobenthic families with panmictic populations identified in this study. *Hippocampus reidi* can be found in estuaries and mangroves (e.g. Aylesworth et al., 2015; Rosa et al., 2007); therefore, its presence below the plume is not surprising. Members of the Apogonidae, in turn, frequently swim in the water column (Riginos & Leis, 2019) and therefore might have a more developed dispersal capacity than other CRFs.

Major fluctuations in sea level must have also allowed some intermittent genetic exchange through the corridor under the Amazon plume during the early Pleistocene, despite the already relatively high sedimentation rate and freshwater discharge of the Amazon River. The more recent divergence dates, estimated as having occurred after the emergence of the barrier, and the wide sharing of haplotypes among large reef fishes that also diverged in the early Pleistocene also supports the intermittent barrier hypothesis (Robertson et al., 2006; Rocha, 2003). Interestingly, of the 30 species addressed here and reported under the Amazon freshwater plume (Collette & Rützler, 1977; Moura et al., 2016), 22 are panmictic, of which 21 are LRFs. Of the eight species that show some degree of divergence, four are components of a pair of species separated by the Amazon Barrier. As these species are currently found below the plume, they are likely using the Great Amazon Reef System as a corridor between the Caribbean and Brazilian Provinces, as proposed by Rocha et al. (2002) for *Acanthurus*. Likewise, it is likely that in periods of low sea level the corridor of the Great Amazon Reef System was closed, possibly preventing gene flow between populations on both sides of the barrier and eventually promoting the diversification that gave rise to sibling species. The predominance of LRFs species reported below the Amazon plume strengthens the hypothesis that size is relevant for the use of the corridor of the Great Amazon Reef System. Also, the genetic exchange allowed by this intermittent barrier appears to have a preferential route, since in all cases of shared haplotypes identified the Brazilian lineage haplotype is found in Caribbean populations, but never the opposite. This is likely a result of the northwestern flow of the North Brazil Current (Peterson & Stramma, 1991; Rocha, 2003), and can also indicate secondary contact after speciation (Rocha et al., 2008).

The intense environmental volatility of the Pleistocene/Pliocene horizon also seems to be linked to the recent divergence dates found between sister-species of *Scarus*. A recent biogeographical hypothesis indicates that the colonization of *Scarus* in the Western Atlantic from the Tropical Eastern Pacific occurred at 6.5 Mya (Siqueira et al., 2019). In both species-pairs of *Scarus* included here (*S. taeniopterus* vs. *S. zelindae* and *S. guacamaia* vs. *S. trispinosus*), the divergence dates are recent (~1 Mya) and the sister lineage of the sibling Caribbean-Brazilian clade is a Caribbean-endemic species (Siqueira et al., 2019). This scenario suggests a process of intra-regional diversification in the Caribbean followed by inter-regional diversification (Caribbean vs. Brazil).

A more intriguing and complex biogeographical scenario is necessary to explain the diversification of *Clepticus*, *Scartella*, *Scorpaena* and *Sphoeroides spengleri* in the Atlantic. The phylogenetic analyses of *Clepticus*, *Scartella* and *Sphoeroides spengleri* revealed an unusual biogeographical relationship between the Brazilian Province and the Eastern Atlantic, with this pairs of species being sisters to the Caribbean region (Figures S13, S45 and S83; Beldade et al., 2009; Araujo et al., 2020). This indicates a separation between the Caribbean and South Atlantic lineages promoted by the Amazon-Orinoco Barrier and subsequent isolation of populations off Brazil and the Eastern Atlantic (Araujo et al., 2020; Beldade et al., 2009; Floeter et al., 2008). In the case of *Scorpaena*, it is likely that the Amazon-Orinoco Barrier was responsible for an older cladogenetic event that was subsequently followed by a more recent division between lineages, originating the species that now lives in the tropical Eastern Pacific and the Caribbean (Figure S74; Nirchio et al., 2016). This secondary cladogenetic event was likely promoted by the emergence of the Isthmus of Panama at 3–5 Mya (Bacon et al., 2015; Coates & Stallard, 2013; Leigh et al., 2013; Montes et al., 2012). Interestingly, the population of *Scorpaena* off Brazil is not yet recognized as a distinct species, even though the cladogenetic event that resulted in the isolation of the ancestral population of the genus in the region is older than the one that originated *Scorpaena mystes* in the Pacific. This might be due to a more general pattern proposed by Rocha (2003), where species isolated by the Amazon-Orinoco Barrier in the Western Atlantic seem to be anatomically more similar than those originated by the rise of the Isthmus of Panama.

4.3 | Underestimated reef-fish diversity in the Western Atlantic

The results also indicate that a substantial number of species of reef fishes await formal recognition in the Caribbean and Brazilian Provinces. This lack of proper taxonomic resolution is likely a result of the scarcity of studies dealing with the taxonomy of reef fishes in general but particularly in the Brazilian Province, despite advances made in the last 30 years (e.g. Gasparini et al., 2001; Moura, 1995; Rocha

& Rosa, 1999; Sazima et al., 1997; Sazima et al., 2002). This might also be related to a possibly unconscious bias in species selection in coral reef studies, since CRF species are generally negatively selected in surveys (Ackerman & Bellwood, 2000; Bellwood et al., 2020). A third situation that might explain the lack of a distinctive taxonomic status of isolated populations refers to different views about what a species is. That seems to be the case of the seahorse *Hippocampus erectus*, for instance, whose isolated populations in the Brazilian and Caribbean Provinces were still recognized as a single species in a recent taxonomic review since the genetic distance between them failed to reach a 2% threshold (Lourie et al., 2016). Regardless of all those situations, the recent wave of molecular systematic studies addressing CRFs in the Western Atlantic, including sequences from Brazil, revealed that several species considered widely distributed actually represent species complexes (e.g. Araujo et al., 2020; Dias et al., 2019; Maxfield et al., 2012; Muss et al., 2001; Smith-Vaniz et al., 2018; Volk et al., 2021). This trend will likely be reinforced in future studies, also highlighting the need for more clear taxonomic recognition on the status of distinct populations in the Western Atlantic.

5 | CONCLUSIONS

In summary, we provide evidence that the variability in the effectiveness of the Amazon-Orinoco Barrier is directly related to changes in sedimentation rates, freshwater discharge and fluctuations in sea level through time, given that periods with higher sedimentation and many drops in sea level coincide with periods of greater occurrence of divergences between the Caribbean and Brazilian pairs. Nevertheless, all older (9.4–2.4 Mya) speciation events appear to be more linked to the initial supply of sediment from the newborn Amazon River rather than the fluctuations in sea level, given the milder variations of sea level that occurred between the Middle Miocene and the early Pleistocene. Since its origin, the Amazon River has posed a significant obstacle to the dispersal of Western Atlantic reef ichthyofauna—especially small fishes—which has grown stronger over time.

ACKNOWLEDGEMENTS

Financial support to GSA was provided by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES grant #88882.461696/2019-01), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq grant PROTAX 443302/2020), and grant nos. #88881.624424/2021-01 (CAPES) and #4-22-0314 (Lakeside Foundation) for visiting LAR at the California Academy of Sciences. SRF was supported by CAPES (grant #4551-13-3), and LAR by the California Academy of Sciences Hope For Reefs Initiative. SRF is grateful to his research productivity grant provided by the CNPq (grant 307340/2019-8). We thank A. Lindner (UFSC) and Anderson V. Vasconcellos (Universidade do Estado do Rio de Janeiro) for their comments on the manuscript. Ana C. Petry (NUPEM/UFRJ) helped with the analyses. We are also grateful to Ana Liedke, Brian Bowen, Eric Hoffman and Ricardo Beldade for kindly providing us with the requested sequences. No permits were needed to carry out this work.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data are available in supplementary material.

ORCID

Gabriel S. Araujo https://orcid.org/0000-0002-8415-4853

REFERENCES

Ackerman, J. L., & Bellwood, D. R. (2000). Reef fish assemblages: A re-evaluation using enclosed rotenone stations. *Marine Ecology Progress Series*, *206*, 227–237.

Araujo, G. S., Vilasboa, A., Britto, M. R., Bernardi, G., von der Heyden, S., Levy, A., & Floeter, S. R. (2020). Phylogeny of the comb-tooth blenny genus *Scartella* (Blenniiformes: Blenniidae) reveals several cryptic lineages and a trans-Atlantic relationship. *Zoological Journal of the Linnean Society*, *190*(1), 54–64. https://doi.org/10.1093/zoolinnean/zlz142

Aylesworth, L. A., Xavier, J. H., Oliveira, T. P. R., Tenorio, G. D., Diniz, A. F., & Rosa, I. L. (2015). Regional-scale patterns of habitat preference for the seahorse *Hippocampus reidi* in the tropical estuarine environment. *Aquatic Ecology*, *49*(4), 499–512.

Bacon, C. D., Silvestro, D., Jaramillo, C., Smith, B. T., Chakrabarty, P., & Antonelli, A. (2015). Biological evidence supports an early and complex emergence of the Isthmus of Panama. *Proceeding of the National Academy of Sciences of the United States of America*, *112*(19), 6110–6115.

Beldade, R., Heiser, J. B., Robertson, D. R., Gasparini, J. L., Floeter, S. R., & Bernardi, G. (2009). Historical biogeography and speciation in the Creole wrasses (Labridae, *Clepticus*). *Marine Biology*, *156*(4), 679–687. https://doi.org/10.1007/s00227-008-1118-5

Bellwood, D. R., Hemingson, C. R., & Tebbett, S. B. (2020). Subconscious biases in coral reef fish studies. *Bioscience*, *70*(7), 621–627. https://doi.org/10.1093/biosci/biaa062

Boehm, J. T., Woodall, L., Teske, P. R., Lourie, S. A., Baldwin, C., Waldman, J., & Hickerson, M. (2013). Marine dispersal and barriers drive Atlantic seahorse diversification. *Journal of Biogeography*, *40*(10), 1839–1849.

Bouckaert, R., Vaughan, T. G., Barido-Sottani, J., Duchêne, S., Fourment, M., Gavryushkina, A., Heled, J., Jones, G., Kühnert, D., De Maio, N., Matschiner, M., Mendes, F. K., Müller, N. F., Ogilvie, H. A., du Plessis, L., Popinga, A., Rambaut, A., Rasmussen, D., Siveroni, I., … Drummond, A. J. (2019). BEAST 2.5: An advanced software platform for Bayesian evolutionary analysis. *PLoS Computational Biology*, *15*, e1006650.3.

Bowen, B. W., Bass, A. L., Muss, A., Carlin, J., & Robertson, D. R. (2006). Phylogeography of two Atlantic squirrelfishes (Family Holocentridae): exploring links between pelagic larval duration and population connectivity. *Marine Biology*, *149*(4), 899–913.

Bowen, B. W., Rocha, L. A., Toonen, R. J., & Karl, S. A. (2013). The origins of tropical marine biodiversity. *Trends in Ecology & Evolution*, *28*(6), 359–366. https://doi.org/10.1016/j.tree.2013.01.018

Brandl, S. J., Goatley, C. H., Bellwood, D. R., & Tornabene, L. (2018). The hidden half: Ecology and evolution of cryptobenthic fishes on coral reefs. *Biological Reviews*, *93*(4), 1846–1873.

Briggs, J. C. (1974). *Marine zoogeography*. McGraw-Hill.

Briggs, J. C., & Bowen, B. W. (2012). A realignment of marine biogeographic provinces with particular reference to fish distributions. *Journal of Biogeography*, *39*(1), 12–30. https://doi.org/10.1111/j.1365-2699.2011.02613.x

Chollett, I., & Robertson, D. R. (2020). Comparing biodiversity databases: Greater Caribbean reef fishes as a case study. *Fish and Fisheries*, *21*, 1195–1212. https://doi.org/10.1111/faf.12497

Clement, M., Posada, D., & Crandall, K. (2000). TCS: A computer program to estimate gene genealogies. *Molecular Ecology*, *9*(10), 1657–1659.

Coates, A. G., & Stallard, R. F. (2013). How old is the Isthmus of Panama? *Bulletin of Marine Science*, *89*(4), 801–813.

Collette, B. B., & Rützler, K. (1977). Reef fishes over sponge bottoms off the mouth of the Amazon River. *Proceedings of the Third International Coral Reef Symposium*, *1*, 305–310.

Cowman, P. F., & Bellwood, R. (2013). Vicariance across major marine biogeographic barriers: Temporal concordance and the relative intensity of hard versus soft barriers. *Proceedings of the Royal Society B: Biological Sciences*, *280*(1768), 2013–1541.

Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: More models, new heuristics and parallel computing. *Nature Methods*, *9*(8), 772.

Delrieu-Trottin, E., Brosseau-Acquaviva, L., Mona, S., Neglia, V., Giles, E. C., Rapu-Edmunds, C., & Saenz-Agudelo, P. (2019). Understanding the origin of the most isolated endemic reef fish fauna of the Indo-Pacific: Coral reef fishes of Rapa Nui. *Journal of Biogeography*, *46*(4), 723–733. https://doi.org/10.1111/jbi.13531

Dias, R. M., Lima, S. M. Q., Mendes, L. F., Almeida, D. F., Paiva, P. C., & Britto, M. R. (2019). Different speciation process in a cryptobenthic reef fish from the Western Tropical Atlantic. *Hidrobiologia*, *837*(1), 133–147. https://doi.org/10.1007/s10750-019-3966-z

Figueiredo, J., Hoorn, C., van der Ven, P., & Soares, E. (2009). Late Miocene onset of the Amazon River and the Amazon deep-sea fan: Evidence from the Foz do Amazonas basin. *Geology*, *37*(7), 619–622. https://doi.org/10.1130/G25567A.1

Figueiredo, J., Hoorn, C., van der Ven, P., & Soares, E. (2010). Late Miocene onset of the Amazon River and the Amazon deep-sea fan: Evidence from the Foz do Amazonas basin: REPLY. *Geology Forums*, *38*(7), 213.

Floeter, S. R., Rocha, L. A., Robertson, D. R., Joyeux, J. C., Smith-Vaniz, W. F., Wirtz, P., Edwards, A. J., Barreiros, J. P., Ferreira, C. E., Gasparini, J. L., Brito, A., Falcon, J. M., Bowen, B. W., & Bernardi, G. (2008). Atlantic reef fish biogeography and evolution. *Journal of Biogeography*, *35*(1), 22–47.

Francini-Filho, R. B., Asp, N. E., Siegle, E., Hocevar, L., Lowyck, K., D'Avila, N., Vasconcelos, A. A., Baitelo, R., Rezende, C. E., Omachi, C. Y., Thompson, C. C., & Thompson, F. L. (2018). Perspectives on the great Amazon reef: Extension, biodiversity, and threats. *Frontiers in Marine Science*, *5*(142). https://doi.org/10.3389/fmars.2018.00142

Fricke, R., Eschmeyer, W. N., & Van der Laan, R. (Eds.) (2022). Eschmeyer's catalog of fishes: Genera, species, references. http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmain.asp

Gasparini, J. L., Rocha, L. A., & Floeter, S. R. (2001). *Ptereleotris randalli* n. sp., a new dartfish (Gobioidei: Microdesmidae) from the Brazilian coast. *Aqua, Journal of Ichthyology and Aquatic Biology*, *4*, 109–114.

Gorini, C., Haq, B. U., dos Reis, A. T., Silva, C. G., Cruz, A., Soares, E., & Grangeon, D. (2013). Late Neogene sequence stratigraphic evolution of the Foz do Amazonas Basin, Brazil. *Terra Nova*, *26*(3), 179–185. https://doi.org/10.1111/ter.12083

Haq, B. U., Hardenbol, J., & Vail, P. R. (1987). Chronology of fluctuating sea levels since the Triassic. *Science*, *235*(4793), 1156–1167.

Hilgen, F. J., Lourens, L. J., & Van Dam, J. A. (2012). The Neogene period. In F. M. Gradstein, J. G. Ogg, M. Schmitz, & G. Ogg (Eds.), *The Geologic Time Scale* (pp. 923–978). Elsevier.

Hoorn, C., Bogotá-A, G. R., Romero-Baez, M., Lammertsma, E. I., Flantua, S. G. A., Dantas, E. L., Dino, R., do Carmo, D. A., & Chemale, F. (2017). The Amazon at sea: Onset and stages of the Amazon River from a marine record, with special reference to Neogene plant turnover in the drainage basin. *Global and Planetary Change*, *153*, 51–65. https://doi.org/10.1016/j.gloplacha.2017.02.005

Hoorn, C., Guerrero, J., Sarmiento, G. A., & Lorente, M. A. (1995). Andean tectonics as a cause for changing drainage patterns in Miocene northern South America. *Geology*, *23*(3), 237–240.

Humphries, C. J., Ladiges, P. Y., Roos, M., & Zandee, M. (1988). Cladistic biogeography. In A. A. Myers & P. S. Giller (Eds.), *Analytical biogeography: An integrated approach to the study of animal and plant distributions* (pp. 371–404). Chapman and Hall.

Johnson, W. E., Eizirik, E., Pecon-Slattery, J., Murphy, W. J., Antunes, A., Teeling, E., & O'Brien, S. J. (2006). The late Miocene radiation of modern Felidae: A genetic assessment. *Science*, *311*(5757), 73–77.

Lajeunesse, M. J., & Fox, G. (2015). Statistical approaches to the problem of phylogenetically correlated data. In G. A. Fox, S. Negrete-Yankelevich, & V. J. Sosa (Eds.), *Ecological statistics: Contemporary theory and application* (pp. 261–283). Oxford University Press.

Leigh, E. G., O'Dea, A., & Vermeij, G. J. (2013). Historical biogeography of the Isthmus of Panama. *Biological Reviews*, *89*(1), 148–172.

Leigh, J. W., & Bryant, D. (2015). PopART: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, *6*(9), 1110–1116.

Li, D., & Ives, A. R. (2017). The statistical need to include phylogeny in trait-based analyses of community composition. *Methods in Ecology and Evolution*, *8*, 1192–1199. https://doi.org/10.1111/2041-210X.12767

Lourie, S. A., Pollom, R. A., & Foster, S. J. (2016). A global revision of the Seahorses *Hippocampus* Rafinesque 1810 (Actinopterygii: Syngnathiformes): Taxonomy and biogeography with recommendations for further research. *Zootaxa*, *4146*(1), 1–66. https://doi.org/10.11646/zootaxa.4146.1.1

Luiz, O. J., Madin, J. S., Robertson, D. R., Rocha, L. A., Wirtz, P., & Floeter, S. R. (2012). Ecological traits influencing range expansion across large oceanic dispersal barriers: Insights from tropical Atlantic reef fishes. *Proceedings of the Royal Society of London B*, *279*(1730), 1033–1040.

Maxfield, J. M., Van Tassell, J. L., St Mary, C. M., Joyeux, J. C., & Crow, K. D. (2012). Extreme gender flexibility: using a phylogenetic framework to infer the evolution of variation in sex allocation, phylogeography, and speciation in a genus of bidirectional sex changing fishes (*Lythrypnus*, Gobiidae). *Molecular Phylogenetics and Evolution*, *64*(3), 416–427.

Montes, C., Bayona, G., Cardona, A., Buchs, D. M., Silva, C. A., Morón, S., Hoyos, N., Ramírez, D. A., Jaramillo, C. A., & Valencia, V. (2012). Arc-continent collision and orocline formation: Closing of the Central American Seaway. *Journal of Geophysical Research*, *117*, 1–25.

Moura, R. L. (1995). A new species of *Chromis* (Perciformes: Pomacentridae) from southeastern Brazil, with notes on other species of the genus. *Revue Française D'Aquariologie et Herpetologie*, *21*, 91–96.

Moura, R. L., Amado-Filho, G. M., Moraes, F. C., Brasileiro, P. S., Salomon, P. S., Mahiques, M. M., Bastos, A. C., Almeida, M. G., Silva, J. M., Jr., Araujo, B. F., Brito, F. P., Rangel, T. P., Oliveira, B. C., Bahia, R. G., Paranhos, R. P., Dias, R. J., Siegle, E., Figueiredo, A. G., Jr., Pereira, R. C., … Thompson, F. L. (2016). An extensive reef system at the Amazon River mouth. *Science Advances*, *2*(4), e1501252. https://doi.org/10.1126/sciadv.1501252

Muss, A., Robertson, D. R., Stepien, C. A., Wirtz, P., & Bowen, B. W. (2001). Phylogeography of *Ophioblennius*: The role of ocean currents and geography in reef fish evolution. *Evolution*, *55*(3), 561–572.

Nakamura, G., Richter, A., & Soares, B. E. (2021). FishPhyloMaker: An R package to generate phylogenies for ray-finned fishes. *Ecological Informatics*, *66*, 101481.

Nelson, G., & Platnick, N. I. (1981). *Systematics and biogeography: Cladistics and vicariance*. Columbia University Press.

Nirchio, M., Oliveira, C., Siccha-Ramirez, Z. R., Sene, V. F., Sánchez-Romero, O. R., Ehemann, N. R., Milana, V., Rossi, A. R., & Sola, L. (2016). Cryptic Caribbean species of *Scorpaena* (Actinopterygii: Scorpaeniformes) suggested by cytogenetic and molecular data. *Journal of Fish Biology*, *89*, 1947–1957.

Nittrouer, C. A., & DeMaster, D. J. (1986). Sedimentary processes on the Amazon continental shelf: Past, present and future research. *Continental Shelf Research*, *6*, 5–30.

Peterson, R. G., & Stramma, L. (1991). Upper-level circulation in the South Atlantic Ocean. *Progress in Oceanography*, *26*(1), 1–73.

Pinheiro, H. T., Rocha, L. A., Macieira, R. M., Carvalho-Filho, A., Anderson, A. B., Bender, M. G., Di Dario, F., Ferreira, C. E. L., Figueiredo-Filho, J., Francini-Filho, R., Gasparini, J. L., Joyeux, J.-C., Luiz, O. J., Mincarone, M. M., Moura, R. L., Nunes, J. A. C. C., Quimbayo, J. P., Rosa, R. S., Sampaio, C. L. S., … Floeter, S. R. (2018). South-western Atlantic reef fishes: Zoogeographical patterns and ecological drivers reveal a secondary biodiversity centre in the Atlantic Ocean. *Diversity and Distributions*, *24*(7), 951–965. https://doi.org/10.1111/ddi.12729

R Core Team. (2020). *R: A language and environment for statistical computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rabosky, D. L., Chang, J., Title, P. O., Cowman, P. F., Sallan, L., Friedman, M., Kaschner, K., Garilao, C., Near, T. J., Coll, M., & Alfaro, M. E. (2018). An inverse latitudinal gradient in speciation rate for marine fishes. *Nature*, *559*(7714), 392–395. https://doi.org/10.1038/s41586-018-0273-1

Rambaut, A., Drummond, A. J., Xie, D., Baele, G., & Suchard, M. A. (2018). Posterior summarisation in Bayesian phylogenetics using Tracer 1.7. *Systematic Biology*, *66*(5), 901–904.

Riginos, C., & Leis, J. M. (2019). Do tiny fish rule the reefs? *Science*, *364*(6446), 1128–1130. https://doi.org/10.1126/science.aax8961

Robertson, D. R., & Cramer, K. L. (2014). Defining and dividing the Greater Caribbean: Insights from the Biogeography of Shorefishes. *PLoS One*, *9*(7), e102918. https://doi.org/10.1371/journal.pone.0102918

Robertson, D. R., Karg, F., Moura, R. L., Victor, B. C., & Bernardi, G. (2006). Mechanisms of speciation and faunal enrichment in Atlantic parrotfishes. *Molecular Phylogenetics and Evolution*, *40*(3), 795–807.

Rocha, L. A. (2003). Patterns of distribution and processes of speciation in Brazilian reef fishes. *Journal of Biogeography*, *30*(8), 1161–1171.

Rocha, L. A., Bass, A. L., Robertson, D. R., & Bowen, B. W. (2002). Adult habitat preferences, larval dispersal and the comparative phylogeography of three Atlantic *Acanthurus* (Teleostei: Acanthuridae). *Molecular Ecology*, *11*(2), 243–252.

Rocha, L. A., Lindeman, K. C., Rocha, C. R., & Lessios, H. A. (2008). Historical biogeography and speciation in the reef fish genus *Haemulon* (Teleostei: Haemulidae). *Molecular Phylogenetics and Evolution*, *48*(3), 918–928. https://doi.org/10.1016/j.ympev.2008.05.024

Rocha, L. A., Robertson, D. R., Roman, R., & Bowen, B. W. (2005). Ecological speciation in tropical reef fishes. *Proceedings of the Royal Society, B*, *272*, 573–579.

Rocha, L. A., & Rosa, I. L. (1999). New species of Haemulon (TELEOSTEI: HAEMULIDAE) from the Northeastern Brazilian Coast. *Copeia*, *1999*, 447–452.

Rosa, I. L., Oliveira, T. P. R., Castro, A. L. C., de Souza Moraes, L. E., Xavier, J. H. A., Nottingham, M. C., Dias, T. L. P., Bruto-Costa, L. V., Araújo, M. E., Birolo, A. B., Mai, A. C. G., & Monteiro-Neto, C. (2007). Population characteristics, space use and habitat associations of the seahorse *Hippocampus reidi* (Teleostei: Syngnathidae). *Neotropical Ichthyology*, *5*(3), 405–414.

Roy, K., Jablonski, D., & Valentine, J. W. (2002). Body size and invasion success in marine bivalves. *Ecology Letters*, *5*(2), 163–167. https://doi.org/10.1046/j.1461-0248.2002.00316.x

Sazima, I., Gasparini, J. L., & Moura, R. L. (2002). *Labrisomus cricota*, a new scaled blenny from the coast of Brazil (Perciformes: Labrisomidae). *Aqua, Journal of Ichthyology and Aquatic Biology*, *5*, 127–132.

Sazima, I., Moura, R. L., & Rosa, R. S. (1997). *Elacatinus figaro* sp. n. (Perciformes: Gobiidae), a new cleaner goby from the coast of Brazil. *Aqua, Journal of Ichthyology and Aquatic Biology*, *2*, 33–38.

Siqueira, A. C., Bellwood, D. R., & Cowman, P. F. (2019). Historical biogeography of herbivorous coral reef fishes: The formation of an Atlantic fauna. *Journal of Biogeography*, *46*(7), 1611–1624. https://doi.org/10.1111/jbi.1363

Smith-Vaniz, W. F., Tornabene, L., & Macieira, R. M. (2018). Review of Brazilian jawfishes of the genus *Opistognathus* with descriptions of two new species (Teleostei, Opistognathidae). *Zookeys*, *794*, 95–133. https://doi.org/10.3897/zookeys.794.26789

Toonen, R. J., Andrews, K. R., Baums, I. B., Bird, C. E., Concepcion, G. T., Daly-Engel, T. S., Eble, J. A., Faucci, A., Gaither, M. R., Iacchei, M., Puritz, J. B., Schultz, J. K., Skillings, D. J., Timmers, M. A., & Bowen, B. W. (2011). Defining boundaries for ecosystem-based management: A multispecies case study of marine connectivity across the Hawaiian Archipelago. *Journal of marine biology.*, *2011*, 460173. https://doi.org/10.1155/2011/460173

Tosetto, E. G., Bertrand, A., Neumann-Leitão, S., & Nogueira Júnior, M. (2022). The Amazon River plume, a barrier to animal dispersal in the Western Tropical Atlantic. *Scientific Reports*, *12*, 537.

Vermeij, G. J. (2004). *Nature: An economic history*. Princeton University Press.

Vermeij, G. J., Dietl, G. P., & Reid, D. G. (2008). The trans-Atlantic history of diversity and body size in ecological guilds. *Ecology*, *89*(11), S39–S52. https://doi.org/10.1890/07-0663.1

Veron, J. E. (1995). *Corals in space and time: biogeography and evolution of the Scleractinia* (p. 321). Cornell University Press.

Volk, D. R., Konvalina, J. D., Floeter, S. R., Ferreira, C. E. L., & Hoffman, E. A. (2021). Going against the flow: Barriers to gene flow impact patterns of connectivity in cryptic coral reef gobies throughout the western Atlantic. *Journal of Biogeography*, *48*(2), 427–439. https://doi.org/10.1111/jbi.14010

Ward, N. D., Krusche, A. V., Sawakuchi, H. O., Brito, D. C., Cunha, A. C., Moura, J. M. S., da Silva, R., Yager, P. L., Keil, R. G., & Richey, J. E. (2015). The compositional evolution of dissolved and particulate organic matter along the lower Amazon River—Óbidos to the ocean. *Marine Chemistry*, *177*, 244–256.

BIOSKETCH

Gabriel S. Araujo is a Ph.D. candidate at Federal University of Rio de Janeiro (UFRJ), Brazil, and his main interests are the evolution, biogeography and taxonomy of reef fishes. He and the other authors are part of a multi-institutional group that seeks, through multiple perspectives, to characterize the diversity of the Tropical Atlantic reef ichthyofauna and recognize the underlying mechanisms that produce it (see LBMM, https://lbmm.ufsc.br/).

Authors' contributions: Sergio R. Floeter, Gabriel S. Araujo and Luiz A. Rocha conceived the ideas; all authors collected data; Gabriel S. Araujo and Osmar J. Luiz performed the analyses; Gabriel S. Araujo and Sergio R. Floeter led the writing; all authors revised and approved the final manuscript.Gabriel S. Araujo is a Ph.D. candidate at Federal University of Rio de Janeiro (UFRJ), Brazil, and his main interests are the evolution, biogeography and taxonomy of reef fishes. He and the other authors are part of a multi-institutional group that seeks, through multiple perspectives, to characterize the diversity of the Tropical Atlantic reef ichthyofauna and recognize the underlying mechanisms that produce it (see LBMM, https://lbmm.ufsc.br/).

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Araujo, G. S., Rocha, L. A., Lastrucci, N. S., Luiz, O. J., Di Dario, F., & Floeter, S. R. (2022). The Amazon-Orinoco Barrier as a driver of reef-fish speciation in the Western Atlantic through time. *Journal of Biogeography*, *49*, 1407–1419. https://doi.org/10.1111/jbi.14398