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Molecular identification of *Brachygenys* and *Haemulon* species (Perciformes: Haemulidae) from the Brazilian coast

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The fishes of the Haemulidae family are currently allocated to 19 genera with a worldwide distribution in the tropical and subtropical waters of the world's oceans. *Brachygenys* and *Haemulon* are important genera of reef fish in Brazil, as they occur in large shoals, which are both ecologically and commercially valuable. This study identified the Brazilian species of the genera *Brachygenys* and *Haemulon* using DNA barcodes. While we found only a single lineage in *Brachygenys chrysargyrea*, *Haemulon melanurum*, *H. parra*, and *H. squamipinna*, more than one molecular operational taxonomic unit (MOTU) was identified in *H. atlanticus*, *H. aurolineatum*, and *H. plumieri*, indicating the possible existence of discrete populations or cryptic species.

Keywords: Barriers, DNA barcoding, Marine fish, Species delimitation, Western Atlantic.

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Os peixes da família Haemulidae estão atualmente distribuídos em 19 gêneros, com distribuição mundial em águas oceânicas tropicais e subtropicais. *Brachygenys* e *Haemulon* são importantes gêneros de peixes recifais do Brasil, visto que ocorrem em grandes cardumes, de valores ecológicos e comerciais. Este estudo identificou as espécies brasileiras dos gêneros *Brachygenys* e *Haemulon* usando o código de barras de DNA. Embora apenas uma única linhagem de *Brachygenys chrysargyrea*, *Haemulon melanurum*, *H. parra* e *H. squamipinna* tenha sido encontrada em nosso conjunto de dados, mais de uma unidade taxonômica operacional molecular (MOTU) foi identificada em *H. atlanticus*, *H. aurolineatum* e *H. plumieri*, indicando a possível existência de populações discretas ou espécies crípticas.

Palavras-chave: Barreiras, DNA barcoding, Peixes marinhos, Delimitação de espécies, Atlântico Ocidental.

INTRODUCTION

Haemulidae is composed of 136 fish species in 19 genera (Fricke *et al*., 2021). Two of these genera, *Brachygenys* Poey, 1868 and *Haemulon* Cuvier, 1829, are considered important groups of reef fish found in Brazil, given that they occur in large shoals, which are ecologically and commercially valuable (Rocha *et al*., 2008). The most recent review of the Haemulidae identified 21 species in the genus *Haemulon* (scaled-fin grunts), of which, 16 occur in the western Atlantic, while five species are found in the eastern Pacific (Tavera, Wainwright, 2019; Fricke *et al*., 2021). Menezes *et al*. (2003) recorded the occurrence of nine *Haemulon* species on the Brazilian coast: *Haemulon aurolineatum* Cuvier, 1830, *H. chrysargyreum* (Günther, 1859), *H. melanurum* (Linnaeus, 1758), *H. parra* (Demarest, 1823), *H. plumieri* (Lacepède, 1801), *H. sciurus* (Shaw, 1803), *H. squamipinna* Rocha & Rosa, 1999, *H. steindachneri* (Jordan & Gilbert, 1882) (currently identified as *Haemulon atlanticus* Carvalho, Marceniuk, Oliveira & Wosiacki, 2021 by Carvalho *et al*., 2020), and *H. striatum* (Linnaeus, 1758).

Tavera, Wainwright (2019) reassigned *Haemulon chrysargyreum* to the genus *Brachygenys*, based on morphological and molecular evidence, with the current valid name *Brachygenys chrysargyrea* (Günther, 1859) (Fricke *et al*., 2021). The genus *Brachygenys* (smallmouth grunts) includes only three species, *Brachygenys californiensis* (Steindachner, 1875) and *B. jessiae* (Jordan & Bollman, 1890), which are found in the eastern Pacific (Tavera, Wainwright, 2019), and *B. chrysargyrea* which occurs in the Western Atlantic, where it is restricted to the oceanic islands of Brazil, the Fernando de Noronha and Atol das Rocas archipelagos (Rocha, Rosa, 1999; Fricke *et al*., 2021).

The biological characteristics of the *Brachygenys* and *Haemulon* species, including their ample geographic ranges, ecological features, genetic patterns, and speciation mechanisms, has been the subject of many taxonomic, evolutionary, and phylogenetic studies (*e.g*., Rocha *et al*., 2008; Motta-Neto *et al*., 2011a,b; Sanciangco *et al*., 2011; Liang *et al*., 2012; Tavera *et al*., 2012, 2018; Bernal *et al*., 2017; Tavera, Wainwright, 2019). The formation of marine biogeographic barriers, in particular, the Isthmus of Panama, resulted in the establishment of geminal species (Jordan, 1908), that is, allopatric twin

species in the eastern Pacific and western Atlantic oceans, as in the case of *H. steindachneri* from the eastern Pacific and *H. atlanticus* from the western Atlantic (Carvalho *et al*., 2020).

The principal objective of this study was to identify the *Brachygenys* and *Haemulon* species from Brazil based on the DNA barcode method. We also evaluated the influence of oceanic barriers on the dispersal of the study species.

MATERIAL AND METHODS

Sample collection. Three specimens of *Brachygenys chrysargyrea* and 47 specimens of *Haemulon* (*H. atlanticus* = 15 specimens; *H. aurolineatum* = 17 specimens; *H. melanurum* = 4 specimens; *H. parra* = 5 specimens; *H. plumieri* = 5 specimens; and *H. squamipinna* = 1 specimen) were collected between 2006 and 2019 off the coasts of Brazil, between the northern extreme of the country and the southeastern state of São Paulo (Fig. 1; **S1**).

The species collected were identified based on their morphological characteristics (Lindeman, Toxey, 2002; Marceniuk *et al*., 2017). Barcode sequences of 149 specimens were obtained from the GenBank and BOLD databases (**S1**), and were inserted in the distribution map of the study species, in order to obtain a more ample sample of the different coastal regions of the Atlantic.

A small fragment of muscle tissue was removed from each specimen collected during this study and preserved in 95% ethanol at -20°C, before being deposited in

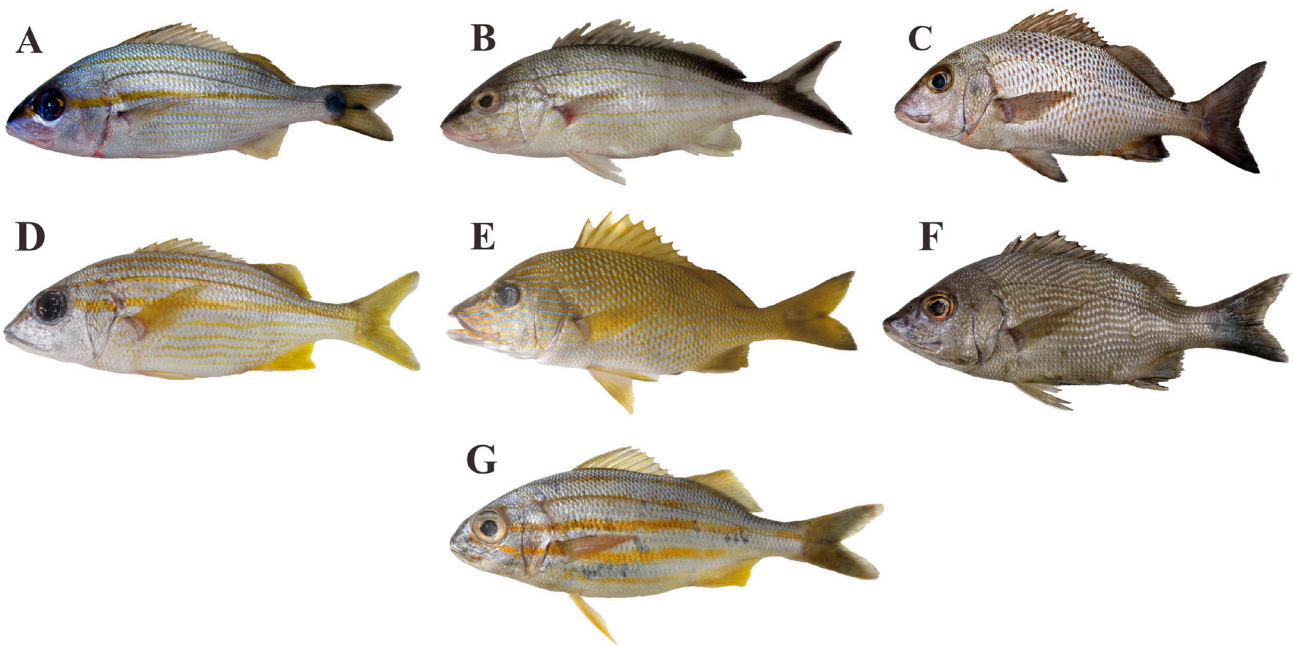

FIGURE 1 | Species of the genera *Haemulon* and *Brachygenys* collected off the coast of Brazil during this study. **A**. *Haemulon aurolineatum* (16.2 cm of Total Length, TL); **B.** *H. melanurum* (18.3 cm of TL); **C.** H. *parra* (21.7 cm of TL); **D.** *H. squamipinna* (15.9 cm of TL); **E.** *H. plumieri* (23.5 cm of TL); **F.** *H. atlanticus* (16.1 cm of TL); **G.** *Brachygenys chrysargyrea* (16.0 cm of TL).

the collection of the Fish Genetics and Biology Laboratory (Laboratório de Biologia e Genética de Peixes, LBP) at UNESP, in Botucatu, São Paulo, and the zoological collection of the Universidade Santa Cecília (AZUSC), in Santos, São Paulo (**S2**).

The species were sampled in accordance with Brazilian legislation, as regulated by the National Council for the Control of Animal Experimentation (CONCEA) and authorized by the Ethics Committee on the Use of Animals (CEUA) of the Biosciences Institute at UNESP through its (protocol 1057/2017).

Extraction of DNA, PCR amplification, and sequencing. The total DNA was extracted from the muscle tissue samples following the protocol proposed by Ivanova *et al*. (2006). Partial sequences of approximately 650 base pairs (bps) of the COI gene were obtained by PCR amplification using the FishF2 and FishR2 primers (Ward *et al*., 2005). The PCR reactions were run in a Veriti® 96-well Thermal Cycler (Applied BiosystemsTM or Mastercycler® EPGradient, Eppendorf) using the following temperature cycle: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 52°C for 30 sec, and extension at 68°C for 1 min, with a final extension at 68°C for 10 min. Each PCR solution comprised of: 7.55 µl of ultrapure water (milli-Q); 1.15 µl of (10X) buffer; 0.5 µl of $MgCl_2$ (50 mM), 0.5 µl of dNTPs (2 mM); 0.25 µl of each primer (5 mM); 0.2 µl of (5 U/µl) *Taq* DNA polymerase PHT (Phoneutria Biotechnologies and Services Ltda., Brazil), and 2 µl of the DNA template (50 – 100 ng/ul), for a final volume of 12.5 µl.

The amplification of the target sequence was confirmed by electrophoresis in 1% agarose gel using Blue Green Loading dye I (LGC Biotecnologia). The amplified PCR products were purified with an ExoSap-IT® (USB Corporation) solution, and the purified products were sequenced using the BigDye Terminator v3.1 Cycle Sequencing Ready Reaction kit (Applied Biosystems). This reaction solution consisted of: 3.9 µl of ultrapure water; 1.05 µl of 5X buffer; 0.7 µl of BigDye Terminator mix; 0.35 µl of the FishF2 or FishR2 primers (10 mM), and 1.0 µl of the purified PCR product (50 ng/µl). The amplification cycle was: 2 min at 96°C, and 35 cycles of 30 sec at 96°C, 15 sec at 54°C, and 4 min at 60°C. The purified PCR products were then precipitated in EDTA 125 nM/sodium acetate/alcohol, and the samples were sequenced automatically using an ABI 3130X1 Genetic Analyzer sequencer (Applied Biosystems™).

Data analysis. The sequences were edited and aligned using the Geneious Pro 4.8.5 software (Kearse *et al*., 2012). The edited sequences were compared with those deposited in the National Center for Biotechnology Information (NCBI) GenBank using the BLASTn tool (Johnson *et al*., 2008). The final matrix had 199 sequences, including 50 obtained in the present study and 149 extracted from GenBank (ncbi.nlm.nih.gov/genbank) or BOLD (boldsystems.org) (**S1**). The end alignment was exported and analyzed to generate trees in the MEGA v 7.0 software (Kumar *et al*., 2018), based on the neighbor-joining (NJ) method using the Kimura -2- Parameter model (K2P) (Kimura, 1980) and the maximum likelihood (ML) method with the best Tamura-Nei model (Kumar *et al*., 2018) and gamma distribution (TRN+G) identified by the PartitionFinder software (Lanfear *et al*., 2012). All trees were tested by bootstrap, with 1000 pseudoreplicates (Felsenstein, 1985).

Species delimitation analyses. Three methods of species delimitation were used: (1) The Automatic Barcode Gap Discovery, ABGD (Puillandre *et al*., 2012) which is based on a pairwise genetic distance matrix (generated in MEGA V7.0) run on the ABGD web server (https://bioinfo.mnhn.fr/abi/public/abgd/abgdweb.html) with the Kimura distance model (K2P) and other parameters at default (Pmin = 0.001; Pmax = 0.1); (2) the Bayesian Poisson Tree Process PTP (Zhang *et al*., 2013) run on the PTP web server (species.h-its.org/ptp), using the best Maximum Likelihood (ML) tree, 10,000 MCMC generations, and a 0.1 burn-in rate as the default settings, and (3) the general mixed Yule-coalescent GMYC (Pons *et al*., 2006; Fujisawa, Barraclough, 2013), run on the GMYC web server (https://species.h-its.org/gmyc/).

This analysis was conducted using the ultrametric gene tree estimated from the birth-death prior and the relaxed lognormal parameters. The number of polymorphic sites, the number of haplotypes, and the haplotype (HD) and nucleotide diversity (Pi) were estimated using DnaSP v5 (Librado, Rozas, 2009), with the median-joining network being produced using the PopArt program (Leigh, Bryant, 2015), for mutational analyses.

RESULTS

Barcode sequences were obtained from 50 specimens, which were complemented with 149 sequences obtained from GenBank and BOLD (**S1**), totaling 199 sequences in the final matrix (184 for *Haemulon* and 15 for *Brachygenys*) representing the different regions of the western Atlantic and eastern Pacific. The barcode sequences obtained here ranged in length from 440 to 558 bps. The overall mean nucleotide frequencies were 23.1% adenine, 26.6% cytosine, 19.6% guanine, and 30.6% thymine. No stop codons, deletions or insertions were found in any of the sequences.

The intraspecific genetic distances (based on the K2P model) ranged from 0.001±0.001, in both *H. melanurum* and *H. parra*, to 0.027±0.005 in *H. plumieri* (Tab. 1). The interspecific values ranged from 0.0746±0.0121 between *H. steindachneri* and *H. atlanticus* to 0.1645±0.0212 between *B. jessiae* and *H. atlanticus* (Tab. 1).

The results of the Maximum Likelihood (ML) analyses and the GMYC species delimitation method indicated the presence of 15 MOTUs in the database, while the ABGD and PTP species delimitation methods identified 13 MOTUs (Fig. 2; **S3**).

A single MOTU was found in *B. californiensis*, *B. chrysargyrea*, *B. jessiae*, *H. atlanticus*, *H. melanurum*, *H. parra*, *H. squamipinna*, and *H. steindachneri* in all analyses. Three MOTUs were identified in *H. aurolineatum* based on to the PTP and ABGD approaches, while the ML and GMYC methods identified four units in the samples of this species. In *H. plumieri*, the PTP and ABGD approaches identified two MOTUs, while the ML and GMYC methods identified three (Fig. 2; **S3**). Given these results, we decided to calculate the genetic distances between these MOTUs using the K2P model (Tab. 2), as described below.

TABLE 1 | Pairwise K2P genetic distances between the *Brachygenys* and *Haemulon* species (below the diagonal) and standard errors (above the diagonal). The numbers in bold type represent the intraspecific K2P genetic distances and their standard errors.

Species	1	2	3	4	5	6	7	8	9	10
1. Brachygenys jessiae	-	0.0188	0.0182	0.0178	0.0185	0.0207	0.0177	0.0206	0.0202	0.0212
2. B. chrysargyrea	0.1376	**0.004±0.001**	0.0169	0.0201	0.0168	0.0199	0.0179	0.0186	0.0186	0.0178
3. B. californiensis	0.1398	0.1269	**0.002±0.001**	0.0156	0.0157	0.0176	0.0176	0.0169	0.0173	0.0164
4. Haemulon aurolineatum	0.1502	0.1643	0.1185	**0.022±0.003**	0.0146	0.0156	0.0157	0.0157	0.0145	0.0155
5. H. plumieri	0.1553	0.1365	0.1258	0.1192	**0.027±0.005**	0.0156	0.0169	0.0161	0.0149	0.0167
6. H. melanurum	0.1608	0.1604	0.1332	0.1128	0.1223	**0.001±0.001**	0.0175	0.0163	0.0180	0.0190
7. H. squamipinna	0.1314	0.1516	0.1394	0.1288	0.1482	0.1374	**0.004±0.002**	0.0124	0.0168	0.0158
8. H. parra	0.1565	0.1425	0.1253	0.1178	0.1342	0.1221	0.0846	**0.001±0.001**	0.0169	0.0145
9. H. steindachneri	0.1554	0.1483	0.1259	0.1041	0.1157	0.1381	0.1306	0.1277	**0.003±0.001**	0.0121
10. H. atlanticus	0.1645	0.1396	0.1150	0.1143	0.1372	0.1498	0.1287	0.1018	0.0746	**0.005±0.002**

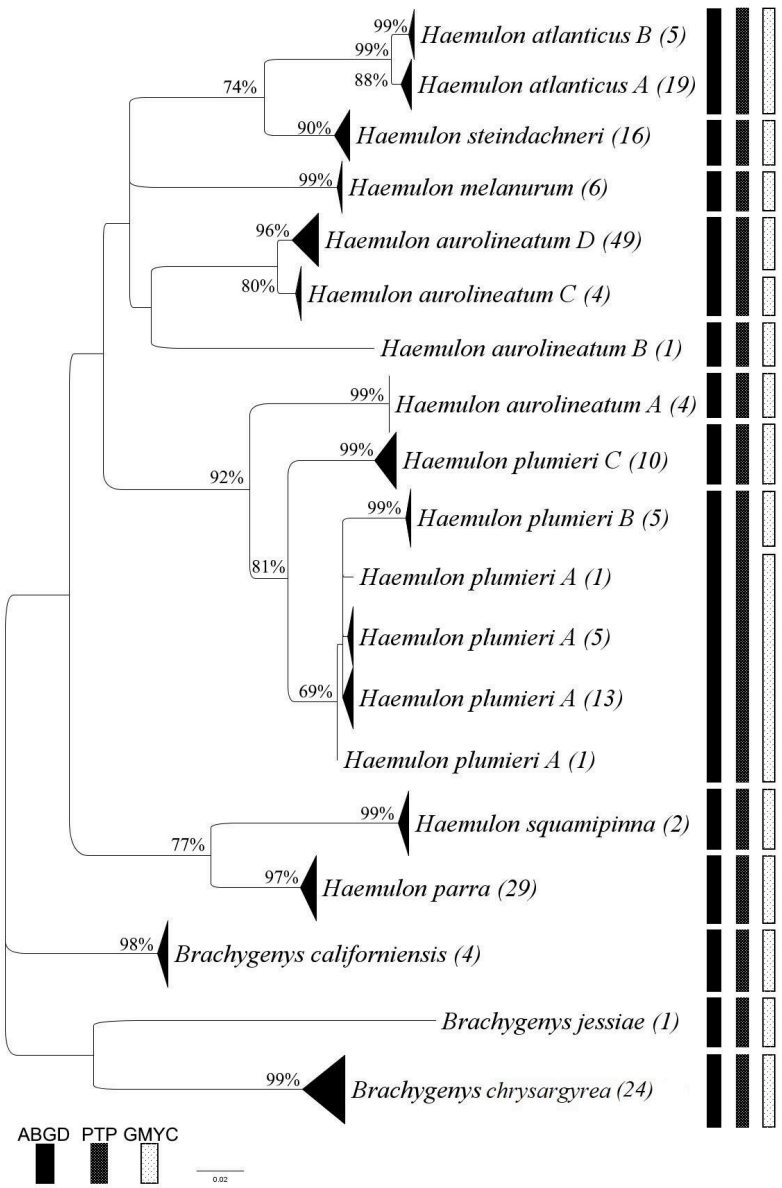

FIGURE 2 | The Maximum Likelihood tree of the *Haemulon* and *Brachygenys* specimens, based on the sequences of the mitochondrial cytochrome c oxidase subunit I gene under the TRN+G model. The numbers at each branch indicate the bootstrap values (1000 pseudoreplicates) and those between parentheses are the number of specimens analyzed. The species delimitation methods were ABGD, PTP, and GMYC (see Material and Methods section).

TABLE 2 | Pairwise K2P distances between *Brachygenys* and *Haemulon* genetic lineages (below the diagonal) and standard errors (above the diagonal). The numbers in bold type represent the intraspecific K2P genetic distances. + Only one sample available. The species identified by letters correspond to the distribution of the specimens as described in the article.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. *Brachygenys jessiae*	-	0.018	0.017	0.019	0.020	0.017	0.019	0.020	0.017	0.020	0.020	0.021	0.021	0.021	0.018	0.018
2. *B. chrysargyrea*	0.137	**0.003**	0.016	0.019	0.018	0.017	0.017	0.019	0.017	0.018	0.018	0.017	0.017	0.017	0.020	0.020
3. *B. californiensis*	0.139	0.126	**0.001**	0.016	0.018	0.015	0.016	0.017	0.017	0.017	0.017	0.016	0.016	0.015	0.016	0.016
4. *Haemulon aurolineatum* A	0.154	0.157	0.127	**0.000**	0.012	0.012	0.012	0.017	0.016	0.016	0.015	0.016	0.017	0.017	0.015	0.014
5. *H. plumieri* C	0.170	0.139	0.139	0.083	**0.001**	0.011	0.010	0.016	0.018	0.017	0.016	0.017	0.017	0.017	0.016	0.017
6. *H. plumieri* B	0.139	0.139	0.121	0.079	0.064+	**0.000**	0.006	0.015	0.017	0.017	0.016	0.019	0.019	0.015	0.015	0.015
7. *H. plumieri* A	0.152	0.134	0.120	0.080	0.048+	0.024+	**0.004**	0.016	0.017	0.017	0.015	0.018	0.018	0.015	0.016	0.016
8. *H. melanurum*	0.160	0.160	0.133	0.133	0.127	0.119	0.120	**0.001**	0.017	0.016	0.018	0.019	0.019	0.015	0.015	0.016
9. *H. squamipinna*	0.131	0.151	0.139	0.138	0.159	0.143	0.143	0.137	**0.003**	0.012	0.017	0.016	0.016	0.016	0.016	0.016
10. *H. parra*	0.156	0.142	0.125	0.129	0.138	0.133	0.132	0.122	0.084	**0.001**	0.017	0.015	0.015	0.016	0.016	0.016
11. *H. steindachneri*	0.155	0.148	0.125	0.120	0.124	0.120	0.110	0.138	0.130	0.127	**0.002**	0.012	0.012	0.015	0.015	0.014
12. *H. atlanticus* B	0.171	0.139	0.121	0.123	0.127	0.154	0.138	0.151	0.128	0.104	0.073+	**0.001**	0.004	0.016	0.015	0.015
13. *H. atlanticus* A	0.162	0.139	0.113	0.131	0.129	0.153	0.136	0.149	0.128	0.101	0.074+	0.012+	**0.001**	0.016	0.016	0.016
14. *H. aurolineatum* B	0.185	0.143	0.120	0.138+	0.135	0.116	0.122	0.110	0.133	0.118	0.114	0.127	0.129	-	0.014	0.014
15. *H. aurolineatum* C	0.151	0.158	0.119	0.116+	0.120	0.109	0.119	0.105	0.131	0.122	0.107	0.118	0.122	0.098+	**0.002**	0.005
16. *H. aurolineatum* D	0.149	0.165	0.117	0.108+	0.125	0.112	0.122	0.111	0.127	0.116	0.102	0.112	0.112	0.106+	0.018+	**0.003**

The three species delimitation methods used in the present study (PTP, ABGD, and GMYC) identified only one *H. atlanticus* MOTU in all the samples analyzed (Fig. 2). However, the ML tree included two lineages, *H. atlanticus* A, with 19 specimens from the Brazilian states of Pará, Ceará, Alagoas, and São Paulo, as well as Colombia, and *H. atlanticus* B (5 specimens from Colombia, Guatemala, and Venezuela). The genetic distance between the *H. atlanticus* A and B lineages was 0.012±0.004. The comparative network analysis of *H. steindachneri*, *H. atlanticus* A, and *H. atlanticus* B further reinforced the presence of three groups (Fig. 3), with 30 mutations separating *H. steindachneri* (Pi = 0.00271; HD = 0.81667) from *H. atlanticus* (Pi = 0.00567; HD = 0.65217), and nine mutations between *H. atlanticus* A Pi = 0.00177; HD = 0.45614) and *H. atlanticus* B (Pi = 0.00155; HD = 0.70000).

In the case of *H. aurolineatum*, the ML and GMYC identified four MOTUs, denominated here as *H. aurolineatum* A (four specimens from Bermuda), *H. aurolineatum* B (one specimen from Bermuda), *H. aurolineatum* C (four specimens from the Gulf of Mexico), and *H. aurolineatum* D, with 49 specimens from Belize, Brazil, Colombia, Jamaica, Venezuela, and the Gulf of Mexico (Fig. 2; **S3**).

The genetic distances between the pairs of these MOTUs ranged from 0.0183±0.049 between *H. aurolineatum* lineages C and D to 0.1382±0.0172 between *H. aurolineatum* lineages A and B (Tab. 2). When the PTP and ABGD methods were applied, however, only three MOTUs were observed - *H. aurolineatum* A, *H. aurolineatum* B, and *H. aurolineatum* C+D (Fig. 2; **S3**). The genetic distances between these MOTUs were 0.1382±0.0173 between *H. aurolineatum* A and B, 0.1089±0.0147 between *H. aurolineatum* A and C+D, and 0.1059±0.0150 between *H. aurolineatum* B and C+D.

There were 37 mutations between *H. aurolineatum* A (Pi = 0.00000; HD = 0.00000) and *H. aurolineatum* D (Pi = 0.00389; HD = 0.72364), five between *H. aurolineatum* C and *H. aurolineatum* D (Pi = 0.00281; HD = 0.83333), and 34 mutations between *H. aurolineatum* B and *H. aurolineatum* C (Pi = 0.00000; HD = 0.00000) (Fig. 3).

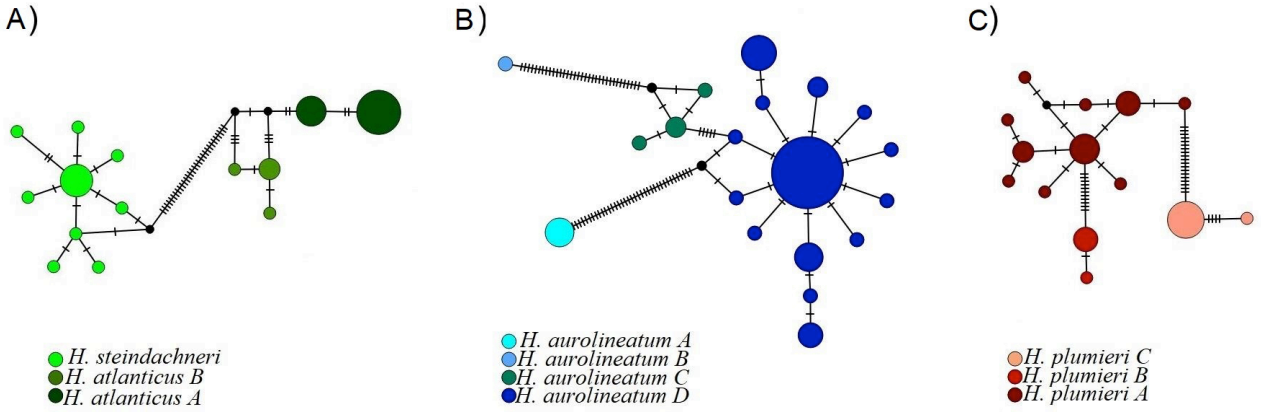

FIGURE 3 | Haplotype networks of the three *Haemulon* species in which multiple MOTUs were identified. The dashes represent mutational steps. The size of the circle representing each haplotype is proportional to the number of individuals with that haplotype. The black dots represent missing haplotypes. **A.** *H. steindachneri* = *Haemulon steindachneri* (eastern Pacific); *H. atlanticus* B = specimens from Colombia, Guatemala, and Venezuela; *H. atlanticus* A = specimens from Brazil and Colombia; **B**. *H. aurolineatum* A = specimens from Bermuda; *H. aurolineatum* B = specimens from Bermuda; *H. aurolineatum* C = specimens from the United States; *H. aurolineatum* D = specimens from Belize, Brazil, Colombia, Jamaica, Venezuela, and the United States; and **C.** *H. plumieri* C = specimens from Brazil and Puerto Rico; *H. plumieri* B = specimens from the United States; *H. plumieri* A = specimens from the Bahamas, Belize, Haiti, Mexico, Puerto Rico, and the United States.

The ML and GMYC analyses identified three MOTUs in *H. plumieri*, identified here as *H. plumieri* A (20 specimens from the Caribbean and Gulf of Mexico), *H. plumieri* B (five specimens from the Gulf of Mexico), and *H. plumieri* C, with 10 specimens from Brazil and the Caribbean (Fig 2; **S3**). The genetic distances between these MOTUs were 0.064±0.011 between *H. plumieri* lineages A and B, 0.048±0.010 between *H. plumieri* A and C, and 0.024±0.006 between *H. plumieri* B and C (Tab. 2). When the PTP and ABGD methods were considered, however, only two MOTUs were observed, one containing *H. plumieri* A+B and the other, *H. plumieri* C (Fig. 2; **S3**). The genetic distance between *H. plumieri* A+B and *H. plumieri* C was 0.052±0.009. There were 11 mutations between *H. plumieri* C (Pi = 0.00194; HD = 0.20000) and *H. plumieri* B (Pi = 0.00097; HD = 0.40000) and 19 mutations between *H. plumieri* C and *H. plumieri* A (Pi = 0.00394; HD = 0.87368) (Fig. 3).

DISCUSSION

Four of the species analyzed, *B. chrysargyrea*, *H. melanurum*, *H. parra*, and *H. squamipinna*, presented extremely low intraspecific distances, and all the different analytical approaches indicated that they represented a single MOTU. In the specific case of *H. squamipinna*, the evidence that the samples represented a single species was expected, given the very restricted distribution of the species off the northeastern coast of Brazil (Rocha, Rosa, 1999). In the other cases, however, the species are much more amply distributed, with *H. melanurum* being found from southeastern Florida to northern Brazil, including the whole of the Caribbean (Menezes *et al*., 2003), while *H. parra* and *B. chrysargyrea* are

distributed from southeastern Florida to southeastern Brazil (Menezes *et al*., 2003).

In all three cases, the distribution of the species straddles the potential barrier formed by the Amazon-Orinoco Plume. In addition, the southern limit of the distribution of *H. parra* in Brazil was originally described as being São Paulo (Menezes *et al*., 2003), and we collected samples in São Paulo during the present study, which indicates that this species also traverses the Vitória Trindade seamount chain, off the eastern coast of Brazil, which was a potential biogeographic barrier during periods of marine regression, in the Quaternary and Tertiary, as observed in other genera and species, such as *Orthopristis ruber* (Cuvier, 1830) (Marceniuk *et al*., 2019), *Macrodon ancylodon* (Bloch & Schneider, 1801) (Santos *et al*., 2006), and *Chaetodipterus faber* (Broussonet, 1782) (Machado *et al*., 2017).

Some of the haemulids from the western Atlantic have ample geographic distributions and have larvae that are able to disperse rapidly on oceanic currents, as well as the ability to migrate vertically in the water column (Majoris *et al*., 2019). These characteristics would maximize the potential for gene flow between the northern and southern populations of these species in the Western Atlantic, which would minimizing the chances of forming isolated groups (Rocha, 2003; Rocha *et al*., 2002, 2005, 2007, 2008). The ample geographic ranges and genetic homogeneity detected here in *H. melanurum*, *H. parra*, and *B. chrysargyrea* may thus also be at least partially due to the swimming capabilities of these fishes.

Some *Haemulon* species have dispersed from the Pacific Ocean to the western Atlantic (Tavera *et al*., 2012), followed by reverse invasions during the occurrence of vicarious events and the formation of the Isthmus of Panama (Stange *et al*., 2018). These historical processes resulted in allopatric speciation, which has given rise to twin species (Jordan, 1908), and both sister and novel lineages (Rocha *et al*., 2007; Luiz *et al*., 2012; Tavera *et al*., 2018; Tavera, Wainwright, 2019). In contrast with the species represented by a single lineage in the western Atlantic, as discussed above, the presence of multiple MOTUs in *H. atlanticus*, *H. aurolineatum*, and *H. plumieri* is an intriguing phenomenon, which cannot be accounted for by either the current barriers to gene flow (such as the Amazon-Orinoco Plume) or ancient processes, such as the isolation of the fauna of the Gulf of Mexico and the southern coast of Brazil (Victoria Trindade seamount chain) during the Quaternary and Tertiary glacial cycles.

Until recently, *H. steindachneri* was believed to occur in both the Pacific and Atlantic oceans, but an ample taxonomic review, supported by cytogenetic and molecular data (Rocha *et al*., 2008; Tavera *et al*., 2012, 2018; Bernal *et al*., 2017, 2019; Motta-Neto *et al*., 2012, 2019; Tavera, Wainwright, 2019), confirmed that *H. steindachneri* is restricted to the Pacific, while a new species, *H. atlanticus*, was described for the Atlantic (Carvalho *et al*., 2020). Ours results also support the separation of the eastern Pacific *H. steindachneri* from *H. atlanticus*, which is found in the western Atlantic (Carvalho *et al*., 2020), with a genetic distance of 0.0746±0.0121 (Tab. 1), and the two species were separated clearly in all analyses.

However, while all three species delimitation methods used here (PTP, ABGD, and GMYC) indicated that only one MOTU was present in the *H. atlanticus* samples, the ML tree identified two groups, denominated here as *H. atlanticus* A (specimens from the Caribbean and the Atlantic coast of South America) and *H. atlanticus* B (specimens from the Caribbean). The genetic distance between these two MOTUs was 0.012±0.004,

which is lower than that usually found between valid marine fish species, *i.e.*, around 2% (Ward, 2009), although it was consistent with that found by Rocha *et al.* (2008) and Carvalho *et al.* (2020), which supports the need for further studies to determine whether this difference is due to the simple isolation of populations or the existence of a cryptic species derived from a recent speciation process (Rocha *et al.*, 2007; Motta-Neto *et al.*, 2011a).

In *H. aurolineatum*, by contrast, some of the analyses identified four MOTUs, that is, *H. aurolineatum* A and B (specimens from Bermuda), *H. aurolineatum* C (specimens from the United States), and *H. aurolineatum* D (specimens from the Gulf of Mexico, Caribbean, and the Atlantic coast of South America), while the other analyses indicated that *H. aurolineatum* C and D constitute a single MOTU (C+D). In *H. plumieri*, some of the analyses identified three MOTUs, that is, *H. plumieri* A (specimens from the Gulf of Mexico and the Caribbean), *H. plumieri* B (specimens from the Gulf of Mexico), and *H. plumieri* C (specimens from the Caribbean and the Atlantic coast of South America), while the other analyses indicated that *H. plumieri* A and B constitute a single MOTU (A+B). Although the genetic distances between some of these MOTU pairs were lower than 2%, many were higher, and reached up to 13%.

In recent years, advances in sequencing technology have supported a substantial increase in the DNA sequences available in databases, such as GenBank, for biodiversity studies. This includes fish, and these advances have contributed to the identification of species from groups with major taxonomic disagreements (Porter, Hajibabaei, 2018; Leray *et al.*, 2019).

However, some authors have questioned the validity of the discrimination of fish species based on DNA sequences, given the frequent misidentification of the sequences available in these databases due to taxonomic inconsistencies, sampling errors, contamination, and hybridization, which reduces their reliability for comparison with other sequences (Locatelli *et al.*, 2020; Pentinsaari *et al.*, 2020). This problem was identified in the case of the five *H. aurolineatum* GenBank sequences from Bermuda, which we believe have been identified mistakenly as *H. aurolineatum*, given their considerable genetic distance from the other specimens analyzed, as well as the lack of published reports of this species in the region of the Bermuda archipelago.

Studies of reef fish have identified a number of processes that may have influenced the present-day intra- and inter-specific structuring observed in some groups found in the western Atlantic (Santos *et al.*, 2006; Rodríguez-Rey *et al.*, 2014; Silva *et al.*, 2014, 2015; Ashe *et al.*, 2015; Souza *et al.*, 2015; Bernal *et al.*, 2018, 2019).

These processes include the glacial cycles of the Pleistocene and Miocene, when the Vitória Trindade seamount chain, off the eastern coast of Brazil, isolated the fish fauna of southern Brazil, with a similar process of isolation occurring in the Gulf of Mexico. Another important event was the uplifting of the Andes, around 8 million years ago, which reconfigured the continental drainages of South America, establishing the transcontinental flow of the Amazon River to the Atlantic Ocean.

All these processes may have caused alterations in the gene flow of reef fish populations. In the specific cases of *H. atlanticus*, *H. aurolineatum*, and *H. plumieri*, however, the well-known barriers, such as the Amazon-Orinoco plume and the Victoria Trindade seamount chain would not account for the differentiation of the MOTUs found herein, which implies the influence of alternative phenomena, such as other paleogeographical

events or even parametric or sympatric speciation events resulting from processes of ecological specialization.

The DNA barcode method contributed to the identification of the Brazilian reef fish fauna of the genera *Brachygenys* and *Haemulon*, and how barriers and ocean currents may influence the population dynamics of these species in the Western Atlantic. Our results indicated that some *Haemulon* species have been able to traverse the barrier of the Amazon plume and that the action of ocean currents may contribute to the dispersion of these species. However, the evolution of the populations of *H. plumieri*, *H. atlanticus*, *H. steindachneri*, and *H. aurolineatum* may have been influenced by variations in oceanographic conditions and barriers resulting in the formation of distinct MOTUs, as identified here, that have enriched the diversity of the reef fish species found off Brazil.

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Najila Nolie Catarine Dantas Cerqueira: Conceptualization, Formal analyses, Methods, Writing–original draft, Writing–review and editing.

Matheus Marcos Rotundo: Conceptualization, Data curation, Writing–review and editing.

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Vanessa Paes da Cruz: Conceptualization, Software, Writing–original draft, Writing–review and editing.

Fausto Foresti: Conceptualization, Project administration, Resources, Writing–review and editing.

Claudio Oliveira: Conceptualization, Project administration, Resources, Supervision, Writing–original draft, Writing–review and editing.

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ETHICAL STATEMENT

The species were sampled in accordance with Brazilian legislation, as regulated by the National Council for the Control of Animal Experimentation (CONCEA) and authorized by the Ethics Committee on the Use of Animals (CEUA) of the Biosciences Institute at UNESP through its (protocol 1057/2017).

COMPETING INTERESTS

The authors declare no competing interests.

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