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RESEARCH ARTICLE

# Hierarchical Genetic Differentiation and Population Structure in the Semiterrestrial Crab *Armases Rubripes* (Brachyura, Sesarmidae)

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## ABSTRACT

Estuaries play a crucial role in maintaining marine biodiversity and providing key ecosystem services, but they are increasingly impacted by local anthropization and global climate change. Developing effective management strategies requires a deep understanding of the genetic structure and diversity of marine species, including how their populations are connected across their distributional range. Here, we used cytochrome oxidase 1 (COI) data to test the hypothesis of significant genetic structuring in the semiterrestrial crab *Armases rubripes* along its distribution, from the Caribbean to the Western South Atlantic coastline. Patterns of genetic diversity, population structure, and demographic history were assessed, and phylogenetic analysis was performed. Our results support this hypothesis, revealing a potential cryptic species complex, with species-level divergence between the Caribbean and Brazilian regions. These two regions exhibit exclusive haplotypes, with up to 16 mutational steps between them, and were recovered as well-supported clades in our phylogenetic reconstructions and high genetic distances that are consistent with interspecific differentiation. We also uncovered unexpected genetic structure within the Brazilian coast, revealing distinct South and North groups among our sampling sites. The analysis of historical demography suggests that Caribbean clade remained stable over time, while Brazilian South group showed signs of recent demographic expansion. Overall, our results suggest that *A. rubripes* is likely a species complex, with Caribbean and Brazil representing separate evolving lineages.

## 1 | Introduction

Genetic structure of marine organisms is shaped by factors that regulate gene flow among and within populations. These factors include present and historical events that shape environmental heterogeneity (e.g., marine currents, temperature, salinity, ice ages, and geographic isolation), ecological barriers (e.g., food availability, predation and interspecific or intraspecific competition), and species-specific dispersal potential (Marochi, Masunari, and Schubart 2017; Buranelli and Mantelatto 2019; Marochi et al. 2022). In many marine species, dispersal potential is influenced by larval behavior (e.g., retention time, light responses), physical factors (e.g., predation and optimal settlement habitat), and planktonic larval duration (PLD) (Cowen and Sponaugle 2009). Simulation and empirical studies have shown that longer PLD can lead to enhanced dispersal potential, increasing the likelihood of gene flow among widespread populations (Siegel et al. 2003; Silva et al. 2010; Marochi et al. 2022). However, this relationship is complex and its importance has to be nuanced (Bowen et al. 2006; Selkoe and Toonen 2011).

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Frequent gene flow among populations promotes genetic homogeneity throughout the species distribution, while larval retention—driven by environmental, behavioral, or physical barriers—can result in increased differentiation among isolated populations due to effects of genetic drift and local adaptation (Taylor and Hellberg 2003; Thurman et al. 2021). This genetic heterogeneity has a "patchwork" pattern (Levins 1968), where genetic variation among populations is expected to be greater than within populations. Over long timescales, restricted gene flow and sustained genetic divergence between populations can lead to speciation (Endler 1977; Thurman et al. 2021). Thus, studies evaluating genetic diversity and population structure are essential to clarify these evolutionary processes.

In the Western Atlantic coast, genetic structure has been found in marine species with long PLDs (e.g., Lessios et al. 2003; Taylor and Hellberg 2003), including several decapod crustaceans (Terossi and Mantelatto 2012; Ituarte et al. 2012; Hamasaki et al. 2015; Laurenzano et al. 2016). Genetic differentiation is most evident in species with widespread distributions when comparing Caribbean and South American populations (Laurenzano et al. 2013; Buranelli and Mantelatto 2019; Thurman et al. 2021; Marochi et al. 2022). This structuring is largely explained by ocean currents splitting populations between ocean basins, as well as historical biogeographic events (e.g., ice ages and sea level changes) acting as barriers to connectivity (Barber et al. 2000; Marochi et al. 2022). Other possible barriers to gene flow in decapod crustaceans along the Western Atlantic include the freshwater discharge from the Amazon and Orinoco rivers in northeastern Brazil and Venezuela, the Central South Equatorial Current (CSEC) and the Cabo Frio upwelling off the coast of Rio de Janeiro state (23° S), Brazil. However, decapod populations along the South American coast show relatively low genetic structure in mitochondrial genes (Oliveira-Neto et al. 2008; Laurenzano et al. 2013; Marochi, Masunari, and Schubart 2017; Marochi et al. 2022), indicating that these later barriers do not prevent gene flow across most of the Atlantic South America coast. Thus, comparing genetic variation of widely distributed species can provide insights into biogeographic patterns and evolutionary history.

Estuaries are part of an ecosystem continuum that is essential for the maintenance and productivity of freshwater and marine biodiversity (Beck et al. 2001; Elliott and McLusky 2002). Unfortunately, near-shore ecosystems are facing increased degradation threats due to local anthropization and global climate change (Gillanders et al. 2011; Statham 2012). To establish well-informed and effective conservation measures, it is necessary to understand population genetic diversity and connectivity along the species' distribution, identifying areas of high diversity (Palumbi 2003; Shanks et al. 2003). Therefore, it is imperative to develop a detailed understanding of species-specific life histories and the connectivity and genetic diversity of their populations.

The semi-terrestrial sesarmid crab *Armases rubripes* (Rathbun 1897) has a widespread distribution in the neotropics (Melo 1996), from Nicaragua (11° N) to the Rio de la Plata estuary, between Uruguay and Argentina (37° S) (Figure 1) (Luppi et al. 2003), with a gap in the available records between the Orinoco and Amazon estuaries (Capítoli et al. 1977; Melo 1996; Luppi et al. 2003; Rodrigues et al. 2021). This is maybe an information gap due to a lack of sampling, as these regions have suitable areas for the species to occur. The species occurs in a variety of coastal habitats, from oligohaline to polyhaline waters, including mangroves (either on the sediment or in burrows), bromeliads, areas near river mouths, and is often found nearby bulrush *Scirpus californicus*, and in salt marshes in proximity to *Spartina* sp. (Capítoli et al. 1977; Fischer et al. 1997; Lima et al. 2006). The larval duration from hatching to megalopa can last 20 to 37 days, and the optimal salinity for larval development is 30 psu (Luppi et al. 2003). Previous phylogenetic studies revealed significant differences between populations of *A. rubripes* from Southeast Brazil and Venezuela, suggesting the occurrence of a potential species complex (Thiercelin 2016). However, Thiercelin (2016) used only two individuals, and evaluating the genetic diversity of additional populations may shed light on this question. The species also exhibits high intraspecific morphological variability (personal observation), which could reflect underlying genetic differentiation among populations. Similar patterns of high morphological variability are observed in other sympatric *Armases* species (Marochi, Masunari, and Schubart 2017; Marochi et al. 2018). Recently, the Sesarmidae *Aratus pisonii* (H. Milne Edwards, 1837) that had a transisthmian distribution, due to its presence in mangroves of the Western Atlantic and Eastern Pacific, was split into two species due to morphological and genetic differences between the populations from the Pacific and the Atlantic (Thiercelin and Schubart 2014). *A. rubripes* has the widest geographic distribution among American Sesarmidae crabs, yet no genetic structure investigations have been done. Hence, the species features a compelling model for studying the influence of geographic barriers on genetic patterns. Our study aimed to investigate the genetic diversity and phylogeographic patterns of *A. rubripes* across its distribution range, assessing a possible case of species complex with higher resolution than previous work. To test the hypothesis of significant genetic structuring among populations, we analyzed the genetic differentiation of cytochrome oxidase 1 (*COI*), comparing Brazilian populations with specimens from the Caribbean (Costa Rica, Colombia, and Venezuela). We hypothesized that *A. rubripes* exhibits substantial genetic differentiation between Brazilian and Caribbean populations, consistent with a species complex scenario.

## 2 | Materials and Methods

### 2.1 | Sampling

The specimens were collected from six Brazilian sampling sites, as well as from one site each in Costa Rica, Venezuela, and Colombia (Table 1; Figure 1). All individuals from the Brazilian coast are deposited in the Capão da Imbuia Museum, Curitiba, Brazil. The Caribbean specimens are deposited at the Schubart collection, Museum der Nature, Hamburg, Germany (Table 1).

We built the geographic distribution of the species based on the records available on (Global Biodiversity Information Facility:

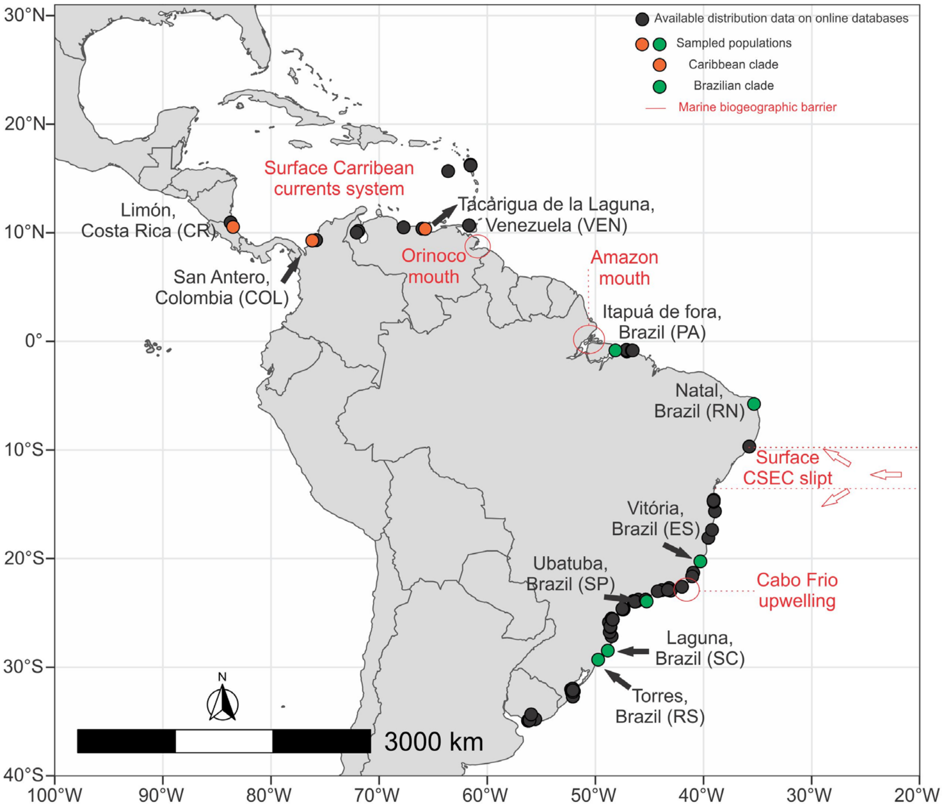

**FIGURE 1** | Sample sites of *Armases rubripes* (orange and green dots) populations from throughout the West Atlantic: Limón, Costa Rica (CR); Tacarigua de la Laguna, Venezuela (VEN); Itapuá de Fora (PA); Natal (RN); Vitória (ES), Laguna (SC) and Torres (RS) from Brazil. Black dots correspond to available distribution data on online databases (gbif, inat, idigbio, obis) and literature.

**TABLE 1** | Sampling locations, coordinates, number of individuals, museum collection numbers of *Armases rubripes* at the Museu de História Natural do Capão da Imbuia (MHNCI), Brazil, and from the Museum der Nature, Germany.

| Location (code) | Coordinates | Number of individuals | Collection *n*° | GenBank *n*° |
|---|---|---|---|---|
| Limón, Costa Rica (CR) | 10°32′ 30.3″N<br>83°30′ 27.7″ W | 12 | Schubart collection | PX909808 - PX909818 |
| San Antero, Colombia (COL) | 9°23′33″ N<br>75°47′11″ W | 1 | Schubart collection | PX909819 |
| Tacarigua de la Laguna, Venezuela (VEN) | 10°18′ 42″ N<br>65°54′ 9″ W | 1 | Schubart collection | PX909820 |
| Itapuá de Fora, Pará, Brazil (PA) | 0°49′ 39.1″S<br>48°9′ 2″ W | 10 | C5711 | PX909821 - PX909830 |
| Natal, Rio Grande do Norte, Brazil (RN) | 5°45′ 45.96″S<br>35°19′ 44.8″ W | 12 | C5713 | PX909852 - PX909863 |
| Vitória, Espírito Santo, Brazil (ES) | 20°15′ 14.1″S<br>40°17′ 33.5″ W | 10 | C5715 | PX909842 - PX909851 |
| Ubatuba, São Paulo (SP) | 23°29′ 23″ S<br>45°09′ 54″ W | 1 | C5716 | PX909864 |
| Laguna, Santa Catarina, Brazil (SC) | 28°28′ 50.8″S<br>48°51′ 77.8″ W | 13 | C5729 | PX909865 - PX909877 |
| Torres, Rio Grande do Sul, Brazil (RS) | 28°28′ 13.2″S<br>48°51′ 30.5″ W | 11 | C5730 | PX909831 - PX909841 |

*Note:* Sampling site codings are used in figures and text.

https://www.gbif.org/) (GBIF 2023); iNaturalist: https://www.inaturalist.org/(using only research level); Integrated Digitized Biocollections: https://www.idigbio.org/and Ocean Biodiversity Information System: https://obis.org/(accessed on 21/02/2023) (Figure 1).

## 2.2 | Molecular Analysis

Mitochondrial DNA (mtDNA) from 71 individuals was extracted from the muscle tissue of the pereiopods or chelae using the Puregene (Gentra Systems) buffer system method. As not all samples successfully amplified the 931 bp fragment, due to older specimens failing to amplify for longer fragments, we amplified three different fragments (326, 706 and 931 bp) from the same COI region. This approach allows us to explore datasets with a larger number of individuals but shorter fragment lengths, and datasets with fewer individuals or sampling sites, but longer alignments containing more genetic information. A 706 base pair (bp) region of the mitochondrial gene cytochrome oxidase subunit I (COI) was amplified using the crustacean specific primers COL1b 5′-CCW GCT GGD GGW GGD GAY CC-3′ and COH1b 5′-TGT ATA RGC RTC TGG RTA RTC-3′, a shorter region of 326 bp using the primers COL3 5′-ATR ATT TAY GCT ATR HTW GCM ATT GG-3′ and COH3 5′-AAT CAR TGD GCA ATW CCR SCR AAA AT-3′, and a longer region of 931 bp using the primers COL8 50-GAY CAA ATA CCT TTA TTT GT-30 5′ 3′ and COH16 5′-CAT YWT TCT GCC ATT TTA GA-3′ (Schubart 2009), by polymerase chain reaction (PCR) (40 cycles: 45 s 94°C/1 min 48°C/75 s 72°C denaturing/annealing/elongation temperatures). Amplification results were checked by running 4 μL of PCR product on 1.5% TBE agarose gel electrophoresis. PCR products were outsourced for sequencing with the primer COL8, COL1b or COL3 to Macrogen Europe Inc. (Amsterdam, the Netherlands). Obtained sequences were edited in Chromas Lite 3.01 (https://technelysium.com.au/wp/chromas/) and aligned using the ClustalW algorithm implemented in MEGA version X (Kumar et al. 2018) with default parameters. All sequences were submitted to GenBank (accession numbers: PX909808 - PX909877).

## 2.3 | Genetic Diversity and Population Structure

Population genetic analyses were performed in DNAsp 5.10 (Librado and Rozas 2009) to assess the number of haplotypes ($h$), number of polymorphic sites ($S$), haplotype diversity (Hd), and nucleotide diversity ($\pi$). Haplotype networks were constructed for the three datasets with different alignment lengths using the R package *pegas* (Paradis 2010). Considering the trade-offs between the number of samples and fragment length, downstream analyses were conducted using only the 706 bp dataset.

To assess levels of genetic differentiation among sampling sites and regions we calculated pairwise Fst values (Weir and Cockerham 1984) using the R package *hierfstat* (Goudet 2005) and performed an AMOVA (Excoffier et al. 1992) with the R package *poppr* (Kamvar et al. 2014). Significance was assessed with 1000 random permutations. AMOVA was performed hierarchically to estimate the genetic variation among regions (Caribbean × Brazil) and among sampling sites within regions. We also performed a discriminant analysis of principal components (DAPC) with the R package *adegenet* (Jombart and Ahmed 2011) using a supervised approach to group definition, considering each sampling site as a separate population. To assess fine-scale structure within the Brazilian coast, we performed another DAPC employing only samples from this region.

To assess the genetic differentiation within the Brazilian region and identify possible genetic discontinuities, we used the R-based computer package GENELAND 4.9.2 (Guillot et al. 2005). This package uses georeferenced populations genetic data and Markov Chain Monte Carlo (MCMC) methods to infer the most likely number of clusters and their geographic distribution under a Bayesian framework. The analysis assumes an unknown number of clusters with equal likelihood. We set the maximum number of clusters (K) to 5, based on haplotype networks results, and used a maximum Poisson process rate equal to the number of individuals in the dataset. The uncorrelated frequency model was applied, and the maximum number of nuclei was set to three times the maximum Poisson rate (as suggested by Guillot et al. 2005). We ran 5,000,000 MCMC iterations, sampling every 1000, and the first 500 samples were discarded as burn-in.

## 2.4 | Phylogenetic Analysis

Maximum likelihood (ML) tree search was conducted for the 326 and 706 bp datasets in RAxML v.8.2.11 (Stamatakis 2014). The best-fit model was selected based on ModelFinder results (Kalyaanamoorthy et al. 2017). Branch support for the ML tree was evaluated via rapid bootstrapping with autoMRE bootstopping, which automatically determines the number of replicates required to stabilize support values. Sequences from three *Armases* species (*A. angustipes*, *A. bennedicti*, and *A. miersii*) were used as outgroups.

## 2.5 | Demographic History

We performed Tajima's D and Fu's $F$ tests to check for potential deviations from neutrality that would indicate historical demographic changes. Observed values were compared against the null distribution generated under the standard neutral model to evaluate significance. Positive values indicate an excess of common variation, suggesting a population bottleneck. On the other hand, negative values indicate an excess of low frequency sites within the population, which might point to a recent population growth (Tajima 1989; Fu 1997). Additionally, we assessed the timing of possible demographic events through a Coalescent Bayesian Skyline Plot (CBSP), implemented in BEAST2 (Drummond et al. 2005). The analysis was performed separately for the Caribbean and the two Brazilian genetic groups (see Results). We applied the GTR model and a strict molecular clock with a mutation rate of 0.0166 per Myr (Schubart et al. 1998). We performed three independent MCMC runs with 10,000,000 generations each, sampling every 10,000 generations, and discarding the first 10% as burn-in. After burn-in removal, replicates were combined with LogCombiner (Rambaut and Drummond 2014).

Convergence was assessed by checking the trace plots on Tracer v1.7 (Rambaut et al. 2018), ensuring an effective sample size (ESS) > 200 for all parameters.

## 3 | Results

### 3.1 | Genetic Diversity and Population Structure

The DAPC analysis using the 706 bp dataset recovered three genetic clusters: individuals from Costa Rica and two clusters from Brazil, namely the North and South groups (Figure 2A). Restricting the analysis to samples from the Brazilian coast, we recovered increased resolution, with nearly 100% of the genetic differentiation within Brazil captured by the first axis, separating North and South groups (Figure 2B). Genetic diversity summary statistics revealed variation among populations of *A. rubripes* (Table 2). Within the Brazilian coast, the South group had lower values of haplotype and nucleotide diversity (0.445 and < 0.001, respectively) when compared to the North group (0.905 and 0.003, respectively). The average haplotype and nucleotide diversity for the entire Brazilian group was 0.870 and 0.003, respectively. In the Caribbean, the haplotype and nucleotide diversity in Costa Rica was 0.800 and 0.001, respectively. The average haplotype and nucleotide diversity for all *A. rubripes* samples was 0.905 and 0.016, respectively.

Genetic distances among populations supported the DAPC-defined structure (Table 3). Distances between North and South Brazilian clusters were low (0.0057), while distances between Brazilian clusters and the Caribbean were 10-fold higher (0.0555–0.0533), indicating substantial regional differentiation. Comparisons with outgroup species revealed much higher distances, averaging 0.1 between Brazilian clusters and *A. angustipes*, *A. benedicti*, and *A. miersii*. The genetic differentiation along the Brazilian coastline is confirmed by GENELAND, which robustly supports the two clusters ($K = 2$) observed in the haplotype network (Figure 6). Pairwise Fst values showed high levels of population genetic structure among Costa Rica and the Brazilian groups (Table 4). Between sampling sites within the Brazilian group, Fst values ranged from −0.004 between Santa Catarina and São Paulo to 0.034 between Rio Grande do Norte and Rio Grande do Sul (Table S1). Hierarchical AMOVA was performed to assess levels of genetic differentiation at different geographical scales (Table 4). Results reveal strong and significant levels of differentiation among regions (91.14%), low variation among sampling sites within regions (5.73%), and no significant genetic variation within sampling sites.

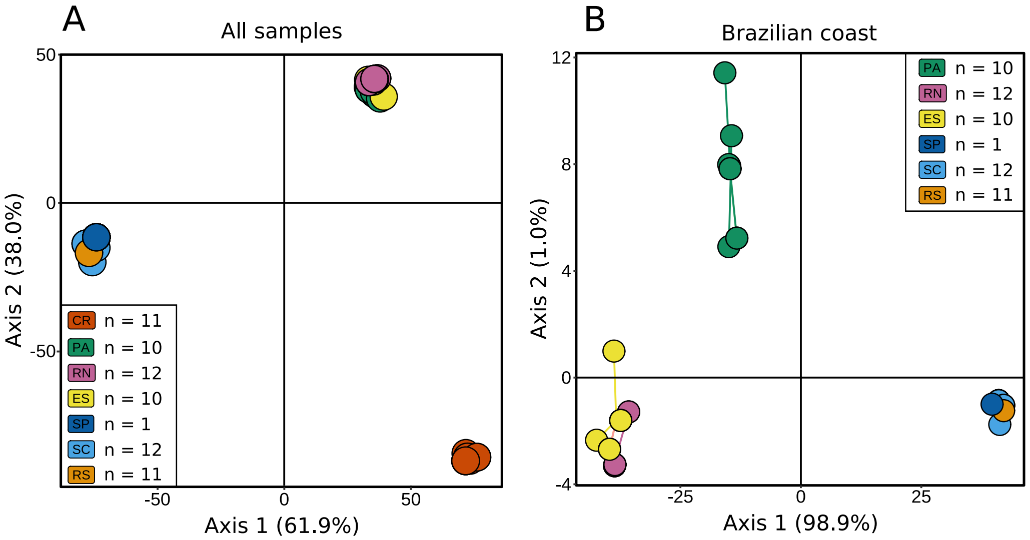

**FIGURE 2** | Discriminant analysis of principal components (DAPC) of *Armases rubripes* derived from COI mtDNA with 706 base pairs. A—considering all sampling sites that successfully amplified for the 706 bp dataset, and B—considering only samples from the Brazilian coast. CR, Costa Rica; ES, Espírito Santo; PA, Pará; RN, Rio Grande do Norte; RS, Rio Grande do Sul; SP, São Paulo; SC, Santa Catarina.

**TABLE 2** | Genetic diversity indices and neutrality tests for each sampling site of *Armases rubripes* based on the COI gene with 706 base pairs.

| | Location | *N* | *h* | *S* | Hd | $\pi$ | Tajima's D | Fu's Fs |
|---|---|---|---|---|---|---|---|---|
| 706 bp | Costa Rica (CR) | 11 | 6 | 5 | 0.800 | 0.001 | −1.46 | −1.44 |
| | Brazil Group | 56 | 21 | 22 | 0.870 | 0.003 | −1.31 | −3.24* |
| | PA + RN + ES (North Group) | 32 | 14 | 15 | 0.905 | 0.003 | −1.29 | −2.07 |
| | SP + SC + RS (South Group) | 24 | 7 | 6 | 0.445 | < 0.001 | −2.08* | −3.17** |
| | *A. rubripes* all sequences | 67 | 27 | 50 | 0.905 | 0.016 | 0.39 | −1.67 |

Abbreviations: *h*, number of haplotypes; Hd, haplotype diversity; *N*, number of individuals; *S*, number of polymorphic sites; $\pi$, nucleotide diversity.
*$p < 0.05$.
**$p < 0.01$.

**TABLE 3** | Mean genetic distances within and between *Armases rubripes* populations and outgroups based on COI gene with 706 base pairs.

| | *A angustipes* | *A benedicti* | *A miersii* | South | North | Caribbean |
|---|---|---|---|---|---|---|
| *A angustipes* | NA | | | | | |
| *A benedicti* | 0.0984 | NA | | | | |
| *A miersii* | 0.0650 | 0.1015 | NA | | | |
| South | 0.1002 | 0.1070 | 0.1119 | 0.0007 | | |
| North | 0.1043 | 0.1113 | 0.1159 | 0.0057 | 0.0033 | |
| Caribbean | 0.1088 | 0.1173 | 0.1130 | 0.0555 | 0.0533 | 0.0017 |

**TABLE 4** | AMOVA results and pairwise Fst values showing levels of genetic structure of *Amases rubripes* based on COI mtDNA 706 basepairs.

| **AMOVA** | | | | |
|---|---|---|---|---|
| **Source of variation** | **Df** | **% of variation** | **Φ-statistic** | ***p*** |
| Among regions | 1 | 91.14 | 0.9688 | 0.001 |
| Among sampling sites within regions | 5 | 5.73 | 0.6478 | 0.001 |
| Within sampling sites | 60 | 3.11 | 0.9114 | 0.128 |
| Pairwise Fst values | | | | |
| | | South | | North |
| North | | 0.025* | | |
| CR | | 0.268* | | 0.251* |

Abbreviation: D.f., degrees of freedom.
*$p < 0.05$.

The haplotype networks built from the three different datasets recovered no shared haplotypes between the two major regions (Caribbean and Brazil). Based on the 326 bp dataset, Costa Rica showed two unique haplotypes that were at least five mutational steps away from Colombia and Venezuela and 21 mutational steps away from the Brazilian haplotypes (Figure 4). Based on the 706 bp, Costa Rica was 32 mutational steps away from the Brazilian haplotypes (Figure 5). Within the Brazilian coast, there was an evident separation of the sampling sites of the South group (RS, SC, SP) from the remaining sampling sites of the North group (ES, PA, RN), with no shared haplotypes between these two regions. Except SP, all remaining sampling sites within the Brazilian coast had at least one private haplotype. Furthermore, PA was the only sampling site that did not share any haplotypes with the remaining Brazilian sites, showing six exclusive haplotypes (Figure 5). The same pattern was recovered with the 936 bp dataset, with Costa Rica being 41 mutational steps away from the Brazilian haplotypes, and no shared haplotypes between the North and South groups of Brazil (Figure S1). Despite not sharing any haplotypes, the most common haplotypes from the North and South groups were separated by only one or two mutational steps (Figures 4 and 5).

## 3.2 | Phylogenetic Analysis

The best-fit model selected by ModelFinder for both the 326 and 706 bp phylogenies was GTR + GAMMA (Tavare 1986), and 300 bootstrap replicates were completed before convergence. In the 706 bp tree, Caribbean and Brazilian samples were recovered as two well-supported (bootstrap support (BS) > 95), reciprocally monophyletic groups (Figure 3). Samples from Costa Rica formed a well-supported clade (BS = 96) in both reconstructions, and samples from Colombia and Venezuela grouped in the 326 bp phylogeny with high support (BS = 98, Figure S1). Within Brazil, the South and North groups did not form reciprocally monophyletic clades, with samples from both populations mixing in both reconstructions (Figure 3 and Figure S1).

## 3.3 | Demographic History

To investigate population dynamics within Brazil and the Caribbean, we ran Bayesian Skyline Plots (BSPs) for each genetic cluster separately (South and North Brazil, and Caribbean) (Figure 7). The South Brazil cluster showed no evidence of population expansion or contraction in the BSP, with coalescent events extending only ~6 kya, consistent with its lower genetic diversity compared to the northern Brazil and Caribbean populations. Despite the limited BSP signal, the southern cluster exhibited significantly negative Tajima's D and Fu's Fs values, suggesting a recent demographic change. The North Brazil cluster displayed signs of a steady, moderate population expansion, although the signal is not pronounced. Neutrality tests were negative but not statistically significant for the North cluster. In contrast, the Caribbean population showed no evidence of

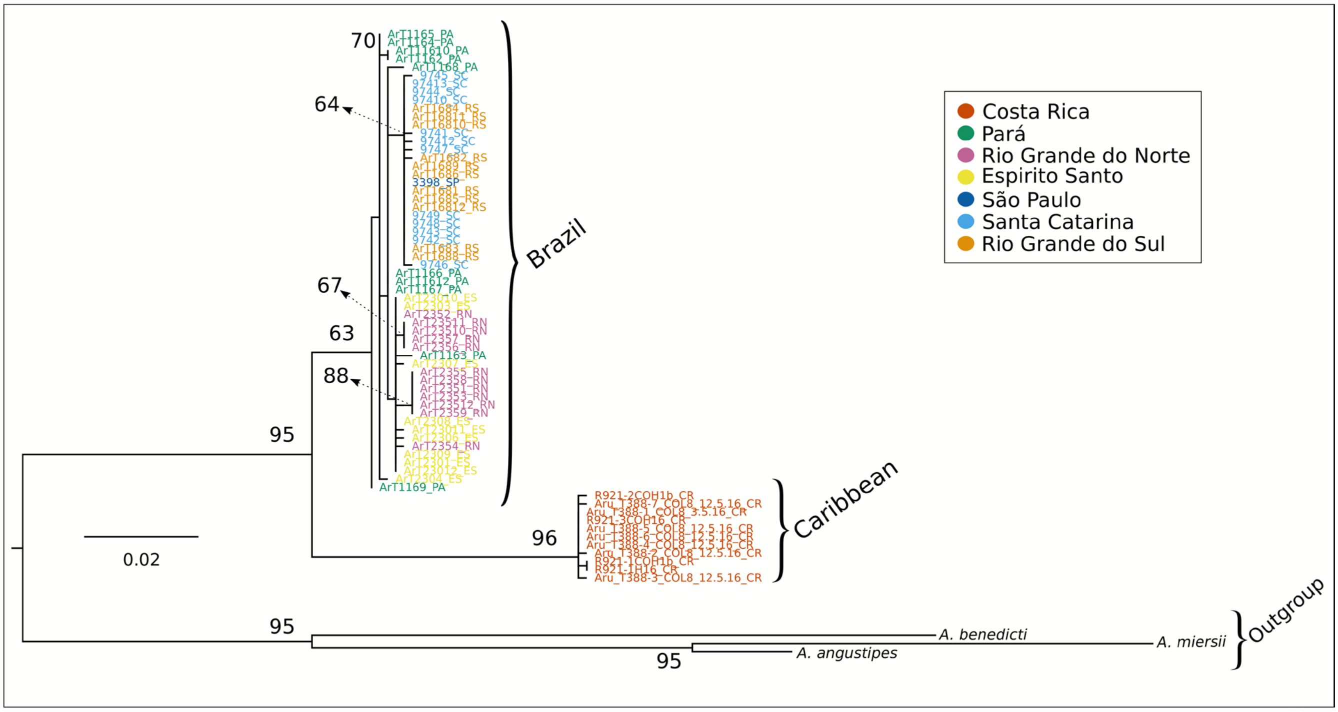

**FIGURE 3** | Maximum likelihood (ML) phylogenetic tree of *Armases rubripes* based on the 706 base pairs of the COI mtDNA. Numbers on branches represent the bootstrap support values.

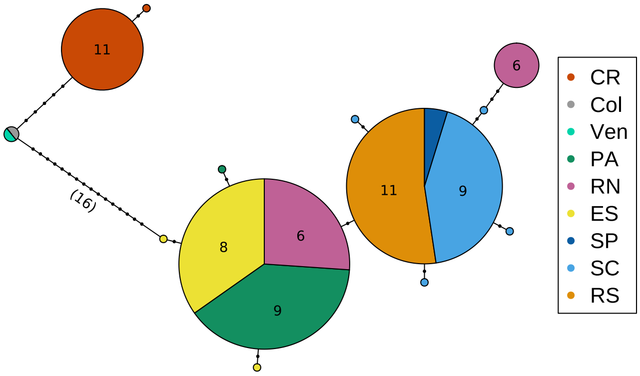

**FIGURE 4** | Maximum parsimony spanning networks of *Armases rubripes* derived from COI mtDNA with 326 base pairs. Colors indicate sampling sites. Black dots represent missing intermediate haplotypes. COL, Colômbia; CR, Costa Rica; ES, Espírito Santo; PA, Pará; RN, Rio Grande do Norte; RS; Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo; VEN, Venezuela.

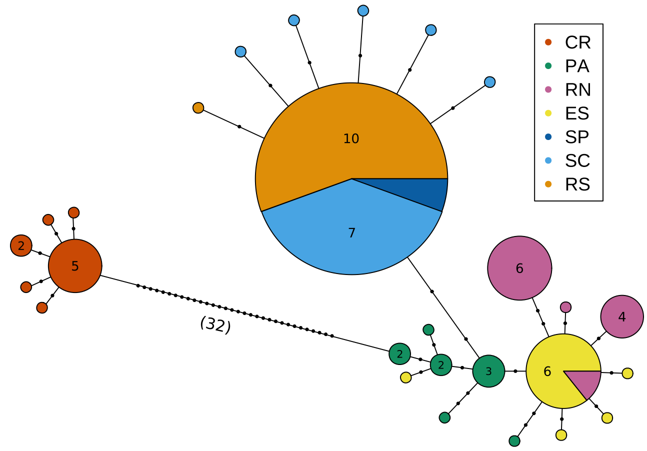

**FIGURE 5** | Haplotype network of *Armases rubripes* derived from COI mtDNA with 706 base pairs. Colors indicate sampling sites. Black dots represent missing intermediate haplotypes. CR, Costa Rica; ES, Espírito Santo; PA, Pará; RN, Rio Grande do Norte; SC, Santa Catarina; RS, Rio Grande do Sul; SP, São Paulo.

recent demographic changes in the BSP, consistent with a stable population size over time.

## 4 | Discussion

### 4.1 | Population Structure

Our results suggest that Armases *rubripes* may represent a cryptic species complex. We recovered high pairwise Fst values between Costa Rica and the Brazilian sampling sites, considerable distance between Caribbean and Brazilian haplotypes, and a high percentage of genetic variation among regions obtained by the AMOVA analysis. Fst values between Caribbean and Brazilian populations (Fst >0.2) were also an order of magnitude greater than those observed among sites along the Brazilian coast (mean Fst <0.02). In our results, Caribbean and Brazilian haplotypes are separated by more than 30 mutational steps in the haplotype network, indicating lack of gene flow and isolation. This pattern is further supported by our phylogenetic analyses (Figure 3), which recovered Caribbean and Brazilian populations as reciprocally monophyletic with high statistical support. We also recovered high genetic distances between Brazilian and Caribbean groups (~5.4%), which is an order of magnitude higher than the mean genetic distance between South and North groups (0.57%). Similar genetic distance values

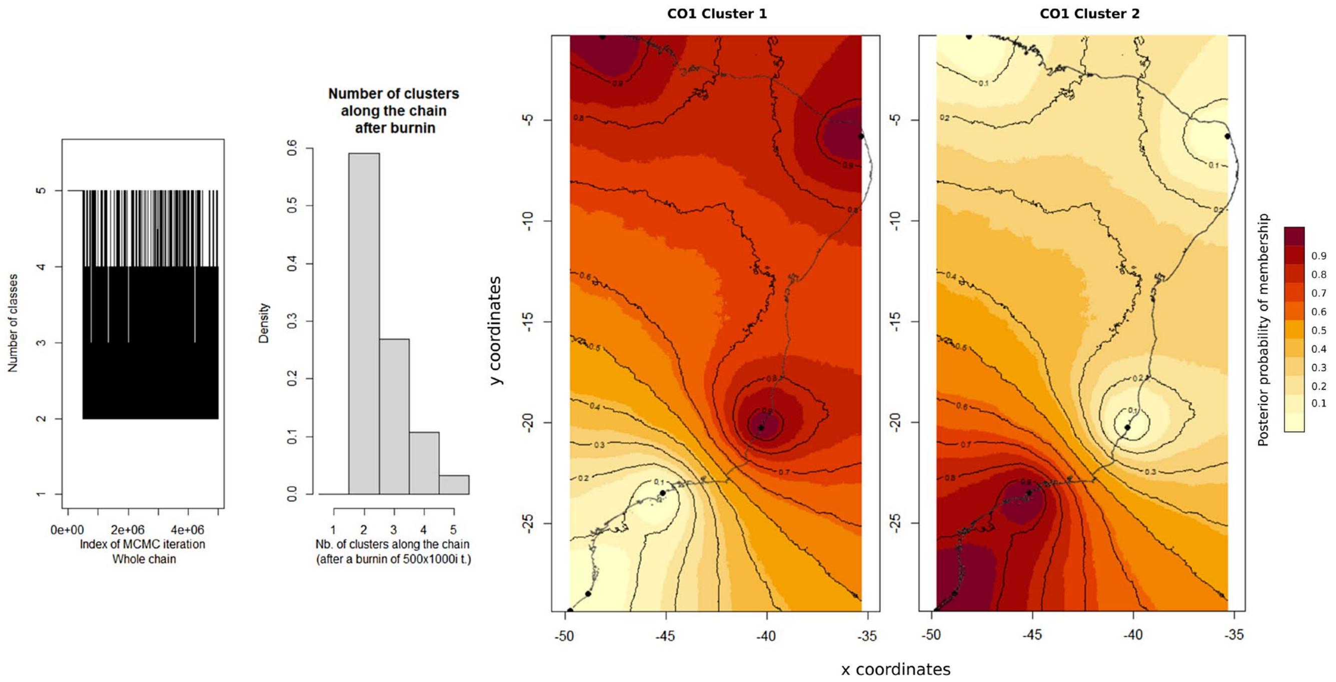

**FIGURE 6** | Number of clusters supported by GENELAND and maps of posterior probability of cluster membership derived from COI mtDNA with 706 base pairs. Black dots indicate the relative position of the sampled sites. Coloration proportional to the posterior probability of population membership to the cluster (darker color indicating higher probability), with isolines of probability. MCMC, Markov chain Monte Carlo.

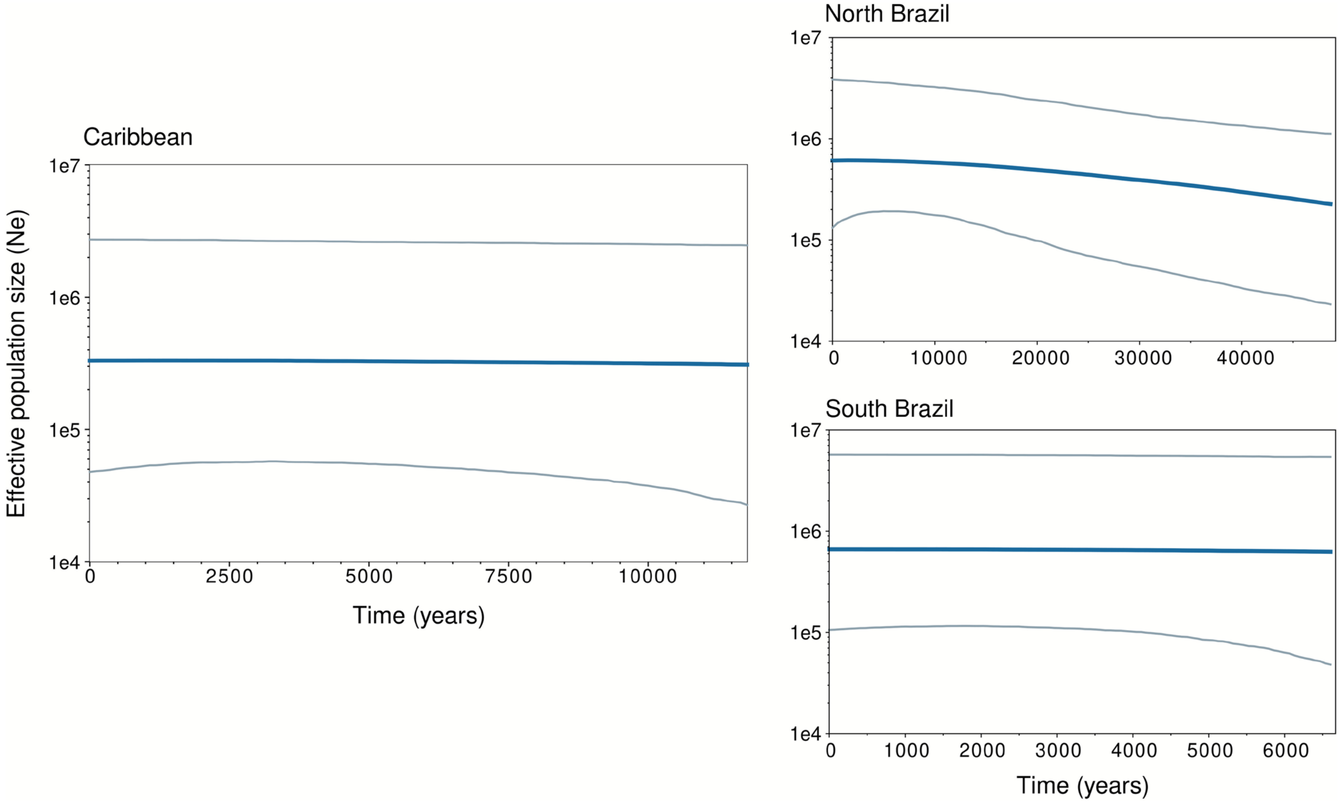

**FIGURE 7** | Bayesian skyline plot built with BEAST v2 and Tracer v1.7 based on 706 base pairs of the COI mtDNA. *Y*-axis represents the effective population size (Ne) and *X*-axis represents time in years. The dark-blue line represents the median estimate, and the light-blue shading represents the 95% posterior probability.

(3.28%) were found between the Sesarmidae species *Aratus pisonii* and *Aratus pacificus* (Thiercelin and Schubart 2014). These patterns are consistent with COI divergence thresholds recovered in decapods, where the mean intraspecific genetic distances rarely exceed 2% (Matzen da Silva et al. 2011). Together, these results provide strong evidence for a potential species-level differentiation (Cracraft 1983; De Queiroz 2007) between these two geographically separated regions, underscoring the need for an integrative taxonomic reassessment of *A. rubripes* across its range.

The genetic divergence between marine species from the Caribbean and Brazilian provinces is well documented in the literature (Laurenzano et al. 2016; Nunes et al. 2017; Araujo et al. 2022; Teschima et al. 2021). The most prominent barrier to dispersal between these regions is the Amazon–Orinoco River plume (Latrubesse et al. 2010), whose extensive freshwater discharge can inhibit the movement of marine migrants (Tosetto et al. 2022). Although *A. rubripes* larvae cannot complete development at salinities below 10, it is plausible that episodic atmospheric events—such as prolonged droughts that increase salinity or strong winds that facilitate short-term transport—allow rapid movement across low-salinity waters (Luppi et al. 2003). Previous studies using the same genetic marker in Sesarmidae and Ocypodidae crabs have shown gene flow between populations north of the Amazon–Orinoco discharge (Trinidad and Tobago, Venezuela) and those south of the barrier (Guyana, northern Brazil) (Buranelli and Mantelatto 2019; Thurman et al. 2021; Marochi et al. 2022). In some Sesarmidae species, long planktonic larval duration (PLD) may enable larvae to reach higher-salinity waters where development can continue (Marochi, Martins, and Masunari 2017) and enable gene flow between populations. *A. rubripes* exhibits a relatively long larval stage—lasting 19–37 days from zoea I to megalopa depending on temperature and salinity—although the duration from megalopa to the first juvenile remains unknown (Luppi et al. 2003). However, increasing evidence suggests that PLD alone is not an optimal proxy for population connectivity in marine species (Selkoe and Toonen 2011). Larval behavior and physiological thresholds, particularly salinity and temperature tolerance, can exert stronger influences on survival, development time and dispersal, ultimately limiting connectivity (Marochi et al. 2024; Martins et al. 2024). Behavioral traits such as active swimming, orientation and habitat recognition via olfactory cues (Kingsford et al. 2002; Montgomery et al. 2006) may further constrain dispersal by promoting local retention.

The major surface currents and microcurrent systems in the Caribbean likely function as physical barriers, contributing to the genetic heterogeneity observed between Caribbean and Brazilian populations (Centurioni and Niiler 2003). These circulation patterns are known to shape population structure in multiple marine invertebrates (Lessios et al. 2003; Kool et al. 2010; White et al. 2010) and in semiterrestrial crabs (Laurenzano et al. 2013; Buranelli and Mantelatto 2019; Thurman et al. 2021; Marochi et al. 2022) by limiting larval exchange between the Caribbean Sea and South America. Thus, the lack of connectivity among *A. rubripes* populations across these regions, as recovered in our analyses, is likely the result of the combined effects of surface currents, larval physiological constraints, behavioral mechanisms, and PLD.

Previous genetic surveys of estuarine crab species along the Brazilian coast have revealed a pattern of genetic homogeneity. For example, panmixia was reported for *Uca rapax* (Ocypodidae), which inhabits open mud or salt flats and estuarine margins with long pelagic larval phases of ~8 weeks, likely promoting dispersal across a broad geographic scale (Laurenzano et al. 2013). Similarly, *Uca maracoani*, which occurs in mesohaline and euryhaline habitats (12–32 psu) along mudflats, mangroves, and riverbanks, exhibited little to no population structure, with an expected larval duration of 20–30 days (Wieman et al. 2014; Marochi et al. 2022). *Ucides cordatus*, a mangrove crab with larvae tolerant to a wide range of salinities, also showed high connectivity across large geographic scales (Britto et al. 2018). Within Sesarmidae, *Aratus pisonii*, an obligate mangrove dweller, and *Armases angustipes*, a habitat generalist with an export larval strategy and prolonged pelagic development in high salinities (> 20 psu), have also been reported to exhibit low levels of genetic structure (Thiercelin and Schubart 2014; Marochi, Masunari, and Schubart 2017). In contrast, we observed genetic differentiation within the Brazilian coast, demonstrating low levels of structure between the North and South groups, and structuring within the north group. This was supported by multivariate clustering analysis, Geneland results based on allele frequency data, and haplotype networks (Table 3, Figures 2–6), although the latter demonstrated only one or two mutational steps separating the two groups. Additionally, the cluster analysis revealed minimal separation between the Para population and the remaining populations within the North group, similar to what was reported for phylopatric reef building species known to have high levels of population differentiation (Adam et al. 2024). Along the Brazilian coast, the most influential current is the South Equatorial Current (SEC), which splits into the northward-flowing Northeast Brazilian Current (NBC) and the southward-flowing Brazil Current (BC) (Peterson and Stramma 1991). Our results demonstrate moderate levels of genetic heterogeneity for *A. rubripes* along the Brazilian coast, but the recovered genetic structure does not seem to be caused by the SEC. The southeastern coast of Brazil harbors regions of considerable temperature stratification in the water column and occurrence of cyclonic meanders in the BC near the coast, such as the Cabo Frio upwelling (Valentin 2001), which has shown to be a barrier for fish species (Volk et al. 2021) and could explain the separation between North and South groups. Nonetheless, it should be noted that the haplogroups represented by each of these populations are separated by only one or two mutational steps, and that these two groups failed to form reciprocally monophyletic groups. Furthermore, genetic distances between South and North are only 0.57%, demonstrating extremely low levels of differentiation.

Unfortunately, the low number of individuals from Colombia and Venezuela limits our ability to provide more in-depth insights concerning the Caribbean region. However, the results derived from the 326 bp dataset suggest low levels of genetic differentiation between the two populations and Costa Rica. Further studies should delve deeper into the structure within the Caribbean clade, which will lead to a more comprehensive understanding of the underlying dynamics of sesarmid crabs in these regions.

## 4.2 | Demographic History and Genetic Diversity

The Last Glacial Maximum (LGM) significantly reduced suitable coastal habitats for tropical and subtropical species and was responsible for major demographic changes in marine taxa worldwide due to sea-level and temperature oscillations (Aris-Brosou and Excoffier 1996; Fu 1997; Yokoyama et al. 2000; Hewitt 2004; Clark et al. 2009). Consistently, demographic studies of marine invertebrates have shown patterns of population expansion after bottlenecks that can be linked to the LGM (Baeza and Fuentes 2013; Baeza et al. 2019; Diringer et al. 2019). In the Brazilian group, Bayesian skyline plots indicate a tendency of population growth in the North group (Figure 7) that is consistent with the onset of the major global deglaciation phase after the LGM (Lambeck et al. 2002), but 95% posterior probability intervals are large and neutrality tests are not significant for this population (Table 2). The South Brazil cluster exhibits low genetic diversity ($\text{Hd} = 0.445$, $\pi < 0.001$) compared to the North group ($\text{Hd} = 0.905$, $\pi = 0.003$), a trend mirrored in the closely related species *Armases angustipes* (Marochi, Martins, and Masunari 2017). Coupled with the significantly negative Tajima's D and Fu's *F* values (Table 2), this would suggest a recent demographic change. However, BSP shows coalescent events only as far back as ~6kya, indicating limited temporal resolution and could imply a demographic change that is not picked up by the BSP analysis. The estimation of the population growth of other semiterrestrial crabs along the Western Atlantic is dated from 14 to 52 kya (Marochi, Masunari, and Schubart 2017; Buranelli and Mantelatto 2019), corroborating our findings.

However, there are examples of populations that were not equally affected by past sea-level fluctuations (see Ludt and Rocha 2015 for a review). Conforming, our demographic inference analysis of the Costa Rica sampling site suggests that this population remained stable over time. This is further corroborated by the neutrality tests, which indicate no significant demographic changes. These results suggest that *A. rubripes* populations in Costa Rica were not significantly affected by recent biogeographic events, a pattern also observed in other crustacean populations in the Caribbean, suggesting demographic stability over time (Baeza and Fuentes 2013; Peres and Mantelatto 2020). Overall, our results indicate that the demographic history of *A. rubripes* is heterogeneous across its range. Future studies incorporating multi-locus data and more comprehensive sampling across the wider Caribbean should provide deeper insights into the recent demographic history of *A. rubripes* in the region.

## 5 | Conclusions

This is the first study to assess the genetic diversity and population structure of the semi-terrestrial crab *Armases rubripes*. Our results revealed multiple layers of genetic differentiation within the species. First, we observed significant genetic structure between populations from the Caribbean and Brazilian regions, with these regions being recovered as well-supported clades in our phylogenetic reconstructions, suggesting a potential cryptic species complex. Second, we identified clear genetic structure within the Brazilian coast, grouping the sampling sites into two clusters: South and North groups. Lastly, an additional substructure is recovered within the North group. Neutrality tests failed to reject the null hypothesis of neutrality for the majority of the sampling sites, with the exception of the South group. Future studies should adopt an integrative approach, combining multilocus and morphological data to confirm the possible species-level divergence between the Caribbean and Brazilian provinces. Additionally, within the Brazilian provinces, assessing the roles of upwelling and ocean currents as potential barriers to gene flow is a promising avenue for future research. Our study not only sheds light on the complex genetic makeup of *A. rubripes*, but also underscores key directions for future investigation into its evolutionary dynamics.

### Author Contributions

M.Z.M., S.M. and C.D.S. conceived the original idea. C.D.S. supervised the project with support from S.M.; M.Z.M. carried out the sampling, extraction, amplification and sequencing with support from N.T. and L.W.; C.L.A. performed the analyses with support from M.Z.M. and N.T.; C.L.A. and M.Z.M. wrote the manuscript. All authors provided critical feedback and helped shape the research, analysis and manuscript.

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### Conflicts of Interest

The authors declare no conflicts of interest.

### Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Haplotype network of *Armases rubripes* derived from COI mtDNA with 931 base pairs. Colors indicate sampling sites. Black dots represent missing intermediate haplotypes. CR: Costa Rica, PA: Pará, RN: Rio Grande do Norte, ES: Espírito Santo, SP: São Paulo and RS: Rio Grande do Sul. **Figure S2:** Maximum likelihood (ML) phylogenetic tree of *Armases rubripes* based on the 326 base pairs of the COI mtDNA. Numbers on branches represent the bootstrap support values. **Table S1:** Pairwise Fst values showing levels of genetic structure of *Amases rubripes* sampling sites based on COIox1 mtDNA 706 basepairs.