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Phylogeographic Structure within the Fiddler Crabs *Leptuca thayeri* and *Uca maracoani* (Brachyura, Ocypodidae) along the Tropical West Atlantic

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Most fiddler crabs have an extended planktonic larval phase, potentially maintaining gene flow among widely separated populations, in the absence of marine barriers. Such marine barriers could be long coastal stretches without suitable habitat, freshwater plumes caused by large river mouths, or strong currents. Typically, fiddler crabs inhabit mangrove habitats, and as mangroves tend to have a patchy distribution, it is important to gather information on the connectivity between neighboring mangroves and recognize local endemisms. To detect potential genetic differentiation among mangrove-dwelling populations of *Leptuca thayeri* and *Uca maracoani* along several thousand kilometers of a tropical coastline, mtDNA sequences of different populations from Brazil and two Caribbean islands were analyzed and compared. As shown in previous studies with fiddler crabs, Brazilian populations are genetically indiscernible, and our data suggest the absence of long-standing gene flow barriers in the two studied species along the Brazilian coast. This includes both sides of the postulated biogeographic barriers corresponding to the split of the Central South Equatorial Current and to the Amazon River freshwater plume. In contrast, conspecific individuals from the Greater Antilles carried different haplotypes, suggesting a biogeographical barrier between Brazil and the Caribbean, apparently having limited gene flow between both regions for extended time periods.

Key words: Cox1 mtDNA, Restricted gene flow, Population genetics, Brazil, Caribbean.

BACKGROUND

Genetic connectivity can be broadly defined as the degree to which populations are considered open or closed by gene flow and dispersal patterns (Hellberg et al. 2002). In most marine invertebrates, planktonic larval development is part of their reproductive strategy, resulting in high dispersal potential (Muñiz-Salazar et al. 2005; Anger 2006). The longer the time span of the planktonic larval phase, the farther the propagules can possibly be transported passively by ocean currents (up to several hundred kilometers) and the higher the

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potential gene flow can be expected among widely separated populations (Silva et al. 2010). This, in turn, can prevent evolution of genetic structuring and local adaptations (Kelly and Palumbi 2010; Bray et al. 2017). In the case of restricted gene flow, the consequent genetic differentiation is not immediately expressed and thus not recognized. Lack of phenotypic divergence may indicate that a species is in morphological stasis or differences among populations are very subtle and require careful diagnosis (Chaklader et al. 2016; Marochi et al. 2017).

Barriers to dispersal in the marine environment may not result from landscape features as in terrestrial ecosystems, but they may occur due to physical and chemical features, such as salinity, temperature, currents patterns, and eddies (Cowen 2002; Banks et al. 2007; Weersing and Toonen 2009; Chapman et al. 2011). For example, the Amazon River discharge in northeastern Brazil and the Orinoco River discharge in Venezuela send great plumes of freshwater into the adjacent ocean, changing the salinity in nearby coastal areas, and thereby potentially hindering the dispersal of organisms that do not tolerate different salinity gradients (Lessios et al. 2001 2003; Hu et al. 2004). The Central South Equatorial Current (CSEC) splits into the North Brazil and South Brazil currents, carrying organisms in opposite directions (Marochi et al. 2017). Also, upwelling and downwelling processes may act as a barrier to dispersal due to temperature gradients and may result in local larval retention (Lessios et al. 2003; Taylor and Hellberg 2003). Thus, despite extensive larval dispersal potential, populations along the coast may present marked genetic differences from each other (disjunctive variation), or show gradual differences (clinal variation), which are not always evident at first, since the same genotype can produce different phenotypes following environmental traits (Scheltema 1975).

Fiddler crabs are distributed in tropical and subtropical coastal areas all over the world, mostly in estuarine areas with mangrove and salt marsh vegetation (Crane 1975; Shih et al. 2016). The postlarval non-migratory nature and low dispersal capacity of fiddler crabs after metamorphosis to benthic stages would result in potential genetic structure between widely separated populations. However, their planktonic larval stages achieve a greater level of genetic exchange among widespread littoral populations. Silva et al. (2010) found no regional genetic or morphological differentiation within a large study area (~3,300 km) and convoluted ocean current circulation patterns in *Austruca occidentalis* (described by Naderloo et al. 2016 as *Uca occidentalis*) along the East African coast. Likewise, a lack or limited genetic structuring was also observed among populations of South American brachyuran crab species along the Atlantic coast (Oliveira-Neto et al. 2007; Laurenzano et al. 2012; Hampton et al. 2014; Wieman et al. 2014; Marochi et al. 2017; Buranelli and Mantelatto 2019). On the other hand, higher genetic differentiation has been reported for Caribbean and Central American populations (Laurenzano et al. 2013; Buranelli and Mantelatto 2019; Peres et al. 2020; Thurman et al. 2021).

Leptuca thayeri (Rathbun, 1900) and *Uca maracoani* (Latreille, 1802) are common and widely distributed neotropical fiddler crab (Crane 1975). *Leptuca thayeri* inhabits burrows in muddy and sandy mangrove areas (commonly among roots) in mesohaline and euryhaline habitats in the mid intertidal zone and is distributed along the Western Atlantic coast from the USA (Florida), Gulf of Mexico, Cuba, Jamaica, Puerto Rico, Guatemala, Venezuela, Trinidad and Tobago, to Brazil (Pará, Maranhão, Piauí, Ceará, Rio Grande do Norte, Paraíba, Pernambuco, Alagoas, Sergipe, Bahia, Espírito Santo, Rio de Janeiro, São Paulo, Paraná, Santa Catarina) (Crane 1975; Thurman et al. 2013; Farias et al. 2014; Mantelatto et al. 2020). The larval duration of *L. thayeri* from hatching to metamorphosis lasts approximately one month, and successful larval development only occurs in salinities higher than 20 ppt (Anger et al. 1989). *Uca maracoani* has been recorded along the Atlantic coast of South America from Trinidad and Tobago, Venezuela, and Guyanas to the south of Brazil (Melo 1996; Wieman et al. 2014), with so far dubious records from the Antilles. *Uca maracoani* inhabits mesohaline and euryhaline habitats (salinity varying from 14 to 32 ppt) on mud flats along bays, estuaries, mangrove areas or riverbanks close to their mouths in the low intertidal zone (Masunari 2006; Wieman et al. 2014). Even if the first zoea and megalopa stages are described morphologically (Negreiros-Fransozo et al. 2009), there is a lack of information in literature regarding the duration of the larval phase and their salinity preferences. However, a similar developmental time, from hatching to metamorphosis, as in other fiddler crab species is expected (≅ 20 to 30 days) (Wieman et al. 2014). The different habitat preferences and optimum salinities during larval development can influence the level of genetic connectivity among populations, and higher genetic structure can be expected in more oligohaline species, showing larval retention mechanisms in estuaries, favored by active larval behavior (*e.g.*, vertical migration) (Cronin and Forward 1986; Kelly and Palumbi 2010; López-Duarte et al. 2011; Bray et al. 2017). To test the hypothesis of high genetic homogeneity among *Leptuca thayeri* and *Uca maracoani* populations, we analyzed the

genetic differentiation, mainly based on the comparison of Brazilian populations with specimens from the Caribbean (Jamaica and Hispaniola).

MATERIALS AND METHODS

Sampling of *Leptuca thayeri* and *Uca maracoani*

Leptuca thayeri specimens were collected from three Brazilian populations in the states of Pará (Marudá), Bahia (Acuípe), and São Paulo (Bertioga). In addition, 11 specimens from Jamaica (Priory, St. Ann) and the Dominican Republic (Luperón). *Uca maracoani* specimens were collected from one Brazilian population in Pará (Marudá), and one population in the Dominican Republic (Sánchez mangroves) (Table 1) (Fig. 1). All individuals sampled were deposited into the Zoologische Staatssammlung München (Table 1).

DNA extraction, amplification, and sequencing

A total of 45 individuals of *L. thayeri* and 20 individuals of *U. maracoani* from five and two sample sites, respectively, were used for genetic analyses (Tables 2 and 3). Genomic DNA was extracted from muscle tissue of pereiopods using the Puregene (Gentra Systems) buffer system method. For both species, DNA amplification from the mitochondrial gene cytochrome oxidase, subunit I (Cox1) was carried out by polymerase chain reaction (PCR) (40 cycles: 45 sec 94°C/1 min 48°C/75 sec 72°C denaturing/annealing/elongation temperatures). For *L. thayeri* and *U. maracoani* an 857 base pair region was amplified using the primers COL1b 5'-CCW GCT GGD GGW GGD GAY CC-3' and COH16 5'-CAT YWT TCT GCC ATT TTA GA-3', and a shorter region of 640 base pairs using the

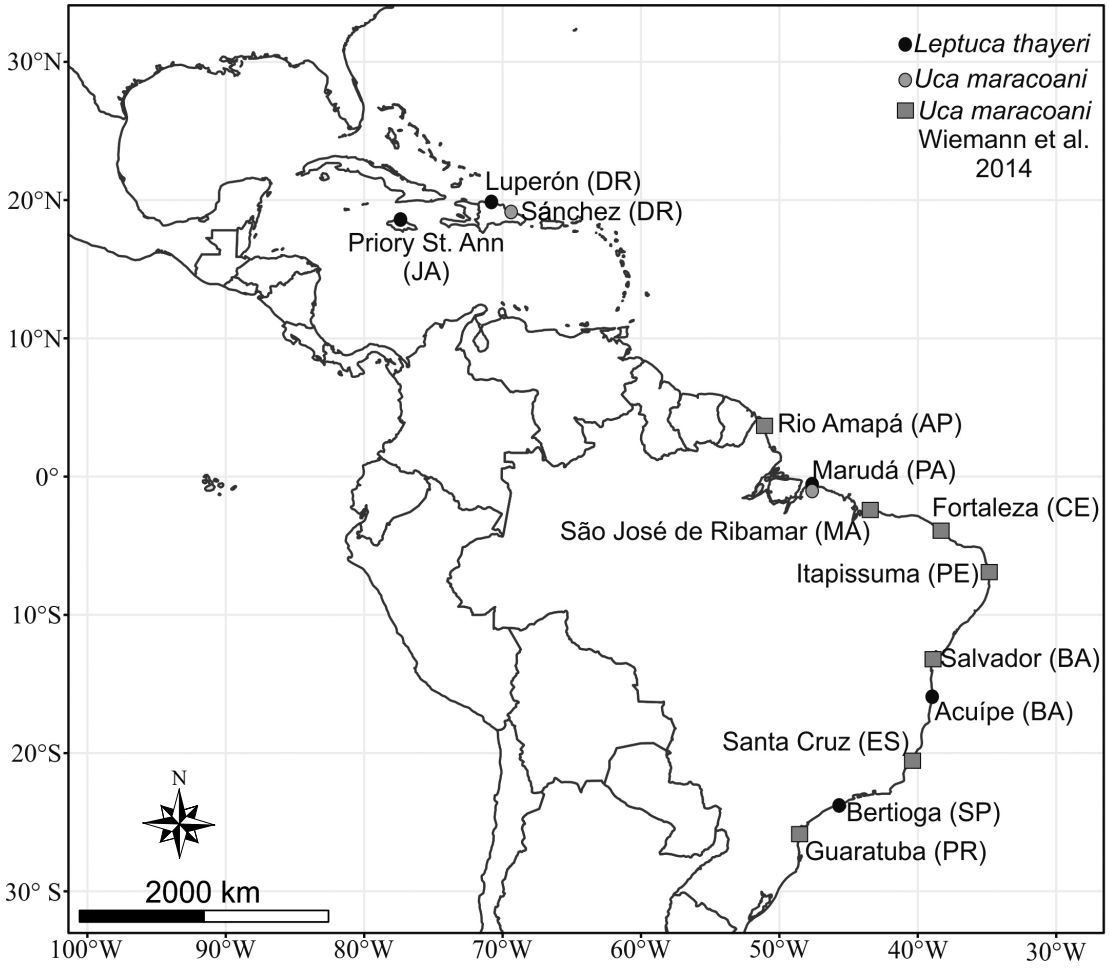

Fig. 1. Sample sites of *Leptuca thayeri* (black dots) and *Uca maracoani* (grey dots) populations from throughout the West Atlantic: Luperón & Sánchez from the Dominican Republic; Priory (St. Ann) from Jamaica; Marudá (Pará), Acuípe (Bahia), Bertioga (São Paulo) from Brazil. Grey squares correspond to localities sampled by Wieman et al. (2014), with sequences of *U. maracoani* in GenBank.

primers COL1b 5'-CCW GCT GGD GGW GGD GAY CC-3' and COH1b 5'-TGT ATA RGC RTC TGG RTA RTC-3' (Schubart 2009). This was necessary, because not all of the older specimens allowed to amplify the longer fragment, and in consequence two different datasets were evaluated, one maximizing the number of individuals (but with a short alignment), the other one maximizing the length of the alignment and exploring new variable positions (but with fewer representatives). 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

Table 1. Sampling locations, coordinates, numbers of individuals, museum collection numbers from the Zoologische Staatssammlung München (ZSM) and newly submitted GenBank accession numbers of *Leptuca thayeri* and *Uca maracoani*

Species	Location	Coordinates	Number of individuals	Collection no.	GenBank no.
Leptuca thayeri	Marudá, PA	0°37'0.23"S 47°37'94.6"W	10	ZSMA20210114	OM938770-OM938779
Leptuca thayeri	Acuípe, BA	15°04'9.92"S 38°59'95.8"W	15	ZSMA20210115	OM938755-OM938769
Leptuca thayeri	Bertioga, SP	23°50"13.7"S 46°09'16.5"W	10	ZSMA20210116	OM938746-OM938754
Leptuca thayeri	Priory, St. Ann, JA	18°26'43"N 77°12'56"W	9	ZSMA20210117	OM938780-OM938789
Leptuca thayeri	Luperón, DR	19°53'48"N 70°57'.33"W	1	ZSMA20210118	ON065543
Uca maracoani	Marudá, PA	0°37'0.23"S 47°37'94.6"W	10	ZSMA20210119	OM938790-OM938799
Uca maracoani	Sánchez mangroves, DR	19°13'30"N 69°37'10"W	11	ZSMA20210120	OM938800-OM938810

Table 2. Genetic diversity indices and neutrality tests for each analysed population of *Leptuca thayeri* and *Uca maracoani* based on regions of different lengths of the Cox1 gene

Species	Sequences length	Location	Population	N	h	S	Hd	π	Tajima's *D* test	Fu's *Fs* test
Leptuca thayeri	609 base pairs	Priory, St. Ann	Jamaica (JA)	9	6	9	0.89	0.003	-1.59*	-1.96*
		Marudá	Pará (PA)	10	7	9	0.933	0.005	0.45	-1.63
		Acuípe	Bahia (BA)	15	11	16	0.933	0.006	-0.7	-4.23*
		Bertioga	São Paulo (SP)	10	7	11	0.933	0.005	-0.43	-1.62
		Greater Antilles		10	6	9	0.844	0.003	-1.69	-1.9
		Pará + Bahia + São Paulo		35	19	23	0.923	0.006	-1.13	-9.28*
		all sequences		45	25	40	0.947	0.011	-0.72	-7.66*
Uca maracoani	606 base pairs	Sánchez	Dominican Republic (DR)	11	1	0	0	0	0	-
		Rio Amapá	Amapá (AP)	11	7	7	0.818	0.002	-1.64*	-4.05*
		Marudá	Pará (PA)	10	7	6	0.911	0.002	-0.97	-3.98**
		São José do Ribamar	Maranhão (MA)	12	10	10	0.955	0.002	-2.04**	-9.06**
		Fortaleza	Ceará (CE)	13	11	12	0.974	0.003	-1.75*	-8.97**
		Itapissuma	Pernambuco (PE)	8	7	8	0.917	0.002	-1.79*	-4.2**
		Salvador	Bahia (BA)	9	9	12	1	0.004	-1.87**	-7.45**
		Santa Cruz	Espírito Santo (ES)	10	7	7	0.911	0.002	-1.26	-3.75**
		Parati	Rio de Janiero (RJ)	10	3	3	0.378	0.0009	-1.56*	-0.45
		Guaratuba	Paraná (PR)	9	6	9	0.893	0.003	-1.72*	-2.21*
		AP + PA + MA + CE + PE + BA + ES + RJ + PR		91	48	45	0.89	0.002	-2.54**	-5.68*
		Dominican Republic		11	1	0	0	0	0	-
		all sequences		103	49	47	0.902	0.0046	-2.16**	-4.94*

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

primer COL1b to Macrogen Europe, Inc. (Amsterdam, the Netherlands). Obtained sequences were edited in Chromas Lite 3.01 (Technelysium Pty Ltd 2005) and manually aligned with BioEdit 5.0 (Hall 1999). Primer sequences and adjacent regions were omitted, resulting in an alignment of 826 or 609 base pairs for *L. thayeri* and 825 or 606 base pairs for *U. maracoani*. The software Artemis (Rutherford et al. 2000) was used to rule out the presence of stop codons, which could indicate the presence of pseudogenes. Sequences were submitted to GenBank (Table 1).

Genetic data analyses

The number of haplotypes, and haplotype (h) and nucleotide (π) diversities were calculated in DnaSP v5 (Librado and Rozas 2009). To assess levels of genetic differentiation among populations, pairwise Φ_{ST} values (Weir and Cockerham 1984; Loh et al. 2001) were calculated with Arlequin ver. 3.11 (Excoffier et al. 2005). A statistical parsimony network was constructed with PopArt (Polzin and Daneshmand 2003; Leigh and Bryant 2015).

The variance between tested groups was assessed by Analyses of Molecular Variance (AMOVA), using Arlequin ver. 3.11. For *L. thayeri* two populations from Caribbean islands (Jamaica and Dominican Republic) and three from Brazil (Pará, Bahia and São Paulo) were compared with the shorter DNA alignment (609 bp). The longer alignment (826 bp) could only be used to compare the northernmost Brazilian population (Pará) with the other northeast and southeast Brazilian populations (Bahia and São Paulo). For *U. maracoani*, one population from the Caribbean (Hispaniola) and one new one from Brazil (Pará) were compared to a relatively large dataset from GenBank (KF666951-KF666995), including Brazilian populations from Amapá, Maranhão, Ceará, Pernambuco, Bahia, Espírito Santo, Rio de Janeiro and Paraná (Wieman et al. 2014).

To examine the population history and to evaluate whether the populations follow the neutrality model at the sampling sites, Tajima's *D*, Fu's *Fs*, and mismatch distribution analyses (Tajima 1989; Fu 1997; Schneider and Excoffier 1999) were carried out using the Arlequin ver. 3.11 software. To test for significant restriction of gene flow, we used a non-parametric permutation procedure (Excoffier et al. 1992), incorporating 10,000 permutations.

RESULTS

Network reconstructions and statistical analyses were based on two alignments of different lengths in order to include sequences from previous studies and GenBank records. This data treatment has the advantage of allowing the inclusion of sequences from more specimens due to the shorter alignment, and thus increases statistical power for distinguishing apparently isolated populations (*e.g.*, Caribbean vs. Brazil), even if the alignment is shorter and may include fewer variable sites. In contrast, fewer sequences in favor of a longer alignment are preferable in the case of no or weak differentiation (*e.g.*, within Brazil), as networks based on a longer alignment allow visualization of more mutations that may have been established between these closely related populations.

For the shorter alignment (609 bp) of *L. thayeri*, 25 different haplotypes were recorded among 45 sequences of the five investigated West Atlantic populations, as compared to 26 different haplotypes in 34 sequences of the three Brazilian populations with the longer alignment (826 bp). The corresponding genetic distances among these haplotypes are depicted in the haplotype networks shown in figure 2. The one based on the shorter alignment clearly demonstrates that Brazilian

Table 3. Genetic diversity indices and neutrality tests for each analysed population of *Leptuca thayeri* and *Uca maracoani* based on the Cox1 gene

Species	Sequences length	Location	Population	N	h	S	Hd	π	Tajima's *D* test	Fu's *Fs* test
Leptuca thayeri	826 base pairs	Marudá	Pará (PA)	10	9	12	0.978	0.005	0.23	-3.97
		Acuípe	Bahia (BA)	15	13	22	0.971	0.006	-1.03	-6.53*
		Bertioga	São Paulo (SP)	9	8	12	0.972	0.004	-0.62	-3.51
		Bahia + São Paulo		24	18	27	0.957	0.005	-1.38	-10.23*
		Pará		10	9	12	0.978	0.005	0.23	-3.97
		L. thayeri all sequences		34	26	32	0.973	0.005	-1.46	-20.31*
Uca maracoani	825 base pairs	Sánchez	Dominican Republic (DR)	11	2	1	0.327	0.0004	-0.1	0.35
		Marudá	Pará (PA)	10	9	8	0.978	0.002	-1.22	-7.35**
		U. maracoani all sequences		21	11	18	0.819	0.007	0.82	-0.22

and Caribbean populations cluster separately, with ten mutational steps (1.48%) in between them (Fig. 2A). From the 45 haplotypes of this shorter alignment, 18 represent single individuals, while the others are shared haplotypes, diverging from one possibly ancestral haplotype H5. This most common haplotype is shared by eight specimens (four individuals from Bahia and two each from São Paulo and Pará). H13 is also found in individuals from all three Brazilian populations (two individuals each from São Paulo and Pará and one from Bahia). In the Caribbean, haplotype H20 is shared by four individuals (three from Jamaica and one from the Dominican Republic).

The longer alignment reinforces the close clustering of the haplotypes from Brazilian populations but suggests a slight distinction of the Pará population (Fig. 2B). 22 out of 34 haplotypes of the 826 bp alignment represent single individuals, while the others are shared haplotypes, diverging from one possibly ancestral haplotype H2. This most common haplotype is shared by five specimens (three individuals from Bahia and two from São Paulo). H7 is found in all Brazilian populations (one individual per population). All these longer haplotypes found in Brazilian populations are closely related, except for a slight deviation of the Pará population not being represented in the most common haplotype (H2) but sharing a unique haplotype instead. This homogeneity stands in sharp contrast to the large and consistent genetic distances to sequences from the Caribbean Province (islands of Hispaniola and Jamaica). Haplotype diversities within the shorter alignment varied from 0.89 to 0.933 and the nucleotide diversity from 0.003 to 0.006, while the longer alignment varied from 0.957 to 0.978 and the nucleotide diversity from 0.004 to 0.006 (Tables 2 and 3).

Statistical analyses based on the shorter alignment did not include the single individual from the Dominican Republic, due to insufficient sample size. Mean pairwise Φ_{ST} values among populations were relatively high (0.64) (Table 4). The majority of pairwise differences between populations were not significant, except for the comparison of Jamaican and the combined Brazilian populations of Pará, Bahia and São Paulo (Table 4). None of the pairwise differences between Brazilian populations based on the longer alignment were significant (Table 4). The AMOVA results showed no significant differences between the two tested groups of the shorter alignment ($P = 0.25$), although the highest amount of variation (76.07%) was in this category. The variation among populations within groups was also not significant ($P = 0.44$). Significant differences were found within populations ($P \leqq 0.001$) and were responsible for 23.87% of the total variation (Table 4). The AMOVA results based on the longer alignment also failed to show significant differences between the two tested groups ($P = 0.3$), accounting for 5.6% of the total variation, while -2.59% of the variation was among populations within groups ($P = 0.66$), and 97.08% of variation within populations ($P = 0.33$). The *L. thayeri* haplotypes from Caribbean and Brazilian populations are significantly different (Fig. 2 and Table 4).

In the short alignment (606 bp) of *U. maracoani*,

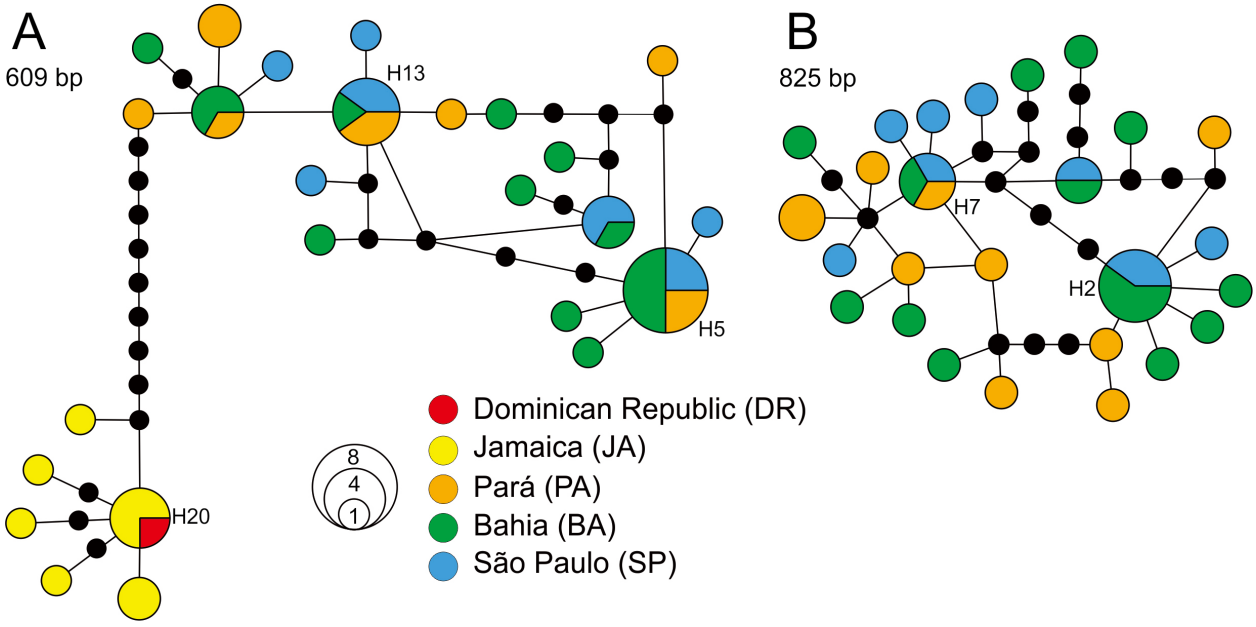

Fig. 2. Haplotype network of *Leptuca thayeri* constructed with Popart, with a connection limit of 95%, derived from Cox1 mtDNA with 609 basepairs (A) and 826 basepairs (B). Black dots represent missing haplotypes (one-step edges); numbered 'H' indicate most common haplotypes.

49 different haplotypes were found among 103 sequences of the ten populations. The haplotype network in figure 3 depicts genetic distances among them, showing that all Brazilian populations cluster in one single clade, five mutational steps away (0.66%) from the Caribbean haplotypes (Fig. 3A). From the 49 haplotypes, 42 are represented by single individuals, while the others are shared, diverging from the possibly ancestral haplotype H7. This most common haplotype is shared by twenty-nine specimens (five individuals from Amapá, eight from Rio de Janeiro, three from Maranhão, three from Pernambuco, three from Espírito Santo, three from Paraná, two from Ceará, one from Pará and one from Bahia). H6 is found in three individuals from Pará, two from Ceará, two from Espírito Santo, one from Maranhão, one from Amapá and one from Pernambuco. H5 is found in four individuals (one each from Amapá, Pará, Maranhão and Rio de Janeiro). H31 is found in two individuals from Pará and one from Bahia. H3 is found in one individual from Amapá and one from Maranhão, and H10 was present in one individual from Maranhão and another from Bahia. In the Caribbean, only a single haplotype (H46) was obtained, suggesting a genetic bottleneck (Fig. 3A). Overall, all haplotypes found in Brazilian populations are closely related. The corresponding haplotype diversities varied from 0.8 to 1 and the nucleotide diversities from 0.002 to 0.004, with the exception of the population of Parati (state of Rio de Janeiro), with values of 0.378 and 0.0009 (Table 2).

In the 826 bp alignment, 8 out of 11 haplotypes represent single individuals from the Pará population (Fig. 3B). No haplotypes were shared between the populations of Pará and the Dominican Republic, which are separated by nine mutations (0.97%). This finding

Table 4. Pairwise differences between sampled populations of *Leptuca thayeri*

Sequence length		São Paulo (10)	Bahia (15)	Pará (10)	Jamaica (9)	
609 base pairs	São Paulo	----------	0.67	0.4	< 0.001*	
	Bahia	-0.0341	----------	0.2	< 0.001*	
	Pará	-0.0089	0.02529	----------	< 0.001*	
	Jamaica	0.7964	0.76801	0.78427	----------	
	Source of variation	*d.f.*	Sum of squares	Variance components	Variation (%)	*p*-value
	Among groups	1	80.109	5.4717	76.07	0.2551
	Among populations within groups	2	3.543	0.0047	0.07	0.4428
	Within populations	40	68.667	1.716	23.87	< 0.001*
	Total	44	153.318	7.19323		
	Fixation indices					
	F_{SC}: 0.00278 (among populations within groups)					
	F_{ST}: 0.7613 (within populations)					
	F_{CT}: 0.7606 (among groups)					
		São Paulo (9)	Bahia (15)	Pará (10)		
826 base pairs	São Paulo	----------	0.67	0.2		
	Bahia	-0.029	----------	0.2		
	Pará	0.0289	0.03	----------		
	Source of variation	*d.f.*	Sum of squares	Variance components	Variation (%)	*p*-value
	Among groups	1	3.466	0.1319	5.6	0.3
	Among populations within groups	1	1.575	-0.0633	-2.69	0.66
	Within populations	31	70.9	2.2871	97.08	0.33
	Total					
	Fixation indices					
	F_{SC}: -0.0284 (among populations within groups)					
	F_{ST}: 0.0291 (within populations)					
	F_{CT}: 0.056 (among groups)					

Φ_{ST} -values below dashed lines; corresponding *P*-values above diagonal; *d.f.*, degrees of freedom; F_{SC}: variance among populations within groups; F_{ST}: variance among populations; F_{CT}: variance among groups defined a priori. Numerals in parentheses denote the sample size of each population. * $P < 0.05$.

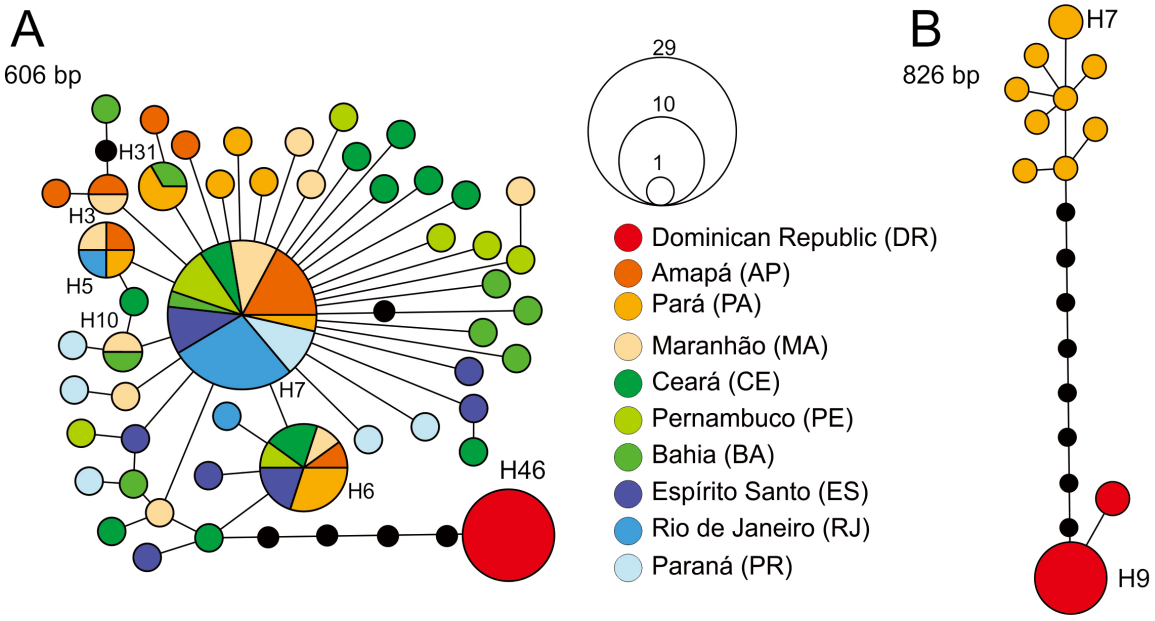

Fig. 3. Haplotype network of *Uca maracoani* constructed with Popart, with a connection limit of 95%, derived from Cox1 mtDNA with 606 base pairs (A) and 825 base pairs (B). Black dots represent missing haplotypes (one-step edges), numbered 'H' indicate most common haplotypes.

Table 5. Pairwise differences between sampled populations of *Uca maracoani*

Sequence length		Dominican Republic (11)	Amapá (11)	Pará (10)	Maranhão (12)	Ceará (13)	Pernambuco (8)	Bahia (9)	Espírito Santo (10)	Rio de Janeiro (10)	Paraná (9)
606 base pairs	Dominican Republic	----------	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*	< 0.001*
	Amapá	0.9037	----------	0.89	0.37	0.51	0.7	0.06	0.83	0.17	0.38
	Pará	0.8841	-0.029	----------	0.91	0.94	0.88	0.13	0.99	0.91	0.23
	Maranhão	8355	0.0041	-0.02	----------	0.47	0.37	0.84	0.75	0.47	0.58
	Ceará	0.8958	0.0011	-0.03	-0.0037	----------	0.99	0.25	0.51	0.67	0.3
	Pernambuco	0.8474	-0.015	-0.02	0.0034	-0.023	----------	0.07	0.011*	0.94	0.28
	Bahia	0.882	0.0487	0.029	-0.0265	0.0126	0.0416	----------	0.29	0.9	0.64
	Espírito Santo	0.9599	-0.022	-0.04	-0.0199	-0.013	0.0076	0.044	----------	0.06	0.53
	Rio de Janeiro	0.8799	0.0185	-0.03	-0.0123	-0.012	-0.0362	0.044	0.0163	----------	0.08
	Paraná	0.8942	-6E-04	0.013	0.0134	0.0134	0.0233	-0.011	0.0079	0.0488	----------
	Source of variation	*d.f.*	Sum of squares	Variance components	Variation (%)	*p*-value					
	Among groups	1	64.29	3.22	80.27	0.09					
	Among populations within groups	8	6.99	0.008	0.22	0.49					
	Within populations	93	72.94	0.78	19.51	< 0.001*					
	Total	102	144.23	4.02							
	Fixation indices										
	F_{SC}: 0.0111 (among populations within groups)										
	F_{ST}: 0.8049 (within populations)										
	F_{CT}: 0.8027 (among groups)										
		Dominican Republic (11)	Pará (10)								
825 base pairs	Dominican Republic	----------	< 0.001*								
	Pará	0.8938	----------								

Φ_{ST} -values below dashed lines; corresponding *p*-values above diagonal; *d.f.*, degrees of freedom; F_{SC}: variance among populations within groups; F_{ST}: variance among populations; F_{CT}: variance among groups define a priori. Numerals in parentheses denote the sample size of each population. * $P < 0.05$.

reinforces the large and consistent genetic distances from South American and Caribbean populations. Haplotype diversities varied from 0.32 to 0.97, and the nucleotide diversities from 0.002 to 0.0004 (Table 3).

All pairwise differences between populations of *U. maracoani* from the Dominican Republic and Brazil were significant in both alignment lengths, while only Pernambuco and Espírito Santo populations were significantly different, for the shorter alignment, when exclusively comparing Brazilian populations (Table 5). Even if the AMOVA results do not confirm these significant differences between Brazilian and the Caribbean populations (the two tested groups) (P = 0.09), this comparison revealed the highest amount of variation (80.27%). The variation among populations within groups was also not significant (P = 0.49) and responsible for 0.22% of variance. Significant differences were found within populations (P < 0.001) and were responsible for 19.51% of the total variation (Table 5).

The demographic history of Brazilian and Caribbean populations of *L. thayeri* and *U. maracoani* was reconstructed using mismatch distributions and neutrality tests. *Leptuca thayeri* populations showed a bimodal distribution of pairwise differences (Fig. 4A), corresponding to the intra-regional differences on one hand and the inter-regional on the other, while the mismatch distribution of Brazilian populations showed a unimodal distribution (Fig. 4B). *Uca maracoani* populations from this study and from Wieman et al. (2014) also showed a bimodal distribution of pairwise differences (Fig. 5), corresponding to the intra-regional differences on one hand and the inter-regional on the other. The majority values of the neutrality tests, when combining all populations of Caribbean and Brazil, were negative for both species (Tables 2 and 3).

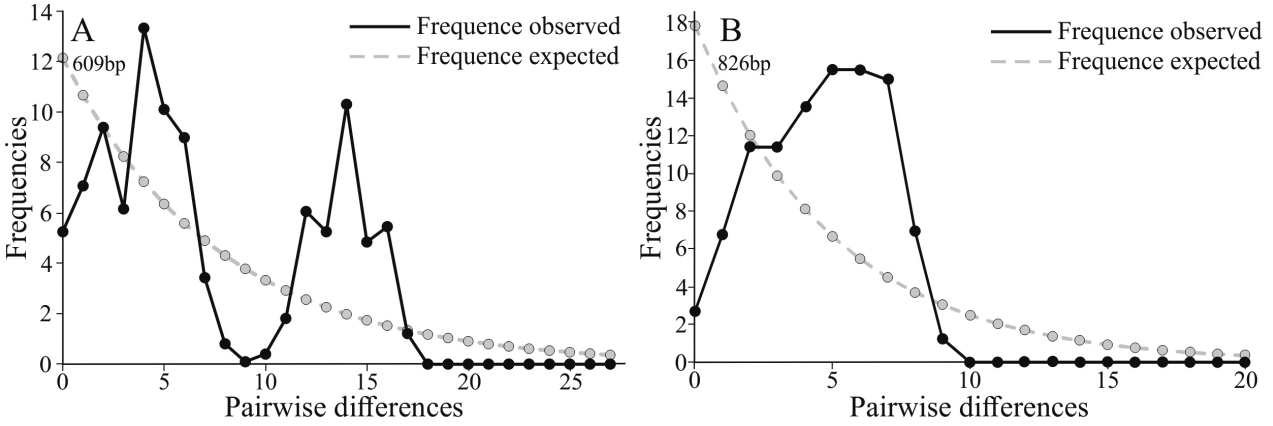

Fig. 4. Mismatch distribution for two Caribbean and three Brazilian populations of *Leptuca thayeri* with 609 basepairs (A) and three Brazilian populations with 826 basepairs (B).

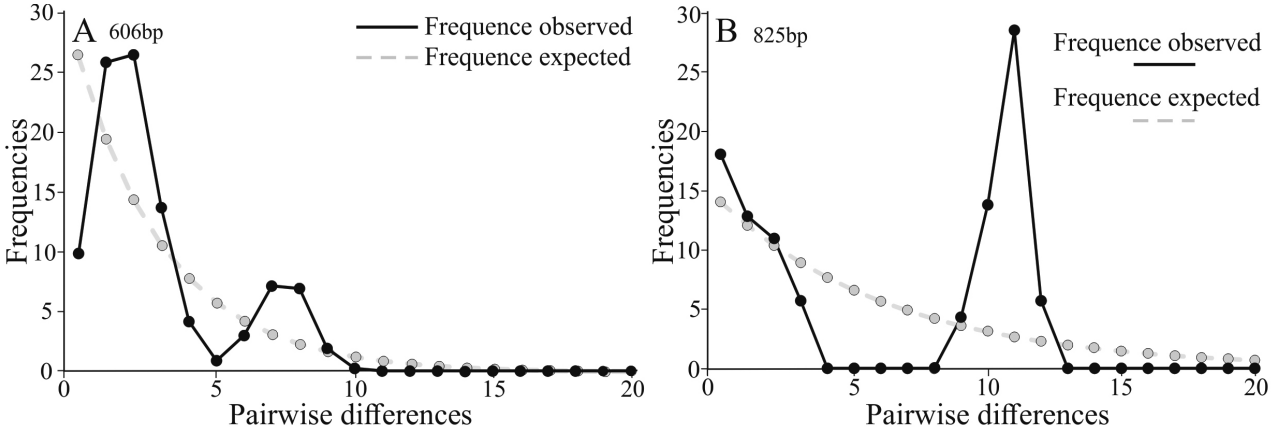

Fig. 5. Mismatch distribution for one Caribbean and nine Brazilian populations of *Uca maracoani* with 606 basepairs (A) and one Brazilian and one Caribbean population with 825 basepairs (B).

DISCUSSION

Prior to this study, the supposed geographic distribution of *Uca maracoani* reached from the south of Brazil (Paraná state) to Venezuela and Trinidad and Tobago in South America, with historical reports from the Dominican Republic and Jamaica (Crane 1975; Melo 1996). However, Barnwell (1986) and Bezerra (2012) pointed out occasional misidentifications with *Uca major* (Herbst, 1782), and the specimens of *U. maracoani* from Jamaica by Sloane (1725) were re-identified as such. Crane (1975) reported the occurrence of a female of *U. maracoani* from Santo Domingo, Dominican Republic, deposited at the American Museum of Natural History (AMNH 2466). Dr. Carl Thurman (pers. communication) examined this specimen and confirmed the identification. However, he assumed an erroneous location, since the individual had an age exceeding one hundred years and his group, and Thurman's group did not sample *U. maracoani* specimens further north than Trinidad and Tobago.

With the current study, we unmistakably confirm the occurrence of *U. maracoani* from the island of Hispaniola in relatively high abundances. Due to the high density of this species in a mangrove fringed embayment near Sánchez (19°13'30"N, 69°37'10"W) on the Samaná Peninsula in the Dominican Republic (Hispaniola), it was a relatively easy task to obtain few specimens and sufficient tissue samples for later use in this study, allowing genetic confirmation of the species identity.

The comparison of DNA sequence data corresponding to the mitochondrial Cox1 gene from the fiddler crab species *L. thayeri* and *U. maracoani*, revealed a marked homogeneity among Brazilian populations. However, South American and Caribbean populations did not share haplotypes and clustered into two non-overlapping groups (Figs. 2 and 3). Even if the overall genetic variance was insufficient to result in statistical significance, the highest variance was detected between groups (Brazil vs. Caribbean), whilst the genetic variance between populations within regions and within populations was low (Tables 4 and 5). These results suggest unrestricted gene flow even among distant areas in Brazil, but the presence of a biogeographic barrier, limiting genetic exchange to Caribbean populations for both species. However, considering the low number of populations evaluated from the Caribbean region, this inference should be interpreted with caution.

Marine invertebrates with an extensive planktonic larval phase usually present a low level of intraspecific genetic differentiation (Scheltema 1971; Berger 1973; Gooch 1975; Crisp 1978). For example, populations of the more southerly distributed *Leptuca uruguayensis* (Nobili, 1901) from north (São Paulo State, Brazil) and south (Mar del Plata, Argentina) of the broad Río de la Plata Estuary appear to be unhindered, despite the large freshwater plume in between (Laurenzano et al. 2012). Likewise, within Brazil, populations of *U. maracoani* from the north (Amapá) to the south (Paraná), populations of *Leptuca leptodactyla* (Rathbun, 1898) and *Minuca rapax* (Smith, 1870) from the north (Pará) to the southeast (São Paulo), and populations of *Minuca burgersi* Holthuis, 1967 from the north (Maranhão) to the south (Santa Catarina) of this large country presented no population structure or differentiation (Wieman et al. 2014; Laurenzano et al. 2016; Buranelli and Mantelatto 2019; Thurman et al. 2021). This agrees with our present mitochondrial DNA datasets of *L. thayeri* and *U. maracoani*, which also failed to reveal statistically significant genetic differentiation between Brazilian populations (Tables 4 and 5), and instead only revealed trends, as in the case of *L. thayeri* from Pará. It provides additional support for the pronounced connectivity between Brazilian estuarine zones. The apparent genetic homogeneity along the 5,400 kilometers of Brazilian coastline considered in this work (ca. 2,900 km between Acuipé and Marudá, and 4,400 km between Guaratuba and Rio Amapá) hints towards the absence of natural barriers for the dispersal of all life stages of the so far investigated fiddler crabs. This means that postulated marine biogeographic barriers, like the coastal upwelling zone, the Amazon River plume, and/or the split of Central vs. South Equatorial marine currents along the northeastern Brazilian coast (for more details, see Rodrigues et al. 2007), do not seem to interfere in larval distribution among the sampled localities at this genetic level (Lessios et al. 2003; Waters and Roy 2004; Marochi et al. 2017, Buranelli and Mantelatto 2019). As our results are consistent with these previous findings, they may be generalized for most other estuarine brachyuran crab species with extended pelagic larval duration along the Brazilian coast, confirming the wide distribution patterns postulated by von Hagen (1970), Holthuis (1959) and Swennen et al. (1982). However, genetic differentiation may still be recognized at more shallow population genetic levels and should be tested with more sensitive methods (*e.g.*, microsatellites), even if it would not be of taxonomic relevance.

Cox1 sequences of *L. thayeri* and *U. maracoani* from the Caribbean Islands show particular genetic patterns, clearly differing from Brazilian ones. Although natural barriers to dispersal are not unequivocally definable, previous studies already revealed genetic structure among Caribbean Island populations and between insular and mainland populations in other marine organisms with extended pelagic larval

durations (Taylor and Hellberg 2003; Laurenzano et al. 2013 2016; Thurman et al. 2021). Our individuals of *L. thayeri* from two of the Greater Antilles (Hispaniola and Jamaica) and of *U. maracoani* from Hispaniola are genetically similar, but different from those of Brazil (1.48 and 0.66%, respectively, for the short alignments), clearly segregated into their own genetic networks (Figs. 2 and 3). A similar pattern was observed in another study dealing with *L. thayeri*, with a different and shorter region of the Cox1 gene and not more than five individuals per populations, comparing 18 Brazilian populations from the extreme north (Amapá) to south (Santa Catarina) with those from Caribbean localities in Mexico, Jamaica, Cuba and from Florida (see Buranelli and Mantelatto 2019). The differences between Brazilian and Caribbean haplotypes found by Buranelli and Mantelatto (2019) (twelve mutation steps corresponding to 1.82% sequence divergence) were in a similar range to those found in our study (ten mutation steps corresponding to 1.48% sequence divergence). Besides a possible larval retention pattern in Caribbean populations, another potential explanation for the genetic differentiation between Brazilian and Caribbean populations is gene flow restriction due to already recognized mainland natural barriers to dispersal, such as the marine currents systems within the Caribbean Sea (Centurioni and Niiler 2003). These currents are known to result in population genetic structure as found in different marine invertebrates (Lessios et al. 2003; Kool et al. 2010; White et al. 2010). However, to test the hypothesis of the role of marine currents systems in the Caribbean Sea for larval dispersal potential, more populations from those areas need to be analyzed and compared with those from Brazil.

Leptuca thayeri has a larval export strategy (Anger et al. 1989), in which the larvae are released during conditions favoring export from their parental habitat (estuaries) and transport towards open waters or along the coastline, thus increasing the chances to be dispersed by currents. It is likely that *U. maracoani* follows the same export strategy, because adults inhabit mesohaline and euryhaline habitats. However, no information is available concerning the best-suited salinity for their larval development. The microhabitat in which both species occur (meso/euryhaline waters) allied with estuarine and coastline geomorphology, could also explain the similar genetic structuring of the two species. Furthermore, a set of abiotic variables (local currents, tide regimes, wind effects, and coast morphology) may also influence the degree of gene flow (Aoki et al. 2008; Robins et al. 2013; Staton et al. 2014).

Species introductions by shipping industry or following escape from aquaculture/aquarist farms can also increase the dispersal potential of marine species. Ships carrying ballast water with plankton can facilitate the dispersal of marine species, especially those with extended planktonic phases, and crab introductions have been documented worldwide (Lavoie et al. 1999; Schubart 2003; Negri et al. 2018). Also, crab introductions after escaping aquaculture/aquaria have been reported as a cause of crab introductions in non-native areas (Magalhães et al. 2005; Magalhães and Costa 2007). These human-mediated introductions can also influence the current and future distribution of fiddler crabs.

The demographic histories of *L. thayeri* and *U. maracoani* indicate a recent population bottleneck, followed by demographic expansions, as denoted by Tajima's *D* and Fu's *Fs* negative values and the bimodal shape of the mismatch distribution (Tables 2 and 3). Temporary historical barriers are likely to be the cause for significant current population structure, as observed in other tropical brachyuran crabs (Felder and Staton 1994; Laurenzano et al. 2016; Marochi et al. 2017). The same pattern of recent population bottleneck followed by demographic expansions was concluded by Buranelli and Mantelatto (2019) for *L. thayeri*. The authors estimate that the population expansion event may have occurred 52 million years ago (Mya). Our *L. thayeri* mismatch distributions are similar to those from Buranelli and Mantelatto (2019), even if the authors showed the distribution of their Brazilian and Carribbean clades separately.

The recent expansion events and possible bottleneck effect in the Caribbean Sea can be related to recent geological events or glaciations. For example, the closing of the Central American Isthmus (~3.5–2.8 Mya) affected ocean currents and sundered the range of marine species, disrupting their gene flow (Lessios 2008), as observed for the sister species *Aratus pisonii* (H. Milne Edwards, 1837) and *Aratus pacificus* (see Thiercelin and Schubart 2014), and *Pachygrapsus transversus* (Gibbes, 1850) and *P. socius* Stimpson, 1871 (see Schubart et al. 2005) in the Atlantic and Pacific oceans. Considerably later, and more likely responsible for the observed effects, repeated expansions and retreats of ice sheets during the last ice ages around 23,000, 41,000, and 100,000 years ago (Rodríguez-Rey et al. 2014; Buranelli and Mantelatto 2019) restricted the areas of suitable habitat for tropical species, due to the lower temperatures. At the end of the respective ice ages, those populations could have expanded their distributions when temperatures became more favorable (Provan and Bennett 2008). The higher genetic diversity observed for both species in the northern populations of Brazil reinforces this idea (possibly also the Caribbean diversity of *L. thayeri*). However, this hypothesis

requires confirmation, and further studies are necessary to support it.

CONCLUSIONS

In this study, we re-investigate the genetic differentiation of two fiddler crab species with extended larval planktonic phases along their West Atlantic distribution, mainly based on the comparison of Brazilian populations with specimens from the Caribbean. We confirm the lack of genetic structure among Brazilian populations of the fiddler crabs *Leptuca thayeri* (based on an unexplored region of the Cox1 gene) and *Uca maracoani* (combining data with a previous study), while also revealing pronounced genetic differences to conspecifics from the Caribbean Islands for both species. An extended larval phase in the marine plankton seems to facilitate gene flow among Brazilian estuaries, supported by an inherent larval export strategy. In contrast, the genetic heterogeneity between Brazilian and Caribbean populations may be due to disjunct distributions or environmental selection. All of these factors have been confirmed as barriers to dispersal in other marine coastal organisms of the Neotropics.

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