








# Filling the gaps: phylogeography of the self-fertilizing *Kryptolebias* species (Cyprinodontiformes: Rivulidae) along South American mangroves

Mateus G. S. Lira<sup>1</sup>  | Waldir M. Berbel-Filho<sup>2</sup>  | Helder M. V. Espírito-Santo<sup>3</sup>  |  
 Andrei Tatarenkov<sup>4</sup>  | John C. Avise<sup>4</sup> | Carlos Garcia de Leaniz<sup>2</sup>  |  
 Sofia Consuegra<sup>2</sup>  | Sergio M. Q. Lima<sup>1</sup> 

<sup>1</sup>Laboratório de Ictiologia Sistemática e Evolutiva, Departamento de Botânica e Zoologia, Universidade Federal do Rio Grande, Programa de Pós-Graduação em Sistemática e Evolução, Natal, Brazil

<sup>2</sup>Department of Biosciences, College of Science, Swansea University, Swansea, UK

<sup>3</sup>Núcleo de Ecologia Aquática e Pesca, Universidade Federal do Pará, Belém, Brazil

<sup>4</sup>Department of Ecology and Evolutionary Biology, University of California, Irvine, California

## Correspondence

Mateus G. S. Lira, Laboratório de Ictiologia Sistemática e Evolutiva, Departamento de Botânica e Zoologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Campus Universitário, BR 101 S/N, Lagoa Nova, Natal, RN, Brazil.  
 Email: mateuslupus@hotmail.com

## Funding information

Conselho Nacional de Desenvolvimento Científico e Tecnológico (BR), Grant/Award Number: 313644/2018-7; Conselho Nacional de Desenvolvimento Científico e Tecnológico, Grant/Award Number: 233161/2014-7; National Geographic Society, Grant/Award Numbers: W461-16, EC-44874R-18

## Abstract

Mangrove killifishes of the genus *Kryptolebias* have been historically classified as rare because of their small size and cryptic nature. Major gaps in distribution knowledge across mangrove areas, particularly in South America, challenge the understanding of the taxonomic status, biogeographical patterns and genetic structuring of the lineages composing the self-fertilizing “*Kryptolebias marmoratus* species complex.” In this study, the authors combined a literature survey, fieldwork and molecular data to fill major gaps of information about the distribution of mangrove killifishes across western Atlantic mangroves. They found that selfing mangrove killifishes are ubiquitously distributed across the Caribbean, Central and South American mangroves and report 14 new locations in South America, extending the range of both the “Central clade” and “Southern clade” lineages which overlap in the Amazon. Although substantial genetic differences were found between clades, the authors also found further genetic structuring within clades, with populations in Central America, north and northeast Brazil generally showing higher levels of genetic diversity compared to the clonal ones in southeast Brazil. The authors discuss the taxonomic status and update the geographical distribution of the Central and Southern clades, as well as potential dispersal routes and biogeographical barriers influencing the distribution of the selfing mangrove killifishes in the western Atlantic mangroves.

## KEYWORDS

genetic structuring, hermaphroditism, *Kryptolebias hermaphroditus*, mangrove rivulus, Rivulidae

\* The authors “Mateus G. S. Lira” and “Waldir M. Berbel-Filho” have contributed equally to this study.

† Funding information

This work was supported by the National Geographic/Waite program (W461-16), the National Geographic Society Early Career grant (EC-44874R-18) and the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (233161/2014-7). Sergio Lima receives research productivity grant issued by CNPq (313644/2018-7). Andrey Tatarenkov and John C. Avise are grateful for the funds provided by University of California at Irvine. Helder Espírito-Santo received a postdoctoral fellowship from Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brasil (CAPES).

## 1 | INTRODUCTION

Information of species geographical distribution and genetic structure is important to guide conservation policies of threatened species and their environment (Allendorf, 2017). Nonetheless, some environments have inherent spatial and temporal complexities, which challenge the access to biological information from its associated organisms. This is the case of mangrove forests that, despite being some of the most

species-rich habitats, are also one of the most threatened (Branoff, 2017; Duke *et al.*, 2007), with biodiversity information often limited by the notorious difficulties of sampling mangrove systems (Blaber & Barletta, 2016; Lugo *et al.*, 2014).

Worldwide, mangrove forests occupy an area of c.  $17 \times 10^6$  ha, of which  $4.5 \times 10^6$  (32.1%) are along the western Atlantic coast, from south USA (29° N) to south Brazil (23° S), also surrounding the Caribbean archipelagos (Blaber & Barletta, 2016; Ferreira *et al.*, 2015). The mangrove killifishes (*Kryptolebias* spp.) are among the rare fish exclusively associated with mangrove forests (Taylor, 2012). *Kryptolebias* is a genus of non-seasonal and small-sized oviparous fishes within the rivulids group (family Rivulidae), composed of seven species (Vermeulen & Hrbek, 2005). Within the genus, the “mangrove killifishes clade” consists of three androdioecious (*i.e.*, populations consisting of males and hermaphrodites) species: *K. marmoratus* (Poey, 1880); *K. hermaphroditus* Costa, 2011; *K. ocellatus* (Hensel, 1868), living in intermittent habitats, as crab burrows, temporary pools or leaf litter (Avisé & Tatarenkov, 2015; Berbel-Filho *et al.*, 2016; Lira *et al.*, 2015; Tatarenkov *et al.*, 2017; Taylor, 2012, Taylor *et al.*, 2008).

The geographical distribution and taxonomic status of mangrove killifishes are complex, particularly in South America (Berbel-Filho *et al.*, 2016; Costa *et al.*, 2010), mainly because of morphological similarities among sympatric species, and major gaps in sampling across mangrove areas (Tatarenkov *et al.*, 2017; Taylor, 2012). *Kryptolebias ocellatus* was first described by Hensel (1868) using a single specimen from Rio de Janeiro, Brazil, followed by *Kryptolebias marmoratus* described by Poey (1880) based on specimens from the USA and/or Cuba. Seegers (1984) suggested that in Rio de Janeiro there were two species, the self-fertilizing (selfing) *K. ocellatus* and a yet undescribed dimorphic androdioecious species, named *Kryptolebias caudomarginatus*. Two decades later, Costa (2011) argued that Hensel's *K. ocellatus* corresponded to *K. caudomarginatus*, which led to reclassification of *K. caudomarginatus* as a junior synonym of *K. ocellatus*. In addition, this study described the species composed of selfing hermaphrodites as *Kryptolebias hermaphroditus* (Costa, 2011). Nonetheless, given the ongoing debate about whether the original species described by Hensel corresponded to either *K. hermaphroditus* (*sensu* Costa, 2011) or *K. caudomarginatus* (*sensu* Seegers, 1984), the discussion about the taxonomic status of the mangrove killifish species is still under discussion, and taxonomic rearrangements are likely to happen with further taxonomical research (Huber, 2017; Tatarenkov *et al.*, 2017, 2020).

Two of the mangrove killifishes, *Kryptolebias marmoratus* and *Kryptolebias hermaphroditus*, are the only known vertebrates capable of self-fertilization (Tatarenkov *et al.*, 2009, 2017). Despite the morphological similarity, these species are readily distinguishable using genetic data, with several phylogenetic studies on mangrove killifishes supporting their sister-species status, forming the “*Kryptolebias marmoratus* species complex” clade (Kanamori *et al.*, 2016; Murphy *et al.*, 1999; Tatarenkov *et al.*, 2009; Tatarenkov *et al.*, 2017; Vermeulen & Hrbek, 2005), which is closely related to the obligate outcrossing species *K. ocellatus*, restricted to southeastern

(in sympatry with *K. hermaphroditus* in Guanabara and Sepetiba bays in Rio de Janeiro state) and southern Brazil (Berbel-Filho *et al.*, 2020).

For a long time, *K. hermaphroditus* was thought to be limited to southeastern Brazil, in Rio de Janeiro (22° S) and São Paulo (23° S) states (Costa, 2011). This geographical distribution contrasted with that of *K. marmoratus*, distributed in Florida, the Bahamas, the Caribbean and parts of Central America (Tatarenkov *et al.*, 2010, 2011, 2012). This distinct distribution formed a geographical gap of over 22° in latitude. Nonetheless, recent studies have expanded the geographic distribution *K. hermaphroditus*, which spans from north of Rio de Janeiro to the Brazilian states of Espírito Santo (19° S) (Sarmiento-Soares *et al.*, 2014), Bahia (14° S) (Silva *et al.*, 2020), Alagoas (10° S) (Costa, 2016), Rio Grande do Norte (05° S) (Lira *et al.*, 2015) and Pará (0.9 N) (Guimarães-Costa *et al.*, 2017).

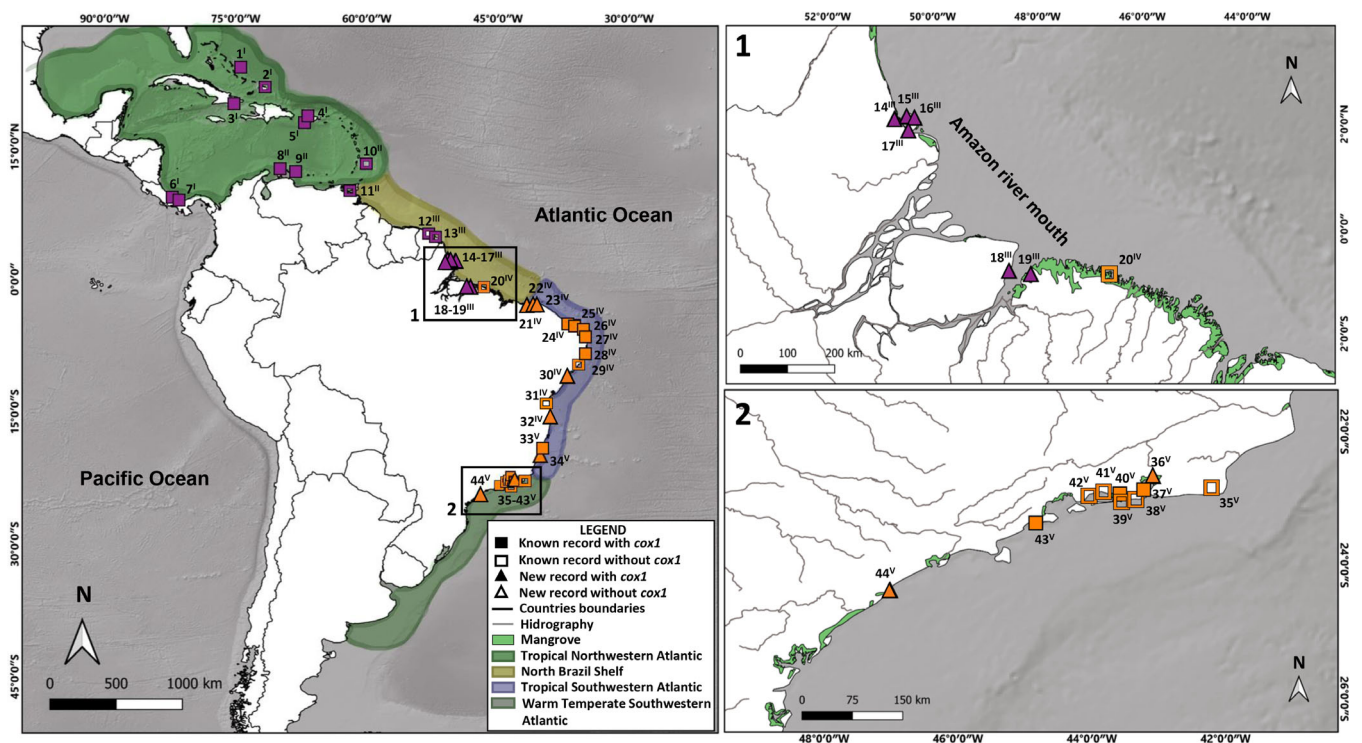
mtDNA and microsatellite data indicate that there is a clear genetic differentiation within the *K. marmoratus* species complex along the Atlantic coast, with three major clades: a “Northern” clade in Florida, northern Cuba, Bahamas, Belize and Honduras corresponding to *K. marmoratus*; a “Southern” clade along the Brazilian coast corresponding to *K. hermaphroditus*; and a “Central clade” in Panama, the Dutch Caribbean, Puerto Rico, Turks and Caicos, and southern Cuba (Tatarenkov *et al.*, 2017). Despite being in geographical proximity (and sometimes in sympatry) with *K. marmoratus* (Tatarenkov *et al.*, 2018, 2020), specimens from the “Central clade” are phylogenetically more related to *K. hermaphroditus* (1% genetic distance at *cox1* mtDNA gene) (Tatarenkov *et al.*, 2017).

Yet, the data for large portions of the South American coast, especially between east of Venezuela and northeast Brazil (c. 5678 km along the coast), as well as in between the northeastern and southeastern portions of the Brazilian coast (c. 2400 km along the coast), is limited and raises questions about the geographical boundaries between mangrove killifish clades of the “*Kryptolebias marmoratus* species complex,” as well the potential environmental factors and geographical barriers influencing their dispersal and gene flow. To address this knowledge gap, the authors combined a literature survey with fieldwork and molecular analysis of new samples to: (a) identify the distribution limits of the Central and Southern clades of the “*Kryptolebias marmoratus* species complex,” including their potential contact zone; and (b) evaluate the levels of mtDNA genetic structuring and diversity within and between these clades.

## 2 | MATERIALS AND METHODS

### 2.1 | Literature, database and collection surveys

To identify the major sampling gaps of the Central and Southern clades, the authors surveyed all literature on the species distribution (Berbel-Filho *et al.*, 2019, 2020; Guimarães-Costa *et al.*, 2017; Lira *et al.*, 2015; Sarmiento-Soares *et al.*, 2014; Tatarenkov *et al.*, 2017, 2018), as well as online databases for sampling records (GBIF; www.gbif.org) and scientific collections (CRIA - SpeciesLink; http://splink.cria.org.br/), using the keywords “*Rivulus ocellatus*” (the previously



**FIGURE 1** Sampling points for populations of the “*Kryptolebias marmoratus* species complex” from the Central and Southern clades based on data extracted from literature surveys and fieldwork. Sampling localities’ numbers are listed in Table 1. Lines along the coast correspond to the boundaries for the marine provinces proposed by Spalding *et al.* (2007). The locations of the Central clade are represented with symbols in purple, whereas the orange symbols correspond to the locations of the Southern clade

valid nomenclature for *K. hermaphroditus* before Costa, 2011) and “*Kryptolebias hermaphroditus*,” only taking into account occurrence records since the year 2000 made available with geographic coordinate information.

In total, the authors gathered information from 44 sampling localities for specimens of the “*Kryptolebias marmoratus* species complex” in Central and South America, corresponding to the Central and Southern clades. In all, 30 (69.19%) occurrence records on localities came from literature and databases and 14 (31.81%) from the authors’ new fieldwork collections (155 individuals sampled), seven of these records were from conservation units in Brazil (Table 1). Some samples represent the first record of *Kryptolebias* in several Brazilian states (Amapá, Piauí, Ceará and Sergipe) filling most of the previous distribution gaps in the north and east coast of Brazil, including another record in Bahia State (Figure 1; Table 1).

## 2.2 | Fieldwork

After the identification of the major sampling gaps on the distribution of mangrove killifishes based on previous data, fieldwork trips were undertaken between 2017 and 2019 to sample two gaps previously identified by Tatarenkov *et al.* (2017), such as the limits between the Central clade (south of Trinidad and Tobago) and the Southern clade

(northeast Brazil), and the large gap found within the Southern clade [in Brazil, between Alagoas (09° S) and Espírito Santo (19° S)]. Fish were collected using hand-nets in mangrove microhabitats and euthanized using clove oil, following the recommended ethical procedures (CEUA/UFRN 056.064/2017). Fin clips and small specimens were preserved in 99% ethanol for molecular analysis, which were sent for sequencing through the licence required from the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SISGEN), in compliance with the provisions of Law No. 13,123/2015 and its regulations, under registration number N° RB7FC3C. Individuals were fixed in formalin 10% and deposited at the ichthyological collection of Universidade Federal do Rio Grande do Norte. All fieldwork was carried out under the licence SISBIO 57145.

## 2.3 | DNA extraction and amplification

DNA extraction from 155 individuals collected along 26 sampling sites in Brazil (Figure 1) was performed using Qiagen® DNeasy blood and tissue extraction kit following the manufacturer’s protocol. A 615 bp region of the mitochondrial gene cytochrome oxidase subunit I (*cox1*) gene was amplified using primers and amplification protocol described in Tatarenkov *et al.* (2017). Both forward and reverse DNA strands were sequenced and assembled using Geneious v. 9.1.8 (www.

**TABLE 1** Sampling localities and respective sampling codes for the *Kryptolebias marmoratus* species complex

Sample ID	Location	Major area	Latitude	Longitude	Cox1	K	Reference
1. SSV <sup>C</sup>	Oyster Lake	San Salvador Island	24° 06' 39.0" N	074° 27' 47.8" W	3	I	Tatarenkov <i>et al.</i> , 2018
2. T&C <sup>C</sup>	Turks and Caicos	Turks and Caicos	21° 47' 25.2" N	071° 40' 11.9" W	NA	I	Tatarenkov <i>et al.</i> , 2017
3. GIT <sup>C</sup>	Guantanamo Bay	Cuba	19° 54' 38.6" N	075° 11' 44.5" W	1	I	Tatarenkov <i>et al.</i> , 2017
4. PRN <sup>C</sup>	Punta Maracayo	Puerto Rico	18° 29' 28.4" N	066° 47' 40.3" W	1	I	Tatarenkov <i>et al.</i> , 2017
5. PRS <sup>C</sup>	Puerto Rico, Southwest	Puerto Rico	17° 56' 57.0" N	066° 52' 06.0" W	3	I	Tatarenkov <i>et al.</i> , 2017
6. ALM <sup>C</sup>	Almirante Bay	Panama	09° 21' 12.2" N	082° 15' 31.5" W	4	I	Tatarenkov <i>et al.</i> , 2017
7. CHI <sup>C</sup>	Chiriquí Lagoon	Panama	09° 00' 40.7" N	081° 47' 27.1" W	12	I	Tatarenkov <i>et al.</i> , 2017
8. ARB <sup>C</sup>	Parkietenbos Dumpsite	Aruba	12° 29' 31.5" N	070° 00' 30.8" W	4	II	Tatarenkov <i>et al.</i> , 2017
9. BNR <sup>C</sup>	Lac Bay	Bonaire	12° 06' 37.7" N	068° 13' 02.4" W	5	II	Tatarenkov <i>et al.</i> , 2017
10. BAR <sup>C</sup>	Graeme Hall Swamp	Barbados	13° 04' 21.0" N	059° 34' 38.0" W	NA	II	Robins, 2019
11. TET <sup>C</sup>	Mangrove in Trinidad and Tobago	Trinidad e Tobago	10° 03' 36.0" N	061° 53' 48.6" W	NA	II	Millen, 2019
12. CAU <sup>C</sup>	Mangrove in Caussade	French Guiana	05° 06' 33.9" N	052° 40' 08.0" W	NA	III	Robert Solène, 2019
13. MAT <sup>C</sup>	Mangrove in Matoury	French Guiana	04° 50' 20.1" N	052° 18' 27.3" W	NA	III	Robert Solène, 2019
14. AMC <sup>C</sup>	Mangrove in Amapá State	N Brazil	02° 04' 32.30" N	050° 36' 34.70" W	4	III	This study
15. MJN <sup>C</sup>	<b>ESEC Maracá-Jipiôca A<sup>1</sup></b>	N Brazil	02° 04' 54.25" N	050° 29' 55.28" W	4	III	This study
16. MJS <sup>C</sup>	<b>ESEC Maracá-Jipiôca B<sup>1</sup></b>	N Brazil	02° 07' 16.50" N	050° 25' 47.40" W	2	III	This study
17. MJU <sup>C</sup>	<b>ESEC Maracá-Jipiôca C<sup>1</sup></b>	N Brazil	01° 55' 25.28" N	050° 27' 56.70" W	2	III	This study
18. MAR <sup>C</sup>	Mangrove in Marajó Island	N Brazil	00° 48' 3.50" S	048° 32' 35.35" W	6	III	This study
19. VIG <sup>C</sup>	Mangrove in Vigia	N Brazil	00° 51' 39.30" S	048° 07' 39.70" W	4	III	This study
20. AJU <sup>S</sup>	Ajuruteua Beach	N Brazil	00° 51' 05.69" S	046° 36' 00.14" W	NA	IV	Guimarães-Costa <i>et al.</i> , 2017
21. POR <sup>S</sup>	<b>Portinho River, Luís Correia<sup>2</sup></b>	NE Brazil	02° 53' 41.9" S	041° 40' 17.1" W	4	IV	This study
22. COR <sup>S</sup>	Coreaú River, Camocim	NE Brazil	02° 52' 52.9" S	040° 57' 41.5" W	4	IV	This study
23. JER <sup>S</sup>	<b>Mangrove in Jericoacoara<sup>3</sup></b>	NE Brazil	02° 50' 26.6" S	040° 34' 39.5" W	4	IV	This study
24. MAC <sup>S</sup>	Mangrove in Macau	NE Brazil	05° 06' 19.9" S	036° 37' 32.2" W	6	IV	Costa, 2016
25. GRE <sup>S</sup>	Mangrove in Guimarães	NE Brazil	05° 06' 29.1" S	036° 20' 17.6" W	6	IV	Costa, 2016
26. CEA <sup>S</sup>	Ceará-Mirim River, Extremoz	NE Brazil	05° 40' 25.88" S	035° 14' 14.48" W	4	IV	Tatarenkov <i>et al.</i> , 2017
27. CUR <sup>S</sup>	Curimataú River, Baía Formosa	NE Brazil	06° 21' 32.5" S	035° 01' 54.8" W	4	IV	Tatarenkov <i>et al.</i> , 2017
28. MPE <sup>S</sup>	Maracaípe River, Ipojuca	NE Brazil	08° 31' 06.6" S	035° 00' 36.8" W	4	IV	Tatarenkov <i>et al.</i> , 2017
29. MUN <sup>S</sup>	Mundaú Lagon, Maceió	NE Brazil	09° 40' 52.0" S	035° 45' 36.0" W	NA	IV	Costa, 2016
30. SER <sup>S</sup>	Sergipe River, Sergipe	NE Brazil	10° 58' 13.7" S	037° 3' 13.2" W	2	IV	This study
31. TIJ <sup>S</sup>	Tijuípe River, Itacaré	NE Brazil	14° 25' 17.6" S	039° 01' 20.48" W	NA	IV	Silva <i>et al.</i> , 2020
32. SAL <sup>S</sup>	<b>Salgado River, Terra Firme<sup>4</sup></b>	NE Brazil	15° 32' 47.17" S	038° 57' 40.30" W	5	IV	This study
33. COQ <sup>S</sup>	Coqueiral Beach, Aracruz	SE Brazil	19° 56' 3.44" S	040° 7' 48.13" W	5	V	Costa, 2016
34. PIR <sup>S</sup>	Piraquê-Açú River, Aracruz	SE Brazil	19° 57' 9.38" S	040° 9' 55.50" W	3	V	This study
35. ARA <sup>S</sup>	Araruama Lake, Iguaba Grande	SE Brazil	22° 50' 31" S	042° 12' 26" W	NA	V	Costa, 2016
36. IRI <sup>S</sup>	<b>Irirí River, Magé<sup>5</sup></b>	SE Brazil	22° 39' 48.79" S	043° 5' 12.20" W	30	V	This study
37. GNB <sup>S</sup>	Guanabara Bay, Rio de Janeiro	SE Brazil	22° 52' 2.48" S	043° 13' 27.48" W	29	V	Costa, 2016
38. MRP <sup>S</sup>	Marapendí Lake, Rio de Janeiro	SE Brazil	23° 00' 52" S	043° 24' 20" W	NA	V	Costa, 2016

(Continues)

TABLE 1 (Continued)

Sample ID	Location	Major area	Latitude	Longitude	Cox1	K	Reference
39. POT <sup>S</sup>	Portinho River, Rio de Janeiro	SE Brazil	23° 00' 25" S	043° 33' 06" W	NA	V	Costa, 2016
40. GUA <sup>S</sup>	Piracão River, Guaratiba	SE Brazil	23° 00' 06.7" S	043° 34' 51.6" W	30	V	Costa, 2016
41. SEP <sup>S</sup>	Sepetiba Bay, Itaguaí	SE Brazil	22° 54' 01" S	043° 49' 31" W	NA	V	Costa, 2016
42. MAG <sup>S</sup>	Mangaratiba Beach, Mangaratiba	SE Brazil	22° 57' 54" S	044° 02' 36" W	NA	V	Costa, 2016
43. PIC <sup>S</sup>	<b>Fazenda River, Picinguaba<sup>6</sup></b>	SE Brazil	23° 22' 01.0" S	044° 50' 13.4" W	2	V	Tatarenkov <i>et al.</i> , 2017
44. PER <sup>S</sup>	<b>Guaraú River, Peruibe<sup>7</sup></b>	SE Brazil	24° 22' 16.62" S	047° 01' 1.44" W	3	V	This study
Total						200	

Note: The superscript letters C and S in Sample ID correspond to individuals from the Central and Southern clades, respectively. Localities' names in bold represent sampling points within conservation units in Brazil, as follows: 1 – Estação Ecológica Maracá-Jipiôca; 2 – Área de Proteção Ambiental Delta do Parnaíba; 3 – Parque Nacional de Jericoacoara; 4 – Reserva Extrativista de Canavieiras; 5 – Área de Proteção Ambiental de Guapimirim; 6 – Parque Estadual da Serra do Mar – Núcleo Picinguaba; 7 – Estação Ecológica Juréia-Itatins.

geneious.com), and the sequences were deposited in GenBank (accession numbers: MT900628–MT900721).

## 2.4 | Geographical distribution, mtDNA clades, genetic structure and diversity

The final data set for the phylogeographical analysis of genetic structure, diversity and genetic clustering included 45 additional *cox1* sequences retrieved from previous studies (Tatarenkov *et al.*, 2017, 2018) (Supporting Information Table S1). This data set included sequences from individuals of the Central clade collected in Cuba, San Salvador Island, Puerto Rico, Panamá, Aruba and Bonaire. In total, the *cox1* data set contained sequences from 200 individuals (155 from this work, 45 retrieved from GenBank) corresponding to 31 locations of the 44 sampling points (Table 1).

The authors used principal component analysis (PCA) with the *cox1* haplotypes in the R package “adeget” v. 2. 1. 2. (Jombart, 2008). To infer whether a particular mtDNA haplotype belonged to the “Central” or “Southern” clades classified in Tatarenkov *et al.* (2017). According to which cluster of the PCA a particular haplotype was classified to, the authors identified it as belonging to either the “Central” or “Southern” clade. To infer the lineage from the data retrieved from previous literature without molecular data available, the authors defined a geographical criterion, which assumes that if a sampling point is geographically located between two other sampling sites confirmed by molecular identification as belonging to a certain clade, the individuals from that locality were classified as belonging to the same clade. To visualize the genetic relationships among haplotypes, the authors generated a haplotype network using the software POPART v. 4.8.4 (Leigh & Bryant, 2015).

In addition, the authors of this study used Geneland v.4.0.7 (Guillot *et al.*, 2008) to identify spatial genetic structure. Geneland integrates mtDNA and spatial data, allowing for uncertainty in the position of sampled individuals. The most likely number of genetic

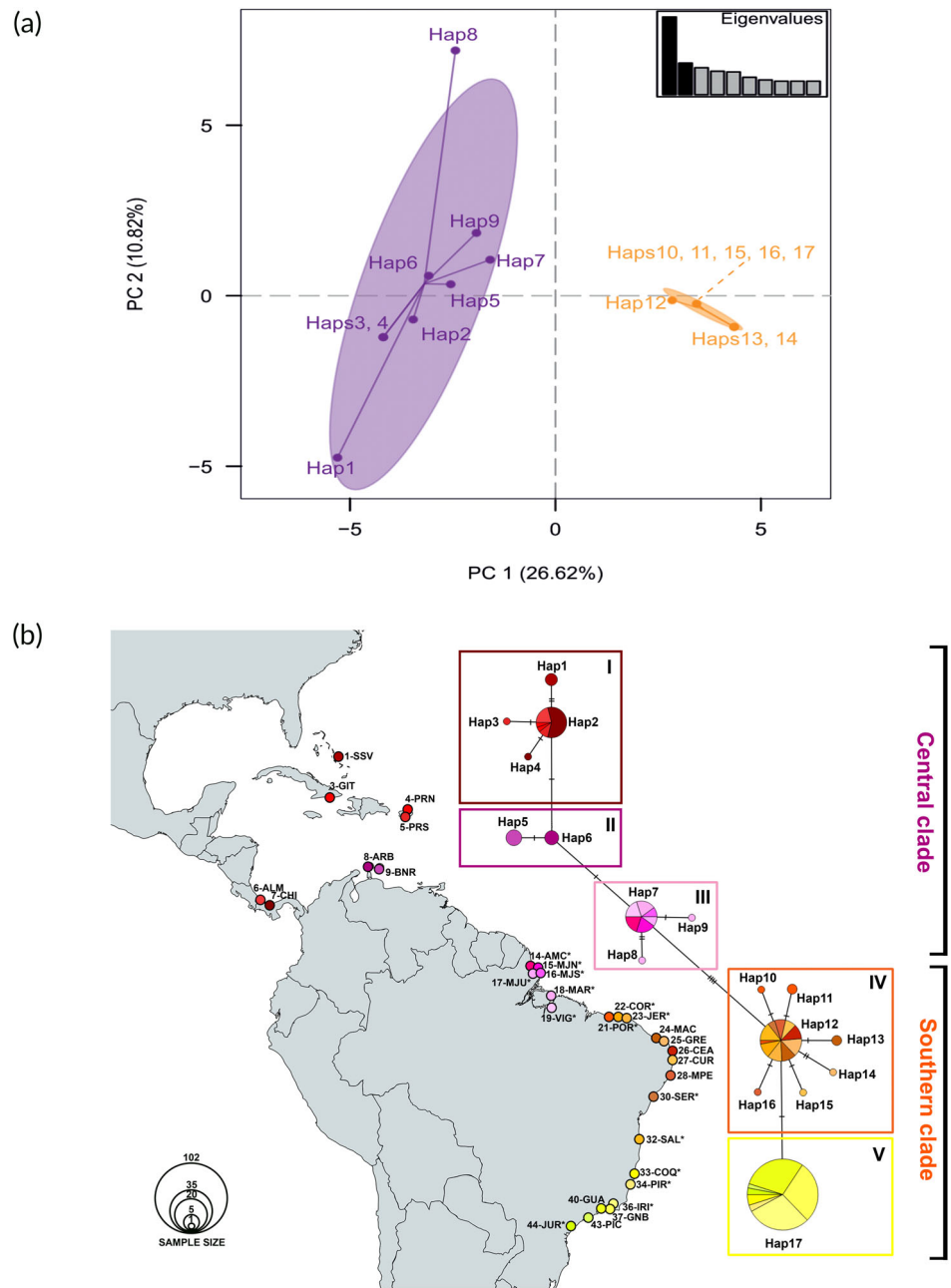
clusters (K) was determined using haplotype data for all 200 individuals with sequences together with the geographical coordinates from their localities. K values ranged from 2 to 10. Nine independent runs were performed, each with 1,000,000 iterations, sampled every 1000 iterations, with a burn-in of 200 iterations.

The authors of this study used Mega v.7 (Kumar *et al.*, 2016) to calculate Kimura-2-Parameter genetic distances between clades (Central and Southern) and among genetic clusters within clades (identified in Geneland). They used Arlequin v. 3.5 (Excoffier & Lischer, 2010) for calculating  $F_{ST}$  values (with 1000 permutations), as well as for metrics of mtDNA genetic diversity per clade and genetic clusters within clades, such as number of haplotypes (H), number of polymorphic sites (S), haplotype (h) and nucleotide diversity ( $\pi$ ). Finally, Arlequin v. 3.5 was also used to run Tajima's and Fu's neutrality tests to detect the possible scenarios of demographic expansion across genetic lineages

The authors used IBD v. 1.52 (Bohonak, 2002) to test for the association between genetic and geographic distances among genetic clusters within major clades as indicated in Geneland. On this software, they ran a Mantel test between the matrices of pairwise  $F_{ST}$  values among genetic clusters and estimated geographic distance in kilometres among genetic clusters. For this latter matrix, the authors selected the centre point of geographical distribution for each genetic cluster and calculated the pair-wise distance among the midpoints of each genetic cluster along the coast.

The authors tested three major hypotheses of genetic structuring using AMOVA with 1000 permutations in Arlequin v. 3.5 (Excoffier & Lischer, 2010). First, the clade division was proposed by Tatarenkov *et al.* (2017), with two major groups with individuals belonging to either the Central or Southern clades. A second hypothesis followed the marine provinces proposed by Spalding *et al.* (2007), represented by four coastal regions along the western Atlantic, which is based on regimes of surface temperature and humidity of ocean currents, environmental factors known to affect the dispersal and gene flow levels of species inhabiting mangrove habitats (Mori *et al.*, 2015). A third

**FIGURE 2** Genetic structure and variation for the “*Kryptolebias marmoratus* species complex” based on *cox1* sequences. (a) First two axis of a principal component analysis (PCA) for *cox1* haplotypes. Individuals were classified as belonging to either the Central (in purple) or Southern clade (in orange) according to which genetic cluster in the PCA their haplotypes belong to. (b) Haplotype network based on 615 bp *cox1* gene from 200 individuals of the “*Kryptolebias marmoratus* species complex” and its geographical distribution. Each colour on the map denotes a sampling location. Only the 31 locations which had *cox1* data were included in the map. Each circle in the haplotype network corresponds to a unique haplotype and its size is proportional to its frequency. Ticks on branches represent mutational steps. Acronyms for sampling points are described in Table 1. Asterisks in acronym names represent new sampling records. Numbered rectangles (from I to V) correspond to the genetic clusters identified using Geneland (see “Results”)



AMOVA followed the genetic clusters suggested by the result of Geneland analysis.

## 3 | RESULTS

### 3.1 | classification and distribution of mtDNA clades

The mtDNA data set consisted of 17 *cox1* haplotypes, 13 (76.47%) of them exclusive to unique localities, whereas the other 4 were found in at least five sampling sites (Figure 2; Supporting Information Table S2). The first two axes of the PCA (explaining 37.44% of the overall variation) revealed two major haplogroups, one containing the haplotypes from the central and other those from the Southern clade (Figure 2a;

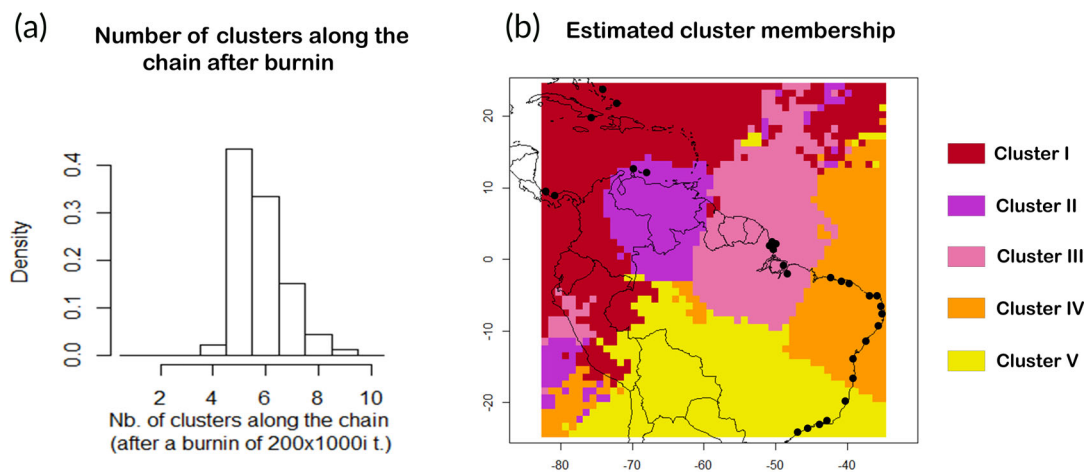
Supporting Information Figure S1). These haplogroups were also observed in the haplotype network, which showed a clear geographical structure (Figure 2b). The Central clade haplogroup contained nine haplotypes (haplotypes 1–9) distributed along 14 localities, from San Salvador Island, Cuba, Puerto Rico, Panama, Aruba, Bonaire and north Brazil, in Amapá and Pará states. The Southern clade haplogroup, restricted to Brazil, was separated by four mutational steps from the Central clade and exhibited eight haplotypes (haplotypes 10–17) along 17 localities, from Piauí to São Paulo states (Figure 2b and Supporting Information Table S2).  $F_{ST}$  was significantly different between the Central and Southern clades ( $P > 0.001$ ;  $F_{ST} = 0.85$ ). K2P genetic distance between Central and Southern clades was 1%.

Despite having fewer individuals sequenced (55 vs. 145), the Central clade showed higher values of genetic diversity when compared to the Southern clade (Table 2). Neutrality tests (Fu  $F_s$  and Tajima's

**TABLE 2** Genetic diversity indexes for (a) mtDNA clade and (b) genetic clusters for the *Kryptolebias marmoratus* species complex

	N	H	S	h	$\pi \times 100$	F's Fu	Tajima's D
(a) Clades							
Central clade	55	9	10	0.74	0.26	-2.00*	-0.81*
Southern clade	145	8	8	0.44	0.09	-4.24*	-1.43*
(b) Genetic clusters							
I. San Salvador/Cuba/Puerto Rico/Panama	24	4	4	0.36	0.10	-0.90*	-1.14*
II. Aruba/Bonaire	9	2	1	0.55	0.09	1.01	1.40
III. North Brazil	22	3	3	0.17	0.04	-1.25*	-1.72*
IV. Northeast Brazil	43	7	7	0.33	0.07	-5.77*	-2.02*
V. Southeast Brazil	102	1	0	0	0	0	0

Note: N, samples; H, number of haplotypes; S, number of polymorphic sites; h, haplotype diversity;  $\pi$ , nucleotide diversity multiplied by 100; F's Fu, statistical neutrality test of Fu and Tajima's D, statistical neutrality test of Tajima. \* $P < 0.05$ .



**FIGURE 3** Geneland clustering results for the “*Kryptolebias marmoratus* species complex” based on *cox1* sequences. (a) Most likely number of genetic clusters after burn-in period according to Geneland analysis from 200 *cox1* sequences from individuals “*Kryptolebias marmoratus* species complex.” (b) Geographical distribution of genetic clusters according to Geneland. Each colour represents a unique genetic cluster, with their respective sampling points within them

D) were negative and significant, indicating that both Central and Southern clades may have undergone recent population expansion, or potential selection at the mitochondrial DNA.

Of the total records, 31 (72.09%) had at least one individual sequenced for *cox1*, allowing mtDNA lineage identification. Following the molecular and geographical criteria, 19 of the sampling sites have individuals who belonged to the Central clade and 25 to the Southern clade. With the new records, the southern limit of the Central clade, previously attributed to Bonaire, is expanded 2630 km south to the Brazilian state of Pará, with sampling records in Barbados, Trinidad and Tobago, French Guiana and Brazil. In Brazil, this lineage is occurring in both sides of the Amazon River, in Amapá and Pará states, respectively (Figure 1).

The results of this study also fill gap in the distribution of the Southern clade along the Brazilian coast. The authors identified mangrove killifish specimens from this clade in the state of Piauí state (02° S) and two sampling sites in the state of Ceará. In the eastern coast of

Brazil, they have now included two new sampling records in Sergipe and Bahia states, besides a few new records in southeastern Brazil (Table 1). With the new record in Peruíbe (São Paulo State), the authors extended the southernmost distribution of the Southern clade (corresponding to *K. hermaphroditus*) by 248 km.

### 3.2 | mtDNA genetic structure and diversity between and within clades

Geneland results indicated further genetic subdivision within Central and Southern clades, with five being the most likely number of genetic clusters (K, in Table 1). Three of those belonged to the Central clade: (a) San Salvador, Cuba, Puerto Rico and Panama; (b) Aruba and Bonaire; and (c) north Brazil (Amapá and Pará states), whereas the other two were within the geographical limits of the Southern clade:

**TABLE 3** Pair-wise  $F_{ST}$  values (below diagonal) and Kimura-2-parameter (K2P) genetic distance (in percentage above diagonal) among genetic clusters indicated in Geneland *Kryptolebias marmoratus* species complex

Genetic cluster	I	II	III	IV	V
Cluster I (San Salvador, Cuba, Puerto Rico and Panama)		0.3	0.4	1.1	1.2
Cluster II (Aruba and Bonaire)	0.68*		0.3	1.0	1.1
Cluster III (north Brazil)	0.82*	0.79*		0.7	0.8
Cluster IV (northeast Brazil)	0.92*	0.92*	0.91*		0.2
Cluster V (southeast Brazil)	0.98*	0.99*	0.99*	0.88*	

Note: Asterisks represent  $P \leq 0.05$ .

(d) northeast Brazil (from Piauí to Bahia states in Brazil) and (e) southeast Brazil (Espírito Santo to São Paulo) (Figure 3; Supporting Information Figure S2). In terms of genetic diversity, the genetic clusters within the Central clade (I and II) have the highest values of genetic diversity, whereas the genetic cluster V (in southeast Brazil) only had one *cox1* haplotype, despite being the one with more individuals sequenced (Table 2). The neutrality tests for genetic clusters I, III and IV were also negative and significant, suggesting geographic or demographic expansion (Table 2). All pair-wise  $F_{ST}$  comparisons among genetic clusters were highly significant ( $P > 0.001$ ) (Table 3). Average  $F_{ST}$  values among genetic clusters were 0.89, with the highest values found between genetic clusters within the Central clade (I, II and III) in comparison to the genetic cluster in southeast Brazil (V). The lowest  $F_{ST}$  value was found between the genetic clusters I and II. The average genetic distance values among clades were 0.7, ranging from 1.2 (between genetic clusters I and V) to 0.2 (between northeast and southeast Brazil) (Table 3).

Despite the geographically structured configuration of haplotypes, they did not display isolation-by-distance ( $R^2 = 0.49$ ;  $P = 0.06$ ).

AMOVA results found significant genetic differences ( $P < 0.001$ ) for the three hypotheses tested, with the greatest amount of genetic partition found among the five genetic clusters revealed by Geneland (94.20%), followed by the four marine provinces (89.66%), and finally the two genetic clades (84.48%) (Table 4).

## 4 | DISCUSSION

The results of this study expand the known spatial distribution of mangrove killifishes in South America, with 14 new records covering 5434 km of the Brazilian coast, and 13 of them confirmed with molecular data. This information indicates a broader geographical distribution of both the Central and the Southern clades than previously thought and reveals different levels of mtDNA genetic diversity and structuring both between and within clades.

### 4.1 | Lineage distribution and taxonomic implications

Mangrove killifishes were first described as “rare and unknown in the wild” (Harrington & Kallman, 1968). Nonetheless, it is likely that a combination of factors has contributed to this presumed “rarity.”

Mangrove killifishes, as most killifish species, are small and cryptic and often inhabit mangrove microhabitats (e.g., crab burrows, shallow temporary pools and mangrove leaf litter), which makes them difficult to sample (Barletta *et al.*, 2010; Costa, 1998; Sarmiento-Soares *et al.*, 2014; Taylor, 2012). The results of this study indicate that the selfing *Kryptolebias* species extensively occupy mangroves in Central and South America, even in areas where it has been historically considered absent, and where particular biogeographical barriers (e.g., Amazon river or a gap in distribution of 1100 km along the Brazilian eastern coast) previously suggested by other studies (Costa, 2016; Taylor, 2012) could have constrained its dispersal and colonization.

The Central and Southern clades are identified based on mtDNA and microsatellite differences (Tatarenkov *et al.*, 2017), but their identity as separate lineages, or as different species, still needs to be confirmed given the large gap in geographical and genetic information between Venezuela and northeast Brazil. The data of this study identified, for the first time, the presence of the Central clade from Aruba and Bonaire to Pará in north Brazil. The *cox1* similarity between Central and Southern clades (five in Tatarenkov *et al.*, 2017, four here) as well as the low genetic distance between them indicate that Central and Southern clades are likely to belong to the same species.

If the hypothesis of a single species from south San Salvador to Brazil is confirmed, future taxonomic rearrangements will need to consider the fact that some *Kryptolebias* species (e.g., *Kryptolebias heyei* Nichols 1914; *Kryptolebias bonairensis* Hoedeman 1958) have been described in the Caribbean region before the description of *K. hermaphroditus*. Nonetheless, a full update on the taxonomic status of *K. hermaphroditus* still depends on further molecular evaluation of its original type-material (fixed in formalin), as there is some debate on whether *K. hermaphroditus* (*sensu* Costa, 2011) represents a junior synonym of *K. ocellatus* (Hensel, 1868; Huber, 2017). In either scenario, it is likely that the taxonomic validity of *K. hermaphroditus* will change (*i.e.*, either as *K. ocellatus* or a junior-synonym of one of the Caribbean species). Therefore, the authors conservatively suggest considering the specimens belonging to the Central and Southern clade as *K. hermaphroditus*; nonetheless, they are aware that a formal taxonomic work is urgently needed to elucidate the taxonomic status of the selfing *Kryptolebias* species.

Geographically, the newly discovered southern limit of the Central clade (in Vigia, Pará state in Brazil) is only 180 km North from Ajuruteua (AJU), a sampling site where the Southern clade had previously been identified (Tatarenkov *et al.*, 2017). Extensive hybridization and introgression between individuals from the Northern clade,

**TABLE 4** AMOVA for three levels of hierarchical structure of populations for *K. marmoratus* species complex in the Central and Southern clades, based on *cox1* data

Source of variation	d.f.	Sum of squares	% of variation	Significance (P)
Test 1 (structure: Phylogeny; two clades)				
Among groups ( $F_{CT}$ )	1	201.83	84.48	<0.001
Among populations within groups ( $F_{SC}$ )	29	70.68	13.44	<0.001
Within populations ( $F_{ST}$ )	169	10.33	2.08	<0.001
Total	199	282.85		
$F_{CT}$ : 0.84, $F_{SC}$ : 0.86, $F_{ST}$ : 0.97				
Test 2 (structure: marines provinces by Spalding <i>et al.</i> (2007), four regions)				
Among groups ( $F_{CT}$ )	3	246.84	89.66	<0.001
Among populations within groups ( $F_{SC}$ )	27	25.67	7.37	<0.001
Within populations ( $F_{ST}$ )	169	10.33	2.96	<0.001
Total	199	282.85		
$F_{CT}$ : 0.89, $F_{SC}$ : 0.71, $F_{ST}$ : 0.97				
Test 3 (structure: genetic clusters by Geneland, five clusters)				
Among groups ( $F_{CT}$ )	4	261.90	94.20	<0.001
Among populations within groups ( $F_{SC}$ )	26	10.61	2.85	<0.001
Within populations ( $F_{ST}$ )	169	10.33	2.95	<0.001
Total	199	282.85		
$F_{CT}$ : 0.94, $F_{SC}$ : 0.49, $F_{ST}$ : 0.97				

Note: The marine provinces proposed by Spalding *et al.* (2007) are shown in Figure 1.

corresponding to *K. marmoratus*, and individuals from the Central clade in San Salvador Island and Bahamas has been recently reported, despite the fact that these two clades are genetically more divergent from each other (3% K2P distance at *cox1*) than the Central and Southern clades (1%) (Tatarenkov *et al.*, 2018, 2020). If genetic similarity is a proxy for reproductive isolation (Sánchez-Guillén *et al.*, 2014), it is possible that hybridization and introgression may also happen between individuals from the Central and Southern clades. Based on the data of this study, the authors suggest a possible contact zone between the Central and Southern clades is on the coast of the Pará state, between Vigia and Ajuruteua. Nonetheless, given the inherent limitations of mtDNA data (*e.g.*, matrilineal inheritance), the authors cannot discard the possibility that these clades are already introgressing in north Brazil. Further research is needed to shed light on the distinctiveness, the potential contact zone, as well as degree of gene flow between these clades in the northern portion of South America.

## 4.2 | Phylogeographic patterns and biogeographical barriers

Determining the potential climatic/biogeographical drivers influencing the patterns of genetic structure in mangrove killifishes is particularly challenging given that mangrove killifishes rarely share microhabitats with other fish species (Taylor, 2012), limiting the possibility of tracing phylogeographical parallels between mangrove killifish species and

other fish species. Nonetheless, phylogeographic studies with other mangrove organisms (*e.g.*, plants and crabs) from the same region have generally revealed similar patterns of genetic structuring compared to the ones found for the mangrove killifishes. Overall, the populations of the “*K. marmoratus* species complex” from the Central clade harbour the highest genetic diversity followed by populations in northeast and southeast Brazil, where a single mtDNA *cox1* haplotype was found across an extension of 1236 km. The indication of population expansion in the Southern clade, although it can also be interpreted as evidence of selection in the mtDNA (Rand, 2001), agrees with the absence of genetic differences previously found in southeast Brazil, even using highly variable microsatellite loci (Tatarenkov *et al.*, 2011, 2017). These results reinforce the idea that *K. hermaphroditus* spreads southwards along South American coast and has recently colonized the mangroves in southeast Brazil (Berbel-Filho *et al.*, 2020). Similarly, Pil *et al.* (2011) observed that populations of the mangrove tree *Rhizophora mangle* L. from north Brazil harboured higher levels of genetic diversity than the populations in higher latitudes, suggesting that during the last glacial event, mangrove forests had retracted and were restricted to the equatorial regions (see Hewitt, 2000, 2004) which worked as refuge areas for the western Atlantic mangroves. After the last glaciation, temperature and humidity (two of crucial predictor of mangrove trees distribution) began to rise along with sea levels (Mori *et al.*, 2015; Takayama *et al.*, 2013), providing a gradual expansion of the distribution of *R. mangle* along coastal regions in southern Brazil. Palynological data support southward expansion of *R. mangle* along the Brazilian coast, with records of

mangrove populations from north Brazil dating c. 5–7× older than the records in southeast (Pil *et al.*, 2011). On the contrary, other mangrove-dwelling organisms, such as Decapoda crustaceans [e.g., *Cardisoma guanhumi* Latreille, 1828, *Ucides cordatus* (Linnaeus, 1763), *Uca maracoani* (Latreille, 1802)], form panmictic populations along the Brazilian coast (Buranelli *et al.*, 2019; Moksnes *et al.*, 2014; Oliveira-Neto *et al.*, 2008; Wieman *et al.*, 2013). These differences among genetic patterns of genetic structure among mangrove organisms might be attributed to different dispersive and colonization strategies (Buranelli *et al.*, 2019; Sereneski-Lima *et al.*, 2020). Given the similar mode of long-term dispersal (*via* viviparous propagules in the trees and fertilized eggs for the fish) and the strict ecological association between mangrove plants and its associated organisms (Davis *et al.*, 1995; Richards *et al.*, 2011), the patterns of genetic structuring observed here in “*K. marmoratus* species complex” may more specifically resemble those of mangrove tree themselves, *R. mangle*, *Avicennia* spp. and *Laguncularia recemosa* (L.) than other mangrove-dwelling organisms.

The five genetic clusters revealed by Geneland explained the highest partition of the genetic variation. The geographical limits of these genetic clusters generally match those of the marine provinces (Spalding *et al.*, 2007), another partition shown to significantly influence the genetic structure of the mangrove killifishes along the western Atlantic mangroves. These results reinforce the potential role of humidity and temperature biotic and abiotic variables in structuring genetic variation among mangrove populations (Hodel *et al.*, 2018; Sereneski-Lima *et al.*, 2020). Nonetheless, the actual ecological effects of those ecoregions for the dispersal of mangroves killifishes remain largely unknown. Further research is needed to investigate which and how different environmental factors influence the dispersal capacity of mangrove killifishes, especially *via* fertilized eggs.

## 5 | CONCLUSION

The size of mangrove ecosystems is shrinking at an alarming rate (Romañach *et al.*, 2018), and information about their associated organisms is relatively scarce. This study has helped to clarify the distribution of mangrove killifishes in Central and South American mangroves and revealed contrasting patterns of genetic structure and diversity across their distribution. *K. hermaphroditus*, although cryptic and microhabitat-specific, seems to be ubiquitously distributed along Caribbean, Central and South American mangroves. The new information provided here should be used to re-evaluate the conservation status of *K. hermaphroditus*, currently classified as near-threatened (Brasil, 2014), particularly considering the geographically structured levels of genetic diversity of the species and the marked differences in threats imposed by different human activities (e.g., aquaculture and urbanization) in different areas across western Atlantic mangrove forests.

## ACKNOWLEDGEMENTS

We are grateful to all the people who supported us during fieldwork: in northeast Brazil to the members of the LISE team from Universidade Federal do Rio Grande do Norte; in Bahia to Erik

Tedesco and Aloisio Tedesco; in Pará to Márcio Silva, Youssef Bitar and Leandra Pinheiro; in Amapá to Thais Marianne, Gírlan Dias, Lailson Ferreira and José Luis Barata for fieldwork. We also thank the managers of the conservation units Aruã Fernandes Antunes Caetano (ESEC Juréia-Itatins, São Paulo), Cláudia Camila Faria de Oliveira (Parque Estadual da Serra do Mar – Núcleo Picinguaba, São Paulo), Jerônimo Carvalho Martins (Parque Nacional de Jericoacoara, Ceará), Iranildo Coutinho (ESEC Maracá-Jipioca, Amapá), as well as André Silva for donating the first specimen collected in Bahia State, and to Juçara Valverde and Pedro Habibe for logistic support in Rio de Janeiro and Ceará, respectively. Finally, we are grateful to three anonymous reviewers whose suggestions have improved the quality of this study.

## AUTHOR CONTRIBUTIONS

S.M.Q.L., M.G.S.L., W.M.B.-F. and H.M.V.E.S. conceived and implemented the study. M.G.S.L., W.M.B.-F. and A.T. analysed the data. S.M.Q.L., H.M.V.E.S., J.A., S.C. and C.G.L. provided funding. M.G.S.L. and W.M.B.-F. wrote the paper with input from all the authors.

## ORCID

Mateus G. S. Lira  <https://orcid.org/0000-0001-8588-552X>

Waldir M. Berbel-Filho  <https://orcid.org/0000-0001-6991-4685>

Helder M. V. Espirito-Santo  <https://orcid.org/0000-0001-7256-1868>

Andrei Tatarenkov  <https://orcid.org/0000-0002-0516-5862>

Carlos Garcia de Leaniz  <https://orcid.org/0000-0003-1650-2729>

Sofia Consuegra  <https://orcid.org/0000-0003-4403-2509>

Sergio M. Q. Lima  <https://orcid.org/0000-0001-9365-4879>

## REFERENCES

- Allendorf, F. W. (2017). Genetics and the conservation of natural populations: Allozymes to genomes. *Molecular Ecology*, 26, 420–430.
- Avise, J. C., & Tatarenkov, A. (2015). Population genetics and evolution of the mangrove rivulus *Kryptolebias marmoratus*, the world's only self-fertilizing hermaphroditic vertebrate. *Journal of Fish Biology*, 87, 519–538.
- Barletta, M., Jaureguizar, A. J., Baigun, C., Fontoura, N. F., Agostinho, A. A., Almeida-Val, V. M., ... Correa, M. F. (2010). Fish and aquatic habitat conservation in South America: A continental overview with emphasis on neotropical systems. *Journal of Fish Biology*, 76, 2118–2176.
- Berbel-Filho, W. M., Espirito-Santo, H. M. V., & Lima, S. M. Q. (2016). First record of a male of *Kryptolebias hermaphroditus* Costa, 2011 (Cyprinodontiformes: Cynolebiidae). *Neotropical Ichthyology*, 14, e160024.
- Berbel-Filho, W. M., Garcia de Leaniz, C., Morán, P., Cable, J., Lima, S. M., & Consuegra, S. (2019). Local parasite pressures and host genotype modulate epigenetic diversity in a mixed-mating fish. *Ecology and Evolution*, 9, 8736–8748.
- Berbel-Filho, W. M., Tatarenkov, A., Espirito-Santo, H. M. V., Lira, M. G. S. L., Garcia de Leaniz, C. G., Lima, S. M. Q., & Consuegra, S. (2020). More than meets the eye: Syntopic and morphologically-similar mangrove killifish species show different mating systems and patterns of genetic structure along the Brazilian coast. *Heredity*, 125, 340–352.
- Blaber, S. J. M., & Barletta, M. (2016). A review of estuarine fish research in South America: What has been achieved and what is the future for sustainability and conservation? *Journal Fish of Biology*, 89, 537–568.

- Bohonak, A. J. (2002). IBD (isolation by distance): A program for analyses of isolation by distance. *Journal of Heredity*, 93(2), 153–154.
- Branoff, B. L. (2017). Quantifying the influence of urban land use on mangrove biology and ecology: A meta-analysis. *Global Ecology and Biogeography*, 26, 1339–1356.
- Brasil. Ministério do Meio Ambiente. Portaria N° 445, de 17 de dezembro de 2014.
- Buranelli, R. C., Felder, D. L., & Mantelatto, F. L. (2019). Genetic diversity among populations of the Western Atlantic mangrove crab *Ucides cordatus* (Linnaeus, 1763) (Decapoda: Brachyura: Ocypodidae): Evidence for panmixia and useful data for future management and conservation. *Journal of Crustacean Biology*, 39(4), 386–395.
- Costa, W. J. E. M. (1998). Phylogeny and classification of Rivulidae revisited: Origin and evolution of annualism and miniaturization in rivulid fishes (Cyprinodontiformes: Aplocheiloidei). *Journal of Comparative Biology*, 3(1), 34–92.
- Costa, W. J. E. M. (2011). Identity of *Rivulus ocellatus* and a new name for a hermaphroditic species of *Kryptolebias* from south-eastern Brazil (Cyprinodontiformes: Rivulidae). *Ichthyological Exploration of Freshwaters*, 22, 185–192.
- Costa, W. J. E. M. (2016). Colouration, taxonomy and geographical distribution of mangrove killifishes, the *Kryptolebias marmoratus* species group, in southern Atlantic coastal plains of Brazil (Cyprinodontiformes: Rivulidae). *Ichthyological Exploration of Freshwaters*, 27, 183–192.
- Costa, W. J. E. M., Lima, S. M. Q., & Bartolette, R. (2010). Androdioecy in *Kryptolebias* killifish and the evolution of self-fertilizing hermaphroditism. *Biological Journal of the Linnean Society*, 99, 344–349.
- CRIA (Centro de Referência e Informação Ambiental). (2011). Specieslink - simple search. Retrieved from <http://www.splink.org.br/index>
- Davis, W. P., Taylor, D. S., & Turner, B. C. (1995). Does the autoecology of the mangrove *Rivulus* fish (*Rivulus marmoratus*) reflect a paradigm for mangrove ecosystem sensitivities? *Bulletin of Marine Science*, 57(1), 208–214.
- Duke, N. C., Meynecke, J. O., Dittmann, S., Ellison, A. M., Anger, K., Berger, U., ... Dahdouh-Guebas, F. (2007). A world without mangroves? *Science*, 317, 41–42.
- Excoffier, L., & Lischer, H. E. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. *Molecular Ecology Resources*, 10, 564–567.
- Ferreira, A. C., Ganade, G., & Attayde, J. L. (2015). Restoration versus natural regeneration in a tropical mangrove: Effects on plant biomass and crab communities. *Ocean & Coastal Management*, 110, 38–45.
- Guillot, G., Santos, F., & Estoup, A. (2008). Analysing georeferenced population genetics data with Geneland: A new algorithm to deal with null alleles and a friendly graphical user interface. *Bioinformatics*, 24, 1406–1407.
- Guimarães-Costa, A., Schneider, H., & Sampaio, I. (2017). New record of the mangrove rivulid *Kryptolebias hermaphroditus* Costa, 2011 (Cyprinodontiformes: Cynolebiidae) in the Pará state, northern Brazil. *Check List*, 13, 2093.
- Harrington, R. W., & Kallman, K. D. (1968). The homozygosity of clones of the self-fertilizing hermaphroditic fish *Rivulus marmoratus* Poey (Cyprinodontidae, Atheriniformes). *The American Naturalist*, 102, 337–343.
- Hensel, R. F. (1868). Beiträge zur kenntniss der wirbelthiere Südbrasilens. *Archiv für Naturgeschichte*, 34, 323–375.
- Hewitt, G. (2000). The genetic legacy of the quaternary ice ages. *Nature*, 405, 908–913.
- Hewitt, G. (2004). Genetic consequences of climatic oscillations in the quaternary. *Philosophical Transactions of the Royal Society B*, 359, 183–195.
- Hodel, R. G., Knowles, L. L., McDaniel, S. F., Payton, A. C., Dunaway, J. F., Soltis, P. S., & Soltis, D. E. (2018). Terrestrial species adapted to sea dispersal: Differences in propagule dispersal of two Caribbean mangroves. *Molecular Ecology*, 27(22), 4612–4626.
- Huber, J. (2017). Reanalysis of single type of *Rivulus ocellatus* Hensel, 1880 in Berlin museum pending its putative molecular analysis, with the proposal of revalidation of *Rivulus caudomarginatus*. *Killi-Data Series*, 2016–2017, 4–12.
- Jombart, T. (2008). Adegnet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11), 1403–1405.
- Kanamori, A., Sugita, Y., Yuasa, Y., Suzuki, T., Kawamura, K., Uno, Y., ... Sakakura, Y. (2016). A genetic map for the only self-fertilizing vertebrate. *G3: Genes, Genomes, Genetics*, 6, 1095–1106.
- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2016). MEGA7: Molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution*, 33, 1870–1874.
- Leigh, J. W., & Bryant, D. (2015). Popart: Full-feature software for haplotype network construction. *Methods in Ecology and Evolution*, 6, 1110–1116.
- Lira, M. G. S. L., Paiva, R. E. C., Ramos, T. P. A., & Lima, S. M. Q. (2015). First record of *Kryptolebias hermaphroditus* Costa, 2011 (Cyprinodontiformes: Rivulidae) in the extreme north Atlantic Forest mangroves, Rio Grande do Norte state, Brazil. *Check List*, 11, 1–5.
- Lugo, A. E., Medina, E., & McGinley, K. (2014). Issues and challenges of mangrove conservation in the Anthropocene. *Madera y Bosques*, 20, 11–38.
- Millen, B. (2019). Ichthyology Collection - Royal Ontario Museum. Version 18.7. Royal Ontario Museum. Occurrence dataset. <https://doi.org/10.15468/sysbx> accessed via GBIF.org on 2019-12-11. Retrieved from <https://www.gbif.org/occurrence/1211714736>.
- Moksnes, P.-O., Corell, H., Tryman, K., Hordoir, R., & Jonsson, P. R. (2014). Larval behavior and dispersal mechanisms in shore crab larvae (*Carcinus maenas*): Local adaptations to different tidal environments? *Limnology and Oceanography*, 59(2), 588–602.
- Mori, G. M., Zucchi, M. I., Sampaio, I., & Souza, A. P. (2015). Species distribution and introgressive hybridization of two *Avicennia* species from the Western hemisphere unveiled by phylogeographic patterns. *BMC Evolutionary Biology*, 15(61), 1–15.
- Murphy, W. J., Thomerson, J. E., & Collier, G. E. (1999). Phylogeny of the Neotropical killifish family Rivulidae (Cyprinodontiformes, Aplocheiloidei) inferred from mitochondrial DNA sequences. *Molecular Phylogenetics and Evolution*, 13, 289–301.
- Oliveira-Neto, J. F., Pie, M. R., Chammas, M. A., Ostrensky, A., & Boeger, W. A. (2008). Phylogeography of the blue land crab, *Cardisoma guanhumi* (Decapoda: Gecarcinidae) along Brazilian coast. *Journal of the Marine Biological Association of the U.K.*, 88(7), 1417–1423.
- Pil, M. W., Boeger, M. R. T., Muschner, V. C., Pie, M. R., Ostrensky, A., & Boeger, W. A. (2011). Postglacial north-south expansion of populations of *Rhizophora mangle* (Rhizophoraceae) along the Brazilian coast revealed by microsatellite analysis. *American Journal of Botany*, 98(6), 1031–1039.
- Poey, F. (1880). Revisio piscium cubensium. *Anales de la Sociedad Española de Historia Natural*, 9, (243–261).
- Rand, D. M. (2001). The units of selection on mitochondrial DNA. *Annual Review of Ecology and Systematics*, 32(1), 415–448.
- Richards, T. M., Justin, M., Krebs, J. M., & McIvor, C. C. (2011). Microhabitat associations of a semi-terrestrial fish, *Kryptolebias marmoratus* (Poey 1880) in a mosquito-ditched mangrove forest, west-Central Florida. *Journal of Experimental Marine Biology and Ecology*, 401, 48–56.
- Robert Solène, N. (2019). Données d'occurrences Espèces issues de l'inventaire des ZNIEFF. Version 1.1. UMS PatriNat (OFB-CNRS-MNHN), Paris. Occurrence dataset <https://doi.org/10.15468/ikshke> accessed via GBIF.org on 2019-05-10. Retrieved from <https://www.gbif.org/occurrence/2599292155>.
- Robins, R. (2019). UF FLMNH Ichthyology. Version 117.317. Florida Museum of Natural History. Occurrence dataset. <https://doi.org/10.15468/8mjsej> accessed via GBIF.org on 2019-06-25. <https://www.gbif.org/occurrence/624163468>.

- Romañach, S. S., DeAngelis, D. L., Koh, H. L., Li, Y., Teh, S. Y., Barizan, R. S. R., & Zhai, L. (2018). Conservation and restoration of mangroves: Global status, perspectives, and prognosis. *Ocean and Coastal Management*, 154, 72–82.
- Sánchez-Guillén, R. A., Córdoba-Aguilar, A., Cordero-Rivera, A., & Wellenreuther, M. (2014). Genetic divergence predicts reproductive isolation in damselflies. *Journal of Evolutionary Biology*, 27, 76–87.
- Sarmiento-Soares, L. M., Ingenito, L. F., Duboc, L., Martins-Pinheiro, R., Borçato, R., & Silva, J. (2014). Primeiros registros de *Kryptolebias ocellatus* (Hensel)(Cyprinodontiformes, Rivulidae) para riachos de Mata Atlântica no Espírito Santo. *Boletim Sociedade Brasileira de Ictiologia*, 111, 15–19.
- Seegers, L. (1984). Zur revision der *Rivulus*-arten sudost-Brasiliens, mit einer Neubeschreibung von *Rivulus luelingi* n. spp. und *Rivulus caudomarginatus* n. spp. (Pisces: Cyprinodontidae: Rivulinae). *Zoologische Beiträge*, 28, 271–320.
- Sereneski-Lima, C., Baggio, R. A., Pil, M. W., Torres Boeger, M. R., & Boeger, W. A. (2020). Historical and contemporary factors affect the genetic diversity and structure of *Laguncularia racemosa* (L.) Gaertn, along the western Atlantic coast. *Estuarine, Coastal and Shelf Science*, 249, 107055.
- Silva, A. T., Chagas, R. J., Santos, A. C. A., Zanata, A. M., Rodriguez, B. K., Polaz, C. N. M., ... Camelier, P. (2020). Freshwater fishes of the Bahia state, northeastern Brazil. *Biota Neotropica*, 20(4), e20200969.
- Spalding, M. D., Fox, H. E., Allen, G. R., Davidson, N., Ferdaña, Z. A., Finlayson, M., ... Robertson, J. (2007). Marine Ecoregions of the world: A bioregionalization of coastal and shelf areas. *Bioscience*, 57(7), 573–583.
- Takayama, K., Tamura, M., Tateishi, Y., Webb, E. L., & Kajita, T. (2013). Strong genetic structure over the American continents and transoceanic dispersal in the mangrove genus *Rhizophora* (Rhizophoraceae) revealed by broad-scale nuclear and chloroplast DNA analysis. *American Journal of Botany*, 100(6), 1191–1201.
- Tatarenkov, A., Earley, R. L., Taylor, D. S., & Avise, J. C. (2012). Microevolutionary distribution of isogenicity in a self-fertilizing fish (*Kryptolebias marmoratus*) in the Florida keys. *Integrative Comparative Biology*, 52, 743–752.
- Tatarenkov, A., Earley, R. L., Taylor, D. S., Davis, W. P., & Avise, J. C. (2018). Natural hybridization between divergent lineages in a selfing hermaphroditic fish. *Biology Letters*, 14, 20180118.
- Tatarenkov, A., Earley, R. L., Taylor, D. S., Davis, W. P., & Avise, J. C. (2020). Extensive hybridization and past introgression between divergent lineages in a quasi-clonal hermaphroditic fish: Ramifications for species concepts and taxonomy. *Journal of Evolutionary Biology*, 1–11.
- Tatarenkov, A., Lima, S. M. Q., & Avise, J. C. (2011). Extreme homogeneity and low genetic diversity in *Kryptolebias ocellatus* from south-eastern Brazil suggest a recent foundation for this androdioecious fish population. *Journal of Fish Biology*, 79, 2095–2105.
- Tatarenkov, A., Lima, S. M. Q., Earley, R. L., Berbel, W. M., Vermeulen, F. B. M., Taylor, D. S., ... Avise, J. C. (2017). Deep and concordant subdivisions in the self-fertilizing mangrove killifishes (*Kryptolebias*) revealed by nuclear and mtDNA markers. *Biological Journal of the Linnean Society*, 122, 558–578.
- Tatarenkov, A., Lima, S. M. Q., Taylor, D. S., & Avise, J. C. (2009). Long-term retention of self-fertilization in a fish clade. *Proceedings of the National Academy of Sciences of the U.S.A.*, 106, 14456–14459.
- Tatarenkov, A., Ring, B. C., Elder, J. F., Bechler, D. L., & Avise, J. C. (2010). Genetic composition of laboratory stocks of the self-fertilizing fish *Kryptolebias marmoratus*: A valuable resource for experimental research. *PLoS One*, 5, e12863.
- Taylor, D. S. (2012). Twenty-four years in the mud: What have we learned about the natural history and ecology of the mangrove rivulus, *Kryptolebias marmoratus*? *Integrative and Comparative Biology*, 52, 724–736.
- Taylor, D. S., Turner, B. J., Davis, W. P., & Chapman, B. B. (2008). A novel terrestrial fish habitat inside emergent logs. *The American Naturalist*, 171(2), 263–266.
- Vermeulen, F. B., & Hrbek, T. (2005). *Kryptolebias sepia* n. sp. (Actinopterygii: Cyprinodontiformes: Rivulidae), a new killifish from the Tapanahony River drainage in southeast Surinam. *Zootaxa*, 928, 1–20.
- Wiemann, A. C., Berendzen, P. B., Hampton, K. R., Jang, J., Hopkins, M. J., Jurgenson, J., & Thurman, C. L. (2013). A panmictic fiddler crab from the coast of Brazil? Impact of divergent ocean currents and larval dispersal potential on genetic and morphological variation in *Uca maracoani*. *Marine Biology*, 161(1), 173.

## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Lira MGS, Berbel-Filho WM, Espírito-Santo HMV, et al. Filling the gaps: phylogeography of the self-fertilizing *Kryptolebias* species (Cyprinodontiformes: Rivulidae) along South American mangroves. *J Fish Biol.* 2021;99: 644–655. <https://doi.org/10.1111/jfb.14753>