Received: 30 August 2019 | Revised: 2 January 2020 | Accepted: 13 January 2020

DOI: 10.1111/mec.15357

ORIGINAL ARTICLE

MOLECULAR ECOLOGY WILEY

Common barriers, but temporal dissonance: Genomic tests suggest ecological and paleo-landscape sieves structure a coastal riverine fish community

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Funding information
University of Michigan; Directorate for Biological Sciences, Grant/Award Number: DEB-15-01301

Abstract

Assessments of spatial and temporal congruency across taxa from genetic data provide insights into the extent to which similar processes structure communities. However, for coastal regions that are affected continuously by cyclical sea-level changes over the Pleistocene, congruent interspecific response will not only depend upon codistributions, but also on similar dispersal histories among taxa. Here, we use SNPs to test for concordant genetic structure among four codistributed taxa of freshwater fishes (Teleostei: Characidae) along the Brazilian Atlantic coastal drainages. Based on population relationships and hierarchical genetic structure analyses, we identify all taxa share the same geographic structure suggesting the fish utilized common passages in the past to move between river basins. In contrast to this strong spatial concordance, model-based estimates of divergence times indicate that despite common routes for dispersal, these passages were traversed by each of the taxa at different times resulting in varying degrees of genetic differentiation across barriers with most divergences dating to the Upper Pleistocene, even when accounting for divergence with gene flow. Interestingly, when this temporal dissonance is viewed through the lens of the species-specific ecologies, it suggests that an ecological sieve influenced whether species dispersed readily, with an ecological generalist showing the highest propensity for historical dispersal among the isolated rivers of the Brazilian coast (i.e., the most recent divergence times and frequent gene flow estimated for barriers). We discuss how our findings, and in particular what the temporal dissonance, despite common geographic passages, suggest about past dispersal structuring coastal communities as a function of ecological and paleo-landscape sieves.

KEYWORDS

Atlantic Rainforest, coastal drainages, freshwater fishes, Pleistocene, population turnover, sea-level fluctuations

1 | INTRODUCTION

Spatial congruence in the distribution of species or in their genetic structure has long been recognized as a signal of shared evolutionary history (Bermingham & Avise, 1986; Donoghue & Moore, 2003; Edwards & Beerli, 2000). Such congruence has helped to identify geographical features structuring communities, especially in cases where a physical barrier is not readily evident, such as ephemeral,

climatic and ecological barriers (Avise, 1992; Carnaval, Hickerson, Haddad, Rodriguez, & Moritz, 2009; Edwards, Keogh, & Knowles, 2012), or when genetic discontinuities are a function of dispersal and demographic traits (Irwin, 2002).

Although concordant genetic structure is strong evidence of a shared evolutionary history among co-occurring taxa, the lack of concordance has different possible explanations that can limit the insights genetic tests alone can provide (Papadopoulou & Knowles, 2016). For example, at regional scales, geological constraints tend to prevail over possible species-specific responses (Albert & Carvalho, 2011; Bermingham & Avise, 1986; Burridge, Craw, & Waters, 2006; Chakona, Swartz, & Gouws, 2013). However, for dynamic histories, such as those subject to cyclical climatic changes, complex colonization and extinction dynamics, and hence, incongruence among community members (e.g., Burbrink et al., 2016) pose specific challenges for understanding the processes underlying genetic structuring. This lack of similarity has left researchers with unanswered questions about how different species respond to potential routes of dispersal among currently isolated populations (Massatti & Knowles, 2016).

In our study, we focus on a coastal riverine fish community of the Brazilian Atlantic Rainforest and take advantage of the dispersal constraints imposed upon riverine fishes to test the community response to historical connections among isolated basins that affect species distributions and dispersal in coastal environments (Dias et al., 2014). That is, unlike terrestrial systems in which genetic structure reflects the effects of habitat suitability in the past or present landscape on movement patterns (see He, Edwards, & Knowles, 2013; López-Uribe, Jha, & Soro, 2019), for riverine species, dispersal is restricted to physical connections across riverine basins (Albert, Petry, & Reis, 2011). As such, the degree of genetic structure across isolated basins reflects the extent to which dispersal has been historically limited. Likewise, similarity in the spatial genetic structure across multiple species identifies routes of connectivity that were accessible to multiple members of aquatic communities, although they may, or may not, have been traversed at similar times (i.e., the divergence times associated with similar spatial structure may differ across taxa).

By coupling spatial and temporal tests of congruent genetic structure with consideration of the ecological differences among four focal taxa distributed along the coastal Atlantic Rainforest, we consider how both the paleo-landscape (e.g., past riverine connectivity) and the ecology of the taxa themselves might act as sieves – that is, determine when and which taxa moved between current isolated river basins. As a consequence of repeated population cycles of isolation and reconnection during the Pleistocene (Papadopoulou & Knowles, 2016; Thomaz & Knowles, 2018), coastal areas may be subject to high spatial and/or temporal lineage turnover (e.g., extirpation-isolation-recolonization; Dolby, Ellingson, Findley, & Jacobs, 2018). Such turnover may contribute to the lack of congruent genetic structure. Moreover, even with congruent spatial genetic structure, there might not be temporal congruence because connections among isolated regions were forged repeatedly, and at different times, during periods of low sea level. Specifically, temporary passages (e.g., river captures and/or riverine connections when sea-level retreat; Lima et al., 2017; Thomaz, Malabarba, & Knowles, 2017; Weitzman, Menezes, & Weitzman, 1988) may not be effectively utilized by all species because species-specific ecological differences might make some routes more or less accessible to some taxa.

To address these questions, we studied four codistributed characid taxa (Ostariophysi: Characiformes), commonly known as tetras, distributed along the Brazilian coast that differ ecologically (Figure 1). Specifically, the focal taxa span a spectrum of ecological specialization and differ in their distance from the current coastline (i.e., areas of proposed connections among currently isolated basins; Conti & Furtado, 2009; Thomaz & Knowles, 2018). They include the more generalized taxon *Mimagoniates microlepis* that inhabits lowland and highland rivers, and *Hyphessobrycon boulengeri*, which is restricted to lowland rivers, as well as *Hollandichthys*, which is restricted to rivers surrounded by a dense forest canopy, and the coastal *Bryconamericus* species group (and hereafter referred as *Bryconamericus*) that inhabits the fast-moving waters of rivers on steep slopes (Figure 1; see Supporting Information for taxonomic details). By testing for spatial congruence and assessing the relative timing of divergence in a comparative framework, our study provides insights about the ecological and paleo-landscape sieves that structure this coastal fish community. We also discuss the implications of our results for more general patterns of species distributions and population connections in coastal communities, including a comparison with terrestrial counterparts in the Brazilian Atlantic rainforest.

2 | MATERIALS AND METHODS

2.1 | Sampling and genomic data

Specimens for each of the four species were collected across their entire distributions; collecting expeditions were conducted during different seasons, with collections of the four taxa concentrated during the 2008–2009, and 2013–2015 field seasons. A total of 47 drainages (populations) were sampled across the four taxa, with an average of 23 drainages sampled per species (Table S1). Vouchers and tissues for this study were catalogued in the ichthyology collection at the Universidade Federal do Rio Grande do Sul (UFRGS), Brazil. Detailed information about fieldwork and vouchers specimens can be obtained from each catalogue number using http://splink.cria.org.br/. Collection permits were obtained from the Brazilian government through the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio), under the license #12038 for Dr Luiz R. Malabarba at UFRGS – Brazil. Additional tissues (approximately 10% of the samples) were obtained from the Museu the Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP) and Museu the História Natural Capão da Imbuia (MHNCI; see complete list in Table S2). All specimens

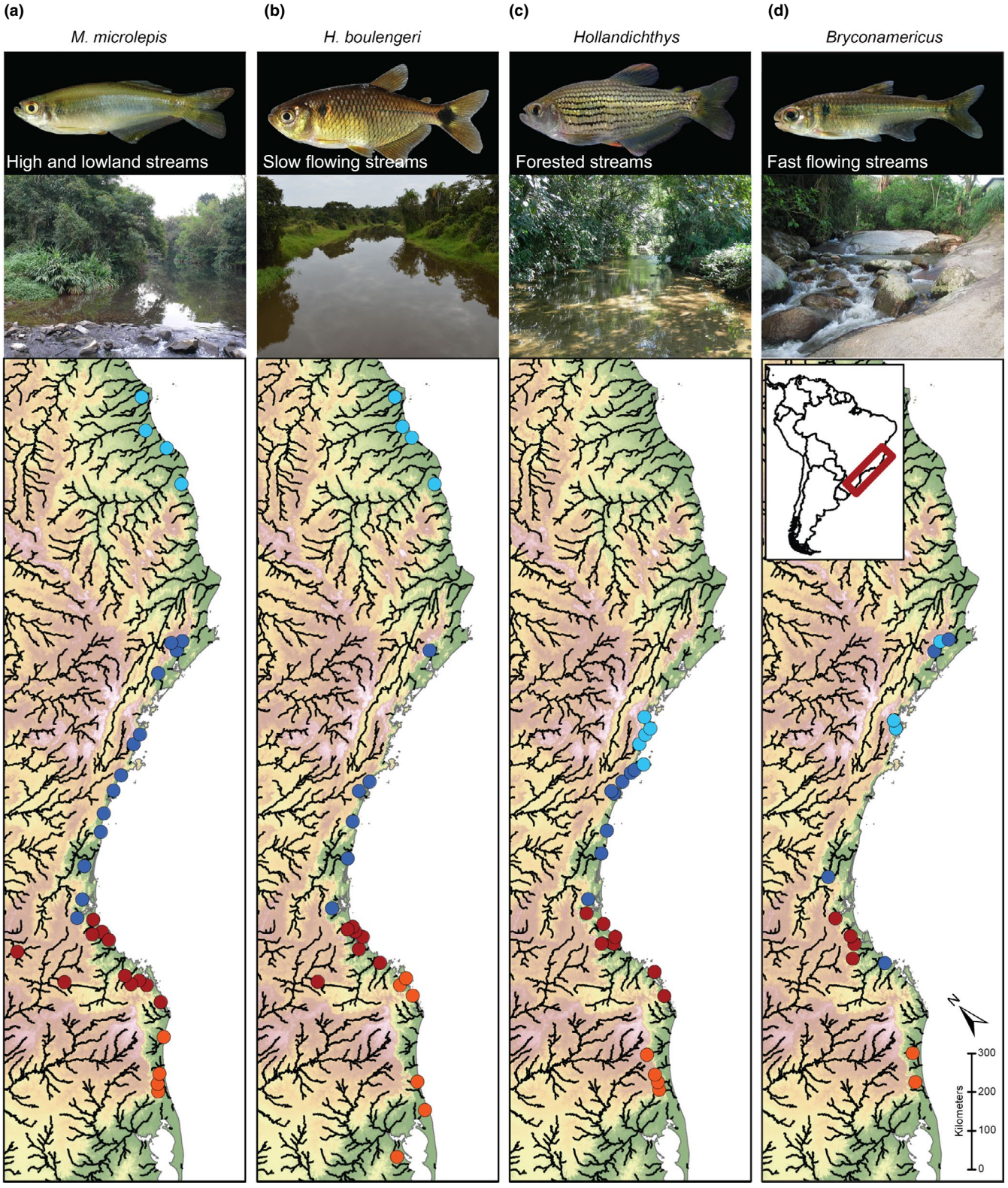

FIGURE 1 Distributional map, specimen and habitat picture of (a) *M. microlepis* (38 mm standard length – SL), (b) *H. boulengeri* (47.8 mm SL), (c) *Hollandichthys* (*H. multifasciatus;* 99.5 mm SL), and (d) *Bryconamericus* (*B. microcephalus;* 57 mm SL) with sampled populations for genomic analyses labeled as coloured dots; see small inset of South America for area of study. Different colours depict main clusters of genetic differentiation obtained with hierarchical analyses among populations of each species (see Figure 2 and results for details) [Colour figure can be viewed at wileyonlinelibrary.com]

and tissues used in this study are in accordance with the Brazilian genetic patrimony rules and indexed in the SISGEN database under the number RF0AF3D.

Six double digest Restriction-site Associated DNA (ddRAD) libraries were constructed: three libraries contained 118 individuals of *Mimagoniates microlepis* for this study (the other 132 individuals sequenced across these libraries were for an unrelated study that is currently unpublished), two libraries containing 136 individuals of *Hyphessobrycon boulengeri*, and one library with 87 individuals of *Bryconamericus*. In addition, two libraries with 182 individuals of *Hollandichthys* were reanalyzed for this study (Thomaz et al., 2017). For some of these nominal taxa, our sampling encompasses more than a single species given taxonomic treatments (see Supporting Information S1 for details). Here, we opt to refer to each taxon by the designations identified above because we note that our results are robust given that the proposed taxonomic revisions all pertain to allopatric lineages, and therefore do not confound our analysis of spatial or temporal congruence/discord (see discussion section for additional detail).

For all the libraries prepared specifically for this study, we followed the protocol of Peterson, Weber, Kay, Fisher, and Hoekstra (2012); the two previously sequenced libraries of *Hollandichthys* followed the Parchman et al. (2012) protocol (see Thomaz et al., 2017 for preparation details), but with the main features are in common with the other protocol (e.g., the enzymes used and size selection). Briefly, genomic DNA was extracted using Qiagen DNeasy kits from tissue samples taken from alcohol preserved body muscle. Between 300 and 400 ng of each DNA sample was double digested with two restriction enzymes (*EcoRI* and *MseI*), followed by a ligation step to add unique barcodes. Samples for each library were pooled and fragments between 350–450 bp were selected using a PippinPrep. A PCR with 10 cycles was used to add Illumina flowcell adapters. All steps described above were followed by a clean-up step using AMPure beads (1.6× ratio; except after Pippin Prep) to remove small DNA fragments such as primers, and by a high sensitivity Qubit quantification assay. Libraries were sequenced on an Illumina HiSeq2500 to generate single-end 150 bp reads (100 bp for *Hollandichthys*) at The Centre for Applied Genomics, Toronto, Canada.

Genomic data were demultiplexed and processed separately for each taxon with the STACKS version 1.41 pipeline (Catchen, Hohenlohe, Bassham, Amores, & Cresko, 2013). For quality filtering, reads with more than one mismatch in the adapter sequence or a barcode distance greater than two (as specified in process radtags) were removed, and individuals with <300 K reads were excluded. To create stacks within each sample, USTACKS was run with a minimum depth of coverage of five and an error bound of $\varepsilon = 0.1$, followed by CSTACKS with a maximum of two mismatches between sequences within a given stack in order to build a catalog of all loci. The stacks of individual samples were matched against the catalogue using SSTACKS with default options. To obtain a vcf output file containing all variable sites from STACKS, we ran the POPULATIONS module with "loose" parameters (i.e., -r 0 -p 2 -m 5 --min_maf 0 --max_obs_het 0.5). We processed this output file in R version 3.3.1 (R Core Team, 2018) to create a whitelist that excluded highly variable positions at the 3' end of all locus and loci with θ-values above the 95th percentile of this distribution, to avoid errors associated with sequencing and assembly (see Figures S1 and S2). Using this whitelist, we reran the POPULATIONS module. All bioinformatics processing with STACKS was performed in the Advanced Research Computing Technology Services at the University of Michigan. We obtained a total of 165 million to 325 million reads per species.

Because of the various requirements of different analyses used to characterize the geographic structuring of genomic variation, such as sensitivity to missing data (Huang & Knowles, 2016a) and for computational feasibility, three data sets were generated varying the amount of missing data and the numbers of individuals. One data set was comprised by one random single SNP per locus with maximum of 50% missing data, and hereafter referred to as the SNP data set (see Table S3 for details), which was used for estimates of population trees; a population refers to all the samples from the same river basin/drainage (or island – see Table S2). The other data set included loci with maximum 25% missing data after filtering and hereafter referred to simply as the genomic data set. Note that for *M. microlepis* we allowed 35% missing data because of the higher levels of missing data that resulted from the addition of individuals in the preparation of the library (specifically, samples from a southern population unrelated to this project were included). The genomic data set was used in most of the analyses including the calculation of summary statistics in the POPULATIONS module of STACKS, whereas a random single SNP per locus were used in the STRUCTURE analysis. Separate data sets, hereafter referred as the reduced data sets, were used in FASTSIMCOAL2 analyses and were generated, when possible, from 20 individuals with the smallest amount of missing data from all the populations separated by each geographic barrier for each taxon (40 individuals in total; see Table S1 for number of individuals used per population), and a single variable SNP per RADtag with <10% missing data (see details below). For all these data sets, individuals with considerably fewer SNPs in comparison to other individuals of the same population were excluded. All filtering steps were performed using the toolset PLINK v.1.90 (Purcell et al., 2007; no filter to screen potentially selected loci was applied given the difficulties of inferring selection under the structured populations). Genomic data are archived on SRA (BioProjectID: PRJNA598706) and all scripts and setting files for programs are available on Dryad under https://doi.org/10.5061/dryad.zkh18936g and on GitHub: https://github.com/ichthya/ThomazKnowles2020_scripts. After applying filters for missing data, genotyping rates ranged from 0.67 to 0.72 for the SNP data set and from 0.85 to 0.92 for the genomic data set across species (see Tables S2 and S3 for information per individuals and per species, respectively).

2.2 | Characterizations of population structure

To examine evolutionary relationships among populations from the drainages along the Brazilian coast, we estimated a population tree (Knowles & Carstens, 2007), accounting for the coalescent variation

associated with random sorting of gene lineages among loci, and incomplete lineage sorting for any given locus, using the program SVDQUARTETS (Chifman & Kubatko, 2014) and as implemented in PAUP* 4.0 (Swofford, 2003) under the multispecies coalescent model with all possible quartets evaluated. Branch support was assessed with 1,000 bootstrap replicates and midpoint rooting was used given the absence of outgroups in our data sets.

Hierarchical STRUCTURE analyses (Pritchard, Stephens, & Donnelly, 2000) were used to evaluate if the probabilistic assignment of individuals in each taxon to genetic clusters showed a species-specific geographic configuration or if there is a general pattern shared among taxa along the Brazilian coast. Specifically, to assess substructure, we performed analyses with the full distribution of a taxon followed by sequential analyses for each of the subsets identified as distinct genetic clusters (see Massatti & Knowles, 2014). The genomic data sets with a single SNP per locus were used, and individuals were not conditioned on any population membership (i.e., population membership was not used as priors). Each data set was analyzed with *K*-values ranging from 1 to 5 or 10 (see Table 1 for specific information for each species). We performed 10 independent runs under the "Admixture" and "Allele Frequencies Correlated" models for 500,000 MCMC iterations following a burnin period of 200,000 iterations for each analysis. The ΔK of Evanno, Regnaut, and Goudet (2005) implemented in STRUCTURE HARVESTER (Earl & vonHoldt, 2012) was used to identify for each taxon the most likely number of genetic clusters. We also considered the likelihood-values for $K = 1$ and 2 to evaluate the lack of geographic structure. The graphical probabilistic assignment of individuals to clusters performed using the CLUMPAK pipeline (Kopelman, Mayzel, Jakobsson, Rosenberg, & Mayrose, 2015).

2.3 | Tests of divergence models

Focusing on the genetic clusters identified among the different taxa based on the phylogenetic tree and STRUCTURE analyses, we performed model comparisons to estimate divergence times and the frequency and strength of connectivity among each geographic barrier for each taxon separately using the composite-likelihood method FASTSIMCOAL2 (Excoffier, Dupanloup, Huerta-Sánchez, Sousa, & Foll, 2013; Excoffier & Foll, 2011) based on the folded joint Site Frequency Spectrum (SFS; i.e., for the minor allele since we did not have information from outgroups to obtain the derived state; Figure S3). With the objective to maximize the number of loci with no missing data and obtain an accurate estimation of allelic frequencies for calculating the SFSs, each SFS was built by subsampling 15 individuals per locus (out of 20 individuals from the reduced data sets; see Table S5 for exceptions) from either side of each geographic barrier using a custom script; the script is available on GitHub: https://github.com/ichthya and is modified from He and Knowles (2016).

Based on each SFS, we estimated parameters under three classes of divergence models: (a) divergence without gene flow (herein called "strict divergence"), and two models of divergence with gene flow, namely (b) divergence with unconstrained gene flow (i.e., gene flow could occur throughout the divergence history, and herein is called "divergence with gene flow"), and (c) divergence with gene flow as a single pulse (herein called "divergence with a pulse of gene flow"). For each of the divergence with gene flow models, symmetrical versus asymmetrical gene flow was modelled (i.e., models with one vs. two migration parameters). The variety of models of gene flow were chosen to accommodate differences in how frequently connections might have been forged between the current isolated river basins, and hence potential differences in how gene flow among populations might have occurred, which is central to testing the hypothesis that species-specific traits might affect how effective a barrier might be (i.e., whether species were more or less likely to remain isolated for extended periods of time despite repeated opportunities for gene flow via historical connections among the current isolated basins). To improve the performance of parameter estimates from the SFS (following recommendations of the program; see Excoffier & Foll, 2011), we calculated an effective population size of one of the two

TABLE 1 Results of hierarchical STRUCTURE analyses, with the full data set (All) and the population subsets (North and South) for each species

Taxa	Level	Loci	Inds.	Gen. rate	*K* tested	First *K*	ΔK	Second *K*	ΔK
Mimagoniates microlepis	All	1,800	113	0.79	10	2	9,054.0	4	20.0
	North	1,042	59	0.87	5	2	5,780.7	4	1,155.1
	South	1,441	54	0.88	5	2	2,110.3	3	2,078.7
Hyphessobrycon boulengeri	All	6,129	134	0.86	10	4	34.3	2	3.0
Hollandichthys	All	6,902	142	0.87	5	2	19,511.8	3	5.9
	North	6,536	83	0.89	5	2	7,272.8	3	2.7
	South	6,335	59	0.87	5	2	12,095.1	3	885.9
Bryconamericus	All	4,276	69	0.95	10	2	10,261.1	3	144.3
	North	4,205	34	0.95	5	2	3,960.4	4	6.0
	South	4,180	28	0.95	5	2	3,331.4	3	998.2

Note: For each analysis (i.e., row), the first and second most probable *K*-values identified using Evanno's method are reported along with the correspondent ΔK. The total number of loci and individuals analyzed are given, as well as the total individual genotyping rate (Gen. rate)

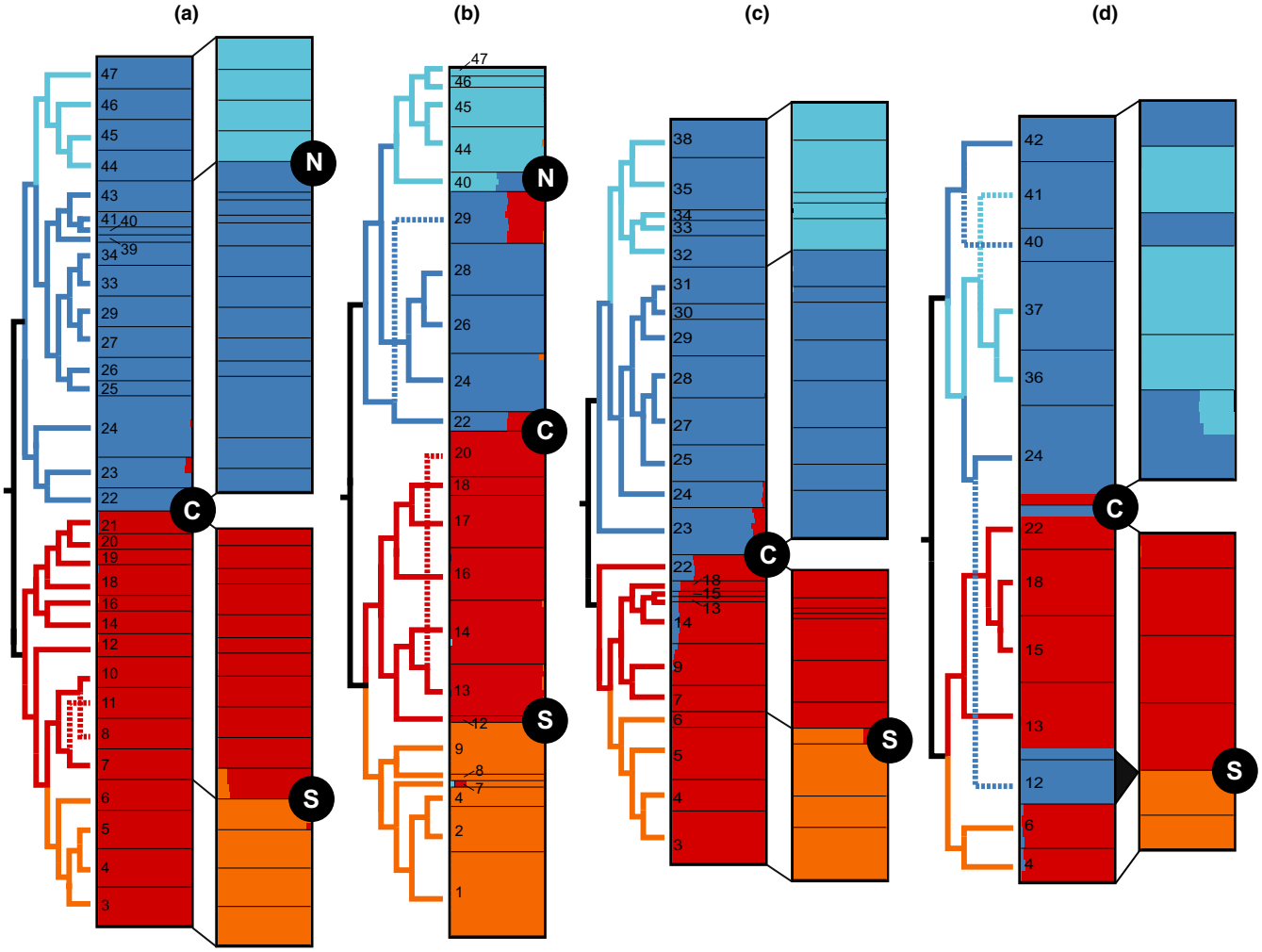

FIGURE 2 Estimates of population relationships and genetic clusters in (a) *M. microlepis*, (b) *H. boulengeri*, (c) *Hollandichthys*, and (d) *Bryconamericus*, from SVDquartets and STRUCTURE analyses. Congruent patterns of divergence are emphasized by black circles with the letter corresponding to the geographic break (*N* = North, C = Central, S = South), which are also highlighted on the distributional maps (see coloured dots in Figure 1). Dashed lines indicate phylogenetic relationships that do not conform strictly to geographic expectation. Note the blue group in South *Bryconamericus* cluster was removed from the hierarchical analysis [Colour figure can be viewed at wileyonlinelibrary.com]

populations (specifically, N_1) directly from empirical data from the nucleotide diversity (π) of fixed and variable sites. The remaining parameters (i.e., N_2, ancestral population size N_{ANC} and divergence time T_{DIV} for all models, gene flow estimates, *MIG* as single parameter or two parameters, as well as the time of gene flow, T_{GF}, in the case of the model with pulsed gene flow) were estimated based on the SFS, with a mutation rate, μ, estimated from the size of a genome (see formula in Lynch, 2010) based on a close relative for each species (see Table 2 and Table S5 for details). To control for the sensitivity of our estimated divergence times to different settings of μ, we also repeated the analyses using the same mutation rate across the species; specifically, we used the mean μ among all four species (2.19E-8). A generation time of one year was used for all species, which is the common generation time in characids (Azevedo, 2010). FASTSIMCOAL2 runs were performed with 40 replicates for each group pair with 100,000 to 250,000 simulations per likelihood estimation based upon a stopping criterion of 0.001, and 10–40 expectation-conditional cycles (ECM). Model comparisons were performed on the basis of their likelihoods using the Akaike Information Criteria (AIC; Akaike, 1974). The power to estimate the parameters was assessed for the most probable model inferred for each geographic barrier and taxon by performing 100 parametric bootstraps of simulated SFS; specifically, data were simulated under the parameters with the highest maximum likelihood and the simulated data sets were analyzed; parameters were estimated from the simulated data sets from 40 runs of each of the 100 simulated SFS, and reported here as the 95% confidence interval.

3 | RESULTS

3.1 | Population genetic structure

Genetic diversity estimates were generally similar across species (see Table S3), ranging from *Bryconamericus*, which showed the highest genetic diversities, to *M. microlepis* and *H. boulengeri* with lower

TABLE 2 Point estimates of demographic parameters for the more probable model of divergence with gene flow (GF) or a pulse of gene flow (Pulse GF) with symmetric (sym.) or asymmetric migration (asym.) for each taxon and shared geographic divisions from FASTSIMCOAL2. Specifically, ancestral population size, N_{ANC}, the population size for the northern population of each division, N_2, the migration rate, MIG as one or two parameters depending on the model, divergence time, T_{DIV}, and the time of the gene flow, T_{GF}, for Pulse GF scenario are reported

Geographic division	Taxa	Model	N_{ANC}	N_1[a]	N_2	T_{DIV}	T_{GF}	MIG	T_{DIV}, T_{GF} (N_1 for fixed μ)
		PRIORS (not bounded)	unif [1e3,1e6]	*Fixed parameter*	unif[1e3,1e6]	unif[1e3,2e6]	unif[1e3,1.2e5] Tadm < TDIV	GF = logunif[1e-5,20]/N2 Pulse GF = unif[1e-8,0.2]	
North	*M. microlepis*	GF (sym.)	12,262 (7,345–17,895)	99,119	17,983 (15,532–22,167)	28,167 (23,634–36,008)	na	6.60E-07 (1.5e-6–2e-8)	29,922 (102,739)
	H. boulengeri	Pulse GF (sym.)	81,988 (4,916–96,052)	80,214	35,084 (29,881–38,740)	143,341 (137,538–210,449)	29,997 (22,455–130,851)	0.00077 (0.00045–0.0011)	133,196, 47,122 (68,493)
Central	*M. microlepis*	GF (sym.)	8,758 (2,131–12,906)	70,485	26,621 (22,040–31,162)	37,170 (31,757–48,590)	na	1.42E-06 (1e-6–2e-6)	41,326 (73,059)
	H. boulengeri	Pulse GF (asym.)	120,106 (11,120–127,751)	50,802	30,536 (28,324–33,609)	233,547 (121,564–259,791)	20,751 (20,163–22,319)	0.945, 0.084 (0.8942–0.9685, 0.058–0.114)	180,095, 17,703 (43,379)
	Hollandichthys[b]	Pulse GF (asym.)	38,053 (23,486–44,788)	98,214	138,302 (127,331–150,833)	108,770 (103,740–130,688)	9,476 (7,936–13,510)	0.047, 0.043 (0.035–0.074, 0.035–0.06)	111,567, 8,581 (100,457)
	Bryconamericus	Pulse GF (asym.)	2,566 (1,171–19,360)	81,933	80,988 (73,847–88,983)	116,180(98,890–127,455)	12,749 (10,910–14,833)	0.008, 0.12 (0.0017–0.0152, 0.089–0.139)	123,032, 16,114 (89,041)
South	*M. microlepis*	GF (sym.)	7,474 (6,164–8,050)	26,432	5,228 (4,669–6,076)	7,897 (7,388–9,673)	na	4.43E-06 (3e-6–6e-6)	7,981 (27,397)
	H. boulengeri	GF (asym.)	78,432 (61,393–77,183)	42,781	30,251 (28,264–32,751)	33,847 (32,239–37,948)	na	1.2e-6, 2.1e-6 (6.8e-7–1.6e-6, 1.4e-6–2.7e-6)	30,695 (36,530)
	Hollandichthys[b]	Pulse GF (asym.)	19,099 (15,553–19,419)	42,411	5,205 (4,787–5,946)	14,253 (13,616–16,956)	1,092 (1,033–1,418)	0.0037, 0.0614 (0.0009–0.007, 0.049–0.081)	14,795, 1,206 (43,379)
	Bryconamericus	Pulse GF (asym.)	14,669 (1,579–21,333)	42,017	66,473 (61,265–70,657)	88,731 (85,052–111,817)	3,734 (1,551–42,769)	0.0013, 0.0077 (0.0001–0.0476, 0.0047–0.0919)	93,952, 4,765 (45,662)

Note: The population size of the southern population per geographic division, N_1, was calculated directly from the empirical data (i.e., it is a fixed parameter in the model) to improve the accuracy of the other parameters estimated from the SFS (following the recommendations for the program; see Excoffier & Foll, 2011). Also given are the priors (top row), and the 95% confidence interval for each parameter in parentheses. T_{DIV} and T_{GF} are also shown for a fixed mutation rate (μ = 2.19E-08). Parameters estimated for all the models are reported in Table S5.

[a]Mutation rate (μ) to calculate N_1 was estimated based on genome size available for the taxa or closely related taxa (C-value): *Mimagoniates microlepis* = 2.27e-8 (C-value = 1.53); *Hyphessobrycon reticulatus* = 1.87e-8 (1.15); *Bryconamericus stramineus* = 2.24e-8 (1.64); and *Hollandichthys* ("clade C") = 2.38e-8 (1.5) (Carvalho, Oliveira, Navarrete, Froehlich, & Foresti, 2002).

[b]Divergence times estimated here for *Hollandichthys* differ from Thomaz et al. (2017) because of differences in sampling design for FASTSIMCOAL2 analyses between the studies (i.e., a regional analysis here, as opposed to specific paleodrainage groupings in the 2017 manuscript) and models tested.

diversities. There is also a strong correspondence between geography and genetic differentiation in all four taxa. Specifically, a latitudinal pattern of relatedness is evident from the phylogenetic analyses (Figure 2 and Figure S5), except for a couple of populations of *Bryconamericus* where geographically distant populations were closely related.

Analyses of the full data set in STRUCTURE identified $K = 2$ as the most probable value of K based on ΔK (Evanno et al., 2005) in three taxa (*M. microlepis*, *Hollandichthys*, and *Bryconamericus*), and a $K = 4$ for *H. boulengeri* (Table 1). These results, as with estimated phylogenetic trees, identified a geographic division in the center of the species distributions at the Paranaguá estuary (hereafter referred to as the central division). This central division is apparent in all four taxa, separating a northern and southern region, but in *Bryconamericus* it appears some gene flow has occurred between geographically distant populations (Figure 2d).

Subsequent STRUCTURE analyses performed in the northern and southern regional groups to account for the hierarchical structure identified $K = 2$ as the most probable value in each taxon (no hierarchical analysis was performed for *H. boulengeri*, given $K = 4$); note that the likelihoods for $K = 1$ were substantially smaller than $K = 2$ in all cases (outputs available on Dryad). In the northern region, the two broadly distributed taxa, *M. microlepis* and *H. boulengeri*, share a geographic division above the mouth of the Paraíba do Sul River (hereafter referred to as the northern division). This division is generally coincident with the northern extent of the distribution of *Hollandichthys* and *Bryconamericus*, which have smaller distributional ranges. These two taxa also exhibit substructure within the northern extent of their geographic range (Figure 2c,d), but their limited distributions means that congruence of the northern division can only be evaluated in *M. microlepis* and *H. boulengeri*. Analysis of the region south of the central division identified additional congruent substructure across all four taxa (hereafter referred to as the southern division), but with some spatial uncertainty. Specifically, for *M. microlepis* and *Hollandichthys* the southern division occurs between Araranguá (population 6) and D'Una (population 7) river basins, whereas in *Bryconamericus* the precise position cannot be assigned due to a sampling gap, and in *H. boulengeri* the southern division occurs slightly to the north between the island population of Florianópolis (population 9) and the inland Itajaí river basin (population 12; Figure 1). All the inferred genetic clusters show a correspondence with the clades in the estimated phylogenetic trees (Figure 2).

3.2 | Comparisons of divergence models

For all the taxa and for each geographic barrier, divergence with gene flow (Table 2) provided a better fit than divergence in isolation (Table S4). Whether a model with, or without, pulsed gene flow was inferred as the best fit varied by taxa (Table 2). Specifically, the fit of the divergence with gene flow (rather than pulsed gene flow) model was consistently estimated to be a better fit in *M. microlepis* and in one case for *H. boulengeri*; however, in one case – the North geographic barrier in *M. microlepis* - the fit of the data did not differ substantially between the two different models of gene flow (Table S4).

Estimates of the divergence times for each of the three inferred geographic divisions date to the Upper Pleistocene (<126 kya; Figure 3) in all species, except for the Central and North divisions in *H. boulengeri* (~234 and 143 kya, respectively). However, the timing of divergence differs across taxa, despite spatial congruence of the geographic divisions (Figure 3 and Table 2). Geographic isolation appears to correspond to at least two temporal events for each of the regional divisions when we consider both the point estimates and the confidence intervals for the divergence time estimates (Figure 3), irrespectively of whether genetic divergence occurred with or without gene flow (Table 2 and Table S5). For example, in the northern division, the divergence time in *H. boulengeri* (~143 kya) contrasts with *M. microlepis* (divergence of ~28 kya; Figure 3). Likewise, the most recent estimated divergence times across taxa were ~8 and 14 kya for *M. microlepis* and *Hollandichthys*, suggesting observed genetic differences accumulated very recently across the shared southern division, which contrast with *Bryconamericus* (~89 kya; Figure 3) for this same area. Although the

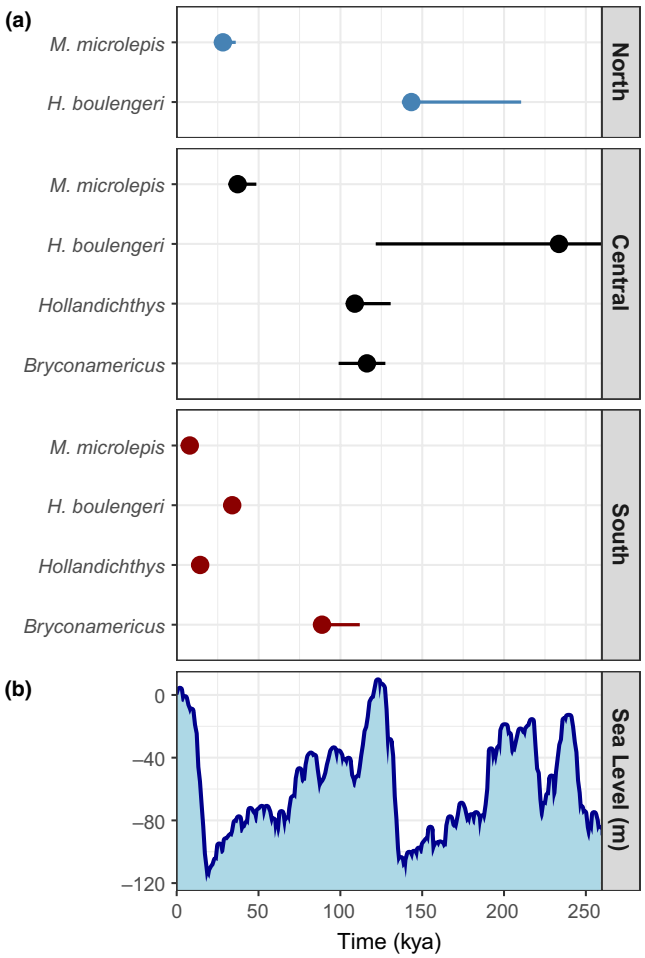

FIGURE 3 Divergence time and 95% confidence interval estimated with FASTSIMCOAL2 for the more probable model inferred per taxon for each geographic division (i.e., North, Central and South; Table 2) along the Brazilian coast with the estimation of sea level for the same time period (Miller, Mountain, Wright, & Browning, 2011) [Colour figure can be viewed at wileyonlinelibrary.com]

timing of divergence reflects when the species became more or less isolated, evaluation of the best fit divergence model indicates the observed genetic differentiation has accumulated with limited gene flow (i.e., divergence models with some gene flow fit the data better; Table 2 and Table S5). When the best model was one in which gene flow occurred as a single pulse, the timing of gene flow is estimated to have occurred sometime close to the Last Glacial Maxima (i.e., always <30 kya; Table 2). Note that even though divergence with gene flow provided the best fit, gene flow was insufficient to overcome the genetic structure associated with the barriers in all cases.

Although the absolute value of the estimated divergences times and times of gene flow pulses presented here might be subject to errors associated with species-specific differences of μ used in the calculations, repeating the analyses using the same mutation rate across taxa demonstrates our results are robust (Table 2). That is, the timing of divergence associated with the barriers differed across taxa (Figure 3), despite spatial congruence in the patterns of geographic isolation among the taxa (Figure 2).

4 | DISCUSSION

Despite shared regional genetic structure across species (Figure 2), differences in the timing of divergences (Figure 3), as well as specifics regarding the limited gene flow that accompanied divergence (Table 2), highlight species-specific dispersal histories. Together the spatial congruence and temporal dissonance reveals the varying degrees of the ephemerality of barriers across landscapes (in this case, isolated coastal riverine basins). Such regional differences in connectivity across paleo-landscapes and among taxa highlight the need for a more nuanced approach for understanding the processes structuring divergence in riverine communities, especially for those characterized by repeated and frequent connections forged by sea-level shifts associated with climatic change. In addition, our work paints a different picture than is frequently envisioned about the effects of climate-induced distributional shifts in the Atlantic Forest (at least for the terrestrial counterparts of the ichthyofauna) where the idea of congruent community response has been popularized. Below we discuss what our findings imply about divergence histories of dynamic landscapes with strict constraints on the geography of dispersal with regards to both (i) the ephemerality of isolation in shaping communities during periods of dramatic climate change, and (ii) expectations for similarity across taxa because of an emphasis on isolated areas, as opposed to dispersal via temporary connections that may be mediated by species-specific ecologies.

4.1 | Ephemeral isolation driven by episodic dispersal

Shared haplotypes and patterns of relatedness between neighboring river basins has classically been used to infer biogeographic histories shaped by past connectivity (e.g., river capture; Lima et al., 2017; Swartz, Chakona, Skelton, & Bloomer, 2014), and has been extended to expectations of congruence among community members (Albert et al., 2011). However, our data shows that a community history shaped by a singular historical event is an oversimplification. In fact, despite the obvious constraints on aquatic dispersal to water, the dispersal and the histories of community members shaped by past connectivity are anything but simple (Figure 3; Table 2).

When we consider shared geographic divisions among taxa, the question becomes what makes these regions standout in terms of their effectiveness as barriers? Two of the three geographic divisions are associated with areas of prominent mountainous relief of granite-gneiss crystalline basement, which agrees with areas associated with paleodrainages boundaries (i.e., the elevated boundary between two areas that drain to different river systems; Thomaz & Knowles, 2018; Weitzman et al., 1988). Specifically, the northern division corresponds with the Cabo Frio Magmatic Lineament, and the southern division with the Serra do Tabuleiro (Villwock, Lessa, Suguiu, Angulo, & Dillenburg, 2005; Zalán & Oliveira, 2005). These geologic features and paleodrainages have notably been invoked as barriers contributing to both speciation and faunal turnover in distributional patterns (Abell et al., 2008; Bizerril, 1994; Dias et al., 2014; Pereira et al., 2013). We note that other paleodrainages have been inferred along the Brazilian coast (Thomaz & Knowles, 2018), but they do not appear to be contributing equally to the regional genetic differentiation across the studied taxa. Additional geological evidence could help explain why some, but not all, paleodrainages are associated with gene divergence. However, one possible explanation may be that the genetic divergence associated with the two specific paleodrainage boundaries detected across the four taxa studied here reflect their stability, especially given that they are associated with prominent geological features that might make them more likely to withstand strong erosion caused by periods of sea level change. On the other hand, the lack of evidence for a role of geologic uplift associated with the central division (Figure 2) is puzzling, but we note that it is positioned in an active tectonic area (i.e., Ponta Grossa Arch; Ribeiro, 2006) with rivers draining to a common outlet based on paleodrainages reconstructions for the LGM (Thomaz & Knowles, 2018). Although this central division has not been identified for structuring communities, high genetic differentiation has been inferred in analyses of population variation in other studies (Thomaz, Malabarba, Bonatto, & Knowles, 2015; Tschá et al., 2017).

Instead of seeking spatial characteristics intrinsic to the shared regional divisions to understand the distribution of genetic divergence, we might also approach the question by asking why the connections forged among some, but not all, contemporary isolated basins have been traversed even more recently than the three divisions identified here. Note that any isolation associated among the basins contained within the inferred regional genetic groups (see Figure 2 and Figure S4) is necessarily more ephemeral (i.e., it is not as old) as the shared regional geographic divisions (Figure 3). It is possible that different degrees of connectivity, or conversely isolation, might relate to bathymetric differences (e.g., continental shelf width and its slope) and/or differences in habitat suitability (distribution

of habitat over time), as is often invoked when studying connectivity in terrestrial communities on islands (Ali & Aitchison, 2014; Papadopoulou & Knowles, 2015, 2016; Shaw & Gillespie, 2016), estuarine fishes (Dolby et al., 2018), and the geographic ranges of freshwater fishes (Carvajal-Quintero et al., 2019). Given the regional structure (Figure 2), we can rule out the possibility that the fish did not have sufficient time to colonize these basins (i.e., each of the species at some point would have been distributed within these regions). This suggests that differences in population persistence, especially given observed distributional gaps within the range of some taxa (Figure 1), might contribute to local, but ephemeral genetic structure. This high turnover is also supported by many freshwater fish species diversity patterns in the area, which is characterized by high levels of endemism (ranging from 67% to 95%; Bizerril, 1994; Reis et al., 2016), with small, disjunct distributions among related taxa separated by some relatively depauperate areas (Ribeiro, Lima, Riccomini, & Menezes, 2006).

In addition to a focus on explaining where geographic barriers might arise, another and relatively understudied question is whether spatial congruence of genetic divergence reflects a single response by the community. To address this question, we can turn to the timing of divergence across species. Overall, the genetic signal recovered here indicates that older events would be erased by the recent connections that happened during the Pleistocene (Figure 3 and Table 2), pointing to the conclusion that there has been a lack of long-term isolation. These findings contrast with previous phylogenetic studies above the species level that have proposed diversification as a result of dispersal events between inland and coastal basins associated with mountain rearrangements during Eocene-Pliocene time period (Ribeiro, 2006; Roxo et al., 2014). However, our evidence of spatial congruence and recency of divergence across coastal barriers indicate that although older geologic events might have contributed to the colonization of the coastal basins (Wendt, Silva, Malabarba, & Carvalho, 2019), temporary connections among the coastal basins promoted during the Pleistocene cycles are the factors shaping the divergence patterns observed in the genomic data. Moreover, the differences in the inferred timing of divergence (i.e., temporal dissonance across species and geographic breaks) point to the episodic nature of when historical connections were traversed, or conversely differences in the effectiveness of barriers, which is a conclusion that is reached whether a common or a species-specific mutation rate are used to estimate divergence times. Nevertheless, there is some temporal clustering (e.g., LGM ~25 kya and ~100 kya; Figure 3), indicating that a null model of random divergence times can be rejected (Bunnefeld, Hearn, Stone, & Lohse, 2018).

Irrespective of the specific cause for differences in the relative ephemerality of genetic structure (i.e., among isolated basins within each of the regional groups; Figure 3), and given that significant genetic structure is also observed within each division (see Figure 2), an inescapable conclusion is that genetic differentiation differs substantially depending upon the geographic setting. Below we discuss what the differences in the ephemerality of isolation across space, as well among taxa, implies about the factors structuring riverine fish communities and communities of the Atlantic Coastal Rainforest of Brazil.

4.2 | Paleo-landscapes and ecological sieves

Although the common spatial genetic structure reinforces the idea that abiotic factors structure freshwater species, and may be attributable to the constraints imposed by riverine environments (Guinot & Cavin, 2015; Tedesco et al., 2012), fishes within a community might exhibit different genetic patterns given their species-specific ecologies associated with different habitats (Waters & Burridge, 2016) or dispersal capabilities (Mather, Hanson, Pope, & Riginos, 2018; Radinger & Wolter, 2014). That is, although historical connections associated with abiotic factors are necessary for any gene flow to occur among the current basins given that they are geographically isolated, they did not necessarily serve as a general conduit for movement of the entire ichthyofauna. Instead, the temporary connections may have acted as taxonomic sieves with respect to realized dispersal. Indeed, the habitat generalist, *M. microlepis*, tends to have relatively young divergence times (Figure 3). It is also the only taxa in which gene flow during the history of divergence associated with the barriers, as opposed to a single pulse of gene flow, was the most probable model (Table 2). In comparison, divergence times were relatively older, and gene flow was limited to a single pulse, in the more specialized taxa that inhabit the highland or the lowland rivers, as well as in the forest habitat specialist (Figure 3 and Table 2). In other words, species-specific differences could reflect the general difference in isolation, or conversely connectivity, such that some temporary passages were more or less accessible to some taxa as a function of dispersal propensities. Under this hypothesis, ecological differences in the fish are causally linked to the relative ephemerality of isolation – that is, ecology acts as a sieve, determining the likelihood of dispersal. Whether the differences observed across regional divisions are consistent with a given dispersal likelihood is an interesting proposition. However, at this point, and given the noted differences in physical characteristics across regional divisions, it is also possible that the paleo-landscapes themselves also contribute to when connections are forged (see also Dolby et al., 2018).

How does the notion of localized and species-specific differences in isolation of these riverine fish compare to our ideas about community responses of the terrestrial counterparts of the Brazilian Coastal Atlantic Rainforest during the Pleistocene? A community-wide effect supporting alternative scenarios have been suggested based on inferred congruence of population histories associated with Pleistocene climatic changes in the Atlantic Forest (Carnaval et al., 2009; Leite et al., 2016; Paz et al., 2018; but see Thomé, Zamudio, Haddad, & Alexandrino, 2014 for discussion about barriers in the region). In contrast, others have argued that in hyperdiverse communities, like the Atlantic Rainforest, congruency in species responses will be highly dependent on the degree of species interactions and ecological fitting (Bunnefeld et al., 2018). Our findings indicate that aquatic organisms may

exhibit species-specific divergence histories, despite being under strong dispersal constraints imposed by riverine environments. Moreover, and perhaps somewhat counterintuitively, our results suggest that an ecological sieve contributes to temporal dissonance in the response of taxa to temporary connections despite spatial congruence, unlike conclusions about shared histories of terrestrial organisms. It may be that differences in processes between riverine and terrestrial systems indeed warrant what might be characterized as different perspectives on the factors structuring divergence. In fact, our population-level findings add to recent evidence that freshwater fishes' species range may be determined by the species' position in the river network, suggesting that theories developed for opens landscapes are inadequate to predict patterns in dendritic landscapes, such as rivers (Carvajal-Quintero et al., 2019). At this point, however, it is not clear whether an emphasis on the stability of regions, as opposed to dispersal during periods of climatic and geologic change, in terrestrial versus riverine systems, respectively, is justified, or whether there might be more commonalities.

The ramifications of the variation in the ephemerality of isolation across space, and among taxa, can be extended to consideration of the speciation process and distribution of diversity. For example, one of the oldest and one of the youngest divergence estimates (i.e., the northern division in *H. boulengeri* and the southern division in *Hollandichthys*, respectively; Figure 3) correspond to the proposed boundaries of putative species recognized by morphological data (Bertaco & Malabarba, 2013; Carvalho, 2006). In addition, for *Bryconamericus*, one species boundary corresponds to the southern division inferred in our study (Hirschmann, Fagundes, & Malabarba, 2017); however, we note the lack of a correspondence between the designation of two other species within this taxon and the regional structure inferred here (i.e., north clusters: populations 40 and 41 for *B. ornaticeps* and population 42 for *B. tenuis* – see Supporting Information S1), which results in a paraphyletic species under the currently proposed nomenclature. It is also notable that the old divergences associated with the central division are not correlated with any obvious morphological differentiation (Bertaco & Malabarba, 2013; Camelier, Menezes, Costa-Silva, & Oliveira, 2018). The variation observed among taxa and geographic divisions could be viewed as evidence of divergence along a speciation continuum, where differentiation might be observed in a limited set of characters in some cases or across multiple traits, as expected as isolation persists (see Huang & Knowles, 2016b). Through this lens, differences among the taxa sampled here would be consistent with differences in the degree of protraction of the speciation process, (Dynesius & Jansson, 2013), and the different lineages or geographic divisions representing differences in the stage of speciation (see Sukumaran & Knowles, 2017), because genetic structure as we show is not equivalent – it is more or less ephemeral depending on the geographic setting and the given species.

Although the strong spatial congruence in divergence patterns across taxa suggests that abiotic factors supersede any taxon-specific differences in their ecologies that might make some barriers more or less effective, the temporal dissonance in divergence times and the extent of gene flow demonstrates how different organisms can differentially perceive the same constraint to dispersal. Overall, these findings highlight how unlikely a unique explanation to fauna diversification it is and the necessity to develop specific predictions at the taxon level. Although time estimates need to be interpreted with caution, the striking recency of events during Pleistocene indicate the role of sea-level changes in the diversification processes in coastal areas. Our work suggests the diversity observed in this hotspot may be the outcome of a complex history of processes that occurred not just millions of years ago, but also includes recent divergence mediated by the vagility of each taxon (e.g., species differ in the extent to which they might capitalize on temporary dispersal routes during Pleistocene sea-level fluctuations). Understanding the response of the organisms to these ephemeral processes, and how they drive population differentiation, is critical to generate expectations on their response to future increases in sea level.

ACKNOWLEDGEMENTS

This work was funded by Rackham Predoctoral Fellowship from the University of Michigan (UM), Hubbs, Carl L. and Laura C. Fellowship from the University of Michigan Museum of Zoology (UMMZ) and an Ichthyology Student Award (also from UMMZ) to A.T.T., and by NSF Dissertation Improvement Grant DEB-15-01301 to L.L.K., and A.T.T. We are very thankful to Professor Luiz R. Malabarba and the Ichthyology Laboratory at the Universidade Federal do Rio Grande do Sul (UFRGS) for all logistics related to fieldwork and specimen curatorial activities. We also thank all the people who contributed to fieldwork – specifically, V. Bertaco, F. Carvalho, T.P. Carvalho, J. Ferrer, A. Hirschmann, F. Jerep, G. Neves, U. Santos, P.C. Silva and J. Wingert, as well as C.A. Lucena and V. Abilhoa from the Museu the Ciências e Tecnologia, Pontifícia Universidade Católica do Rio Grande do Sul (MCP) and Museu the História Natural Capão da Imbuia (MHNCI), respectively, for donating tissues for this study. In addition, we thank M. Kenney for help in laboratory work, Q. He for assistance in analyses, and T.P. Carvalho, R. Pirani, J. Prado, L. Resende-Moreira, and three anonymous reviewers for feedback in early versions of this manuscript.

AUTHOR CONTRIBUTIONS

A.T.T., and L.LK. conceived the study. A.T.T. collected the samples, performed the laboratory work, and analyses. A.T.T., and L.L.K. wrote the manuscript.

DATA AVAILABILITY STATEMENT

RADseq data are archive on Sequence Read Archive (SRA; BioProject ID: PRJNA598706). All post-STACKS processing files that were used as input files and main output files from analyses, plus custom scripts used are available in the Dryad digital repository (Thomaz & Knowles, 2020; https://doi.org/10.5061/dryad.zkh18936g) and on GitHub (https://github.com/ichthya/ThomazKnowles2020_scripts).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Thomaz AT, Knowles LL. Common barriers, but temporal dissonance: Genomic tests suggest ecological and paleo-landscape sieves structure a coastal riverine fish community. *Mol Ecol*. 2020;29:783–796. https://doi.org/10.1111/mec.15357