



**Neotropical freshwater fishes biogeography:
historical and ecological approaches
with insights regarding the conservation.**

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AUGUSTO FROTA

Neotropical freshwater fishes biogeography: historical and ecological approaches with insights regarding the conservation

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*... Tenho pescado os meus peixinhos por todos os cantos deste país <gigante pela própria natureza>... e, não resistindo mais ao <barbarismo> que neste país se pratica e que tende a destruir meus muitos queridos peixes, achei por bem deixar este meu recado através das páginas deste jornal a quem de direito couber a defesa de nossa fauna aquática ...
Penso às vezes, quando será que os governos, a exemplo das estradas de ferro, venham a cuidar de toda margem dos rios, a faixa de terra à margem, que por lei pertence ao Estado...
Penso que quando isso vier a acontecer, o infeliz peixe já será como o dinossauro, uma coisa do passado remoto e só poderá pertencer à história...*

(Abel Barros Frota (querido avô), abril de 1971)

Biogeografia de peixes de água doce neotropicais: abordagens históricas e ecológicas com perspectivas sobre a conservação

RESUMO

Os padrões e processos que ocasionam um sistema de regionalização dos organismos no planeta são hipóteses históricas e ecológicas que a biogeografia busca elucidar. A região Neotropical abriga uma das maiores riquezas de peixes de água doce do mundo, os quais exibem padrões biogeográficos distintos baseados na ecologia, sistemática e evolução. Por intermédio de metodologias dentro do escopo da biogeografia histórica e/ou ecológica, os objetivos deste estudo são apresentar novas abordagens e contribuições ao conhecimento da distribuição geográfica dos peixes de água doce nesta região ao investigar a biogeografia evolutiva de Cnesterodontini e Anablepididae, bem como os padrões espaciais da diversidade beta da ictiofauna na bacia do rio Ivaí, estado do Paraná. Os resultados destacaram cenários bem definidos da formação de barreiras hidrológicas, principalmente entre bacias hidrográficas costeiras e drenagens que fluem para o sistema do rio da Prata na América do Sul. Essas descobertas possibilitaram congruentes relacionamentos de áreas biogeográficas dentro das propostas de diversificação evolutiva à ictiofauna neotropical. Ainda, eventos de dispersão ocasionados por capturas de cabeceiras e mudanças no nível do mar complementaram satisfatoriamente os padrões biogeográficos e os relacionamentos de áreas evidenciados para outras linhagens de peixes neotropicais, apresentando padrões de diversificação semelhantes nas drenagens do Brasil Central e nas drenagens da costa atlântica. Os resultados também evidenciaram forte segregação espacial da ictiofauna ao longo da extensão da bacia do rio Ivaí (sistema do alto rio Paraná), com destaque às influências mútuas de processos baseados em nicho e dispersão. As novas descobertas podem subsidiar referenciais teóricos e metodológicos para os principais mecanismos e padrões específicos às metacomunidades de organismos aquáticos. A aplicação dos resultados aqui obtidos em consonância histórica e ecológica tem grande potencial ao auxílio de um manejo eficaz para a conservação da diversidade de peixes de água doce, seja considerando linhagens com espécies enquadradas em elevado risco de extinção, ou até mesmo bacias hidrográficas de fundamental importância para a manutenção da biodiversidade e funcionalidade ecossistêmica em ambientes aquáticos continentais.

Palavras-chave: Análise Parcimoniosa de Brooks. Áreas prioritárias. Biodiversidade aquática. Interpolação de composição de espécies. Modelagem de dissimilaridade generalizada.

Neotropical freshwater fishes biogeography: historical and ecological approaches with insights regarding the conservation

ABSTRACT

The patterns and processes that cause a system of regionalization of organisms on the planet are historical and ecological hypotheses that biogeography seeks to elucidate. The Neotropical region is home to one of the greatest richness of freshwater fish in the world that exhibit distinct biogeographic patterns based on ecology, systematics, and evolution. Through methodologies within the scope of historical and/or ecological biogeography, the aims of this study are to present new approaches and contributions to the knowledge of the geographic distribution of freshwater fish in this region, investigating the evolutionary biogeography of Cnesterodontini and Anablepidae, as well as the spatial patterns of ichthyofauna beta-diversity in the Ivaí river basin, Paraná State. The results highlighted well-defined scenarios of the hydrological barriers formation, mainly between coastal basins and drainages that flow into the La Plata River system in South America. These findings enabled congruent relationships of biogeographic areas within the proposals for evolutionary diversification to the Neotropical freshwater fishes. Furthermore, dispersal events caused by headwater captures and sea-level changes satisfactorily complemented the biogeographic patterns and area relationships evidenced for other Neotropical fish lineages, showing similar diversification patterns in the Central Brazil drainages and in the Atlantic coast drainages. The results also showed strong spatial segregation of the ichthyofauna throughout the Ivaí River basin (upper Paraná River system) with emphasis on the mutual influences of niche- and dispersal-based processes. These findings can support new theoretical and methodological references for the main mechanisms and patterns specific to the metacommunities of aquatic organisms. Applying the results herein obtained in historical and ecological consonance has great potential to aid an effective management for the conservation of freshwater fish diversity, whether considering lineages with species at high risk of extinction or even river basins of fundamental importance for maintaining biodiversity and ecosystem functionality in continental aquatic environments.

Keywords: Aquatic biodiversity. Brooks Parsimony Analysis. Generalized Dissimilarity Modeling. Priority areas. Species Composition Interpolation.

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SUMMARY

1 INTRODUCTION	13
REFERENCES.....	17
2 EVOLUTIONARY BIOGEOGRAPHY OF CNESTERODONTINI (TELEOSTEI: POECILIIDAE): AREA RELATIONSHIPS AND PRIORITY RANKING FOR CONSERVATION.....	21
2.1 Introduction.....	23
2.2 Materials and methods	26
2.2.1 Data collection	26
2.2.2 Biogeographic areas delimitation	27
2.2.3 Biogeographic analysis	32
2.2.4 Ranking the areas.....	33
2.3 Results	34
2.3.1 Biogeography.....	34
2.3.2 Ranking the areas.....	39
2.4 Discussion	43
2.4.1 Area relationships and implications for the biogeography of Cnesterodontini.....	43
2.4.2 Conservation of Cnesterodontini	46
REFERENCES.....	48
3 EVOLUTIONARY BIOGEOGRAPHY OF THE FRESHWATER FISH FAMILY ANABLEPIDAE (TELEOSTEI: CYPRINODONTIFORMES), A MARINE-DERIVED NEOTROPICAL LINEAGE	60
3.1 Introduction.....	62
3.2 Materials and methods	65
3.2.1 Areas and records	65
3.2.2 Biogeographic analysis	67
3.3 Results	72

3.4 Discussion	80
REFERENCES.....	83
4 SPATIAL DISTRIBUTION PATTERNS AND PREDICTORS OF FISH BETA-DIVERSITY IN A LARGE DAM-FREE TRIBUTARY FROM A NEOTROPICAL FLOODPLAIN.....	90
4.1 Introduction.....	92
4.2 Materials and methods	95
4.2.1 Study area	95
4.2.2 Database preparation.....	98
4.2.3 Species Composition Interpolation	99
4.2.4 Total beta-diversity analysis.....	100
4.2.5 Geo-climatic data	100
4.2.6 Generalized Dissimilarity Modeling.....	101
4.3 Results	102
4.3.1 Species Composition Interpolation	102
4.3.2 Total beta-diversity	103
4.3.3 Generalized Dissimilarity Modeling.....	104
4.4 Discussion	107
4.5 Future perspectives and conclusions.....	111
REFERENCES.....	113
5 CONCLUDING REMARKS	124
APPENDIX LIST.....	126
APPENDIX A - Summary of the geographic distribution data	126
APPENDIX B - Final database with the georeferenced records.	138
APPENDIX C - Matrix file constructed to run with the parsimony analysis.....	148
APPENDIX D - Six equally most parsimonious trees.....	149
APPENDIX E- Geographic distribution of the 108 native fish species	155

1 INTRODUCTION

Studying the geographical distribution of organisms could be the simplest definition of biogeography, however, this apparently easy to define discipline conceals a great complexity that encompasses contents from geology, geography, and biology. Thus, it is not surprising that different researchers attribute different meanings to biogeography (CRISCI *et al.*, 2003). In general, by recognizing distribution patterns, biogeography proposes hypotheses regarding the processes that cause and provide a regionalization system for organisms on the planet. To this end, throughout its history, biogeography has developed with different approaches, but which, essentially, are configured in two major theoretical fields, namely known as historical and ecological biogeography (MORRONE, 2004).

In ecological biogeography, the analysis contents involve shorter periods of time, in a relatively small space and that generally analyze individual or population distribution patterns (MORRONE, 2004). Ecological biogeography must answer questions such as (1) why is a species confined to the region in which it lives? (2) What enables this species to live there and what prevents it from expanding into other areas? (3) What is the role of soil, climate, latitude, and topography in limiting the geographic distribution of organisms? (4) How do we explain species turnover as we move across a mountain or from one environment to another? In this sense, the rise of ecology as a scientific discipline during the beginning of the 20th century provided new approaches to biogeographic studies, in a way that both disciplines (biogeography and ecology) act famously together (COX & MOORE, 2009).

However, the purely ecological explanation becomes insufficient since different sites with similar environmental conditions can be inhabited by very different organisms (MORRONE & CRISCI, 1995). Given this scenario, historical biogeography emerges by proposing that analyses also be directed to the supra-specific distribution patterns, making use of larger spatial and temporal scales and assuming that the factors that produced such patterns are of a historical nature (SANMARTIN, 2012). Thus, historical biogeography studies the distribution of organisms rooted in the assumption that speciation, extinction, and dispersal are the mechanisms by which organisms respond to the spatial and temporal dynamics of the geographic matrix

(LOMOLINO *et al.*, 2006).

There are three general models in historical biogeography to explain distribution patterns: 1) Centers of Origin-Dispersal-Adaptation, 2) Vicariance, and 3) Dispersal-Vicariance. The first model assumes an origin restricted to the ancestor of a taxon, followed by dispersals, arrival in new areas, and adaptations to new conditions. The second model assumes a widely distributed ancestor, which differentiated after the appearance of barriers that isolated populations. The third model contemplates alternative episodes of vicariance and dispersal, in which more real scenarios are evidenced for the explanations of current biotic distributions (MORRONE, 2015) and constitutes an integrative approach within evolutionary biogeography (MORRONE, 2020). In this sense, leading the historical scenarios of biodiversity diversification, cladistic biogeography is based on the premise that there is a clear correspondence between the phylogenetic relationship of taxa with their distribution patterns and geological history (NIHEI, 2016). Therefore, there must be an association between the history of organisms and the history of the planet, suggesting the recognition of general patterns of distribution when analyzing and comparing the cladograms of different phylogenetic proposals with taxa existing in a given spatial region (MORRONE, 2005; PARENTI & EBACH, 2009).

Terrestrial boundaries and saltwater represent strong barriers to dispersal, therefore, river basins can be considered as islands for strictly freshwater organisms (TEDESCO *et al.*, 2012; DIAS *et al.*, 2014). Freshwater fish, precisely because they are limited by geographic barriers of a hydrographic network, constitute an excellent group to investigate ecological and evolutionary processes, that is, these organisms form a peculiar group for good models and studies of ecological and historical biogeography (MYERS, 1947; DE PINNA, 2006; BERR, 2007; LEROY *et al.*, 2019). However, limited taxonomic knowledge at a specific level, inadequate information on the distribution of most species, and scarce or non-existent data on the phylogenetic history of the taxa can limit and bias the accuracy of the hypotheses for the biogeography of freshwater fish (VARI & WEITZMAN, 1990; RIBEIRO *et al.*, 2016). Fortunately, based on modern cladistic methodologies, advances in ichthyology since the 1990s have enabled a great increase in the number and quality of taxonomic review studies and phylogenies of groups of Neotropical fishes, which substantially allowed for the advancement of

the understanding of geographic distributions and, consequently, of the biogeography this highly diversified group of organisms (RIBEIRO *et al.*, 2016).

In numbers, despite summarizing only 1% of the Earth's surface, freshwater environments contain approximately 45% of fish species on the planet (LÉVÊQUE *et al.*, 2007). The Neotropical region *sensu lato* (definition in MORRONE, 2014), in which South America and much of Central America are located, corresponds to the biogeographic domain with the greatest diversity of freshwater fish in the world, with estimates of 9,000 species (BIRINDELLI & SIDLAUSKAS, 2018). Specifically, the South American continent is home to one of the greatest richness of freshwater fish on the planet, with certainty for numbers greater than 740 genera and 5,160 valid species (REIS *et al.*, 2016). Of this biodiversity, many species are small and inhabit mainly low-order watercourses (WINEMILLER *et al.*, 2008; CASTRO & POLAZ, 2020), which are estimated to harbor about 70% of the Neotropical freshwater fish richness (CASTRO & POLAZ, 2020). Despite the high diversity, the consequences of multiple human activities affecting aquatic bodies severely threaten freshwater fish populations. For example, in Brazil alone, approximately 10% (311 species) formally described species were classified into one of three international threat categories, of these, 81% (253 species) are small-sized fish (CASTRO & POLAZ, 2020).

The reasons for such a pronounced diversity are, at the same time, historical and ecological (RIBEIRO *et al.*, 2016) that exhibit distinct biogeographic patterns based on ecology, systematics, and evolution of Neotropical fish (ALBERT *et al.*, 2020). At the large spatial scale, the distribution of fish species is commonly structured by historical events arising from the geological reconfigurations that shaped the spatial networks of river basins (TEDESCO *et al.*, 2005; ALBERT & REIS, 2011; ALBERT *et al.*, 2020). In this sense, the movement of freshwater fish is limited by their inability to cross oceans, mountain ranges, or expansive deserts (BERRA, 2007). In a clear ecological association, fishes have a high induced environmental dependence (OBERDORFF *et al.*, 1995), defined as the spatial structure of the response to habitats in close association with environmental variables, suggesting that at smaller spatial scales the spatial and temporal variables they can be more homogeneous or heterogeneous (MONTGOMERY, 1999), which strongly reflects on the geographic distribution of fish assemblages. Furthermore, the

ability of fish to move in response to environmental changes is limited by the dendritic arrangement of riparian ecosystems, as well as by a variety of physiographic barriers (FAUSCH *et al.*, 2002).

Although the ecological and historical biogeography of Neotropical freshwater fishes has progressed considerably in recent decades, many patterns and processes are still misunderstood to actually decipher the true magnitude of this enormous diversity of fish (RIBEIRO *et al.*, 2016). Understanding such patterns and processes not only ensures the accumulation of scientific knowledge of biodiversity, but can and should become basic requirements to aim for the implementation of mitigation measures for its conservation and, consequently, maintenance of ecosystem services in the face of the greatest environmental crisis of all the times. Thereby, the aims of this study are to present new approaches and contributions to the knowledge of the geographic distribution of freshwater fish in Neotropical environments, using methodologies intrinsically related to historical and/or ecological biogeography.

Specifically, in the three approaches herein carried out, the results obtained through biogeographic analyzes will be presented, which sought to understand: 1) events intrinsically related to the diversification and extinction of lineages widely distributed in the Neotropical region, reporting specific cases of groups with severe extinction threats for many of their species (Cnesterodontini) or with phylogenetic origins from marine ancestors (Anablepidae); 2) the spatial distribution of the ichthyofauna in a hydrographic basin strategically considered as essential to the ecosystem maintenance and fish diversity of the upper Paraná River floodplain, in southern Brazil. In the first approach (section 2), the results showed that dispersal events through headwater capture and sea-level changes are associated with congruences evidenced among cnesterodontins and other fish lineages that exhibit similar diversification patterns in the Central Brazil drainages and in the drainages on the Atlantic coast of South America and, there is a shining priority for the conservation and restoration of the Atlantic Forest to the maintenance of the phylogenetic diversity, species richness, and endangered species of this tribe. In the second approach (section 3), the results indicated that the patterns found for Anablepidae are in accordance with other marine-derived lineages that entered freshwater environments in South America, suggesting that vicariance, dispersal, and extinction events related to marine

transgressions of the Miocene and Quaternary supported the historical relationships among areas. In the third approach (section 4), the results pointed to the spatial separation of the ichthyofauna along the environmental and hydrological gradient of the Ivaí River basin, suggesting that some metacommunity models (e.g., species sorting, mass effect, and patch dynamics) can explain the fish diversity patterns evidenced, and supporting that habitat heterogeneity, environmental gradients, hydrological conditions, and habitat connectivity are fundamental for the maintenance and conservation of fish populations. Overall, the results obtained can establish successful attempts to understand the current dynamics of the geographic distribution of freshwater fish in historical and ecological aspects, allowing practical actions for the conservation and management of fisheries resources, as well as theoretical applications to understanding the association between the patterns of freshwater fish distribution and the processes that shaped them in the Neotropical region.

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2 EVOLUTIONARY BIOGEOGRAPHY OF CNESTERODONTINI (TELEOSTEI: POECILIIDAE): AREA RELATIONSHIPS AND PRIORITY RANKING FOR CONSERVATION

Abstract: Distributional, phylogenetic, molecular, and paleontological data may be integrated to discover biogeographic patterns exhibited by the organisms. Cladistic biogeography uses information on the phylogenetic relationships between the taxa and their geographic distribution to specify a precise sequence of area fragmentation. Brooks Parsimony Analysis (BPA) was performed based on the phylogenetic proposals for Cnesterodontini to hypothesize on the historical relationships among nine areas previously recognized by endemism of freshwater fish from South America. Additionally, we investigated the conservation scenario for cnesterodontins based on biogeographic, phylogenetic, and extinction criteria to establish a priority ranking for the analyzed areas. We found that the area relationships for Cnesterodontini are strongly linked to the historical patterns of evolution of the hydrographic basins, revealing well-defined scenarios of the formation of hydrological barriers between coastal river basins (i.e., Atlantic Slope) and mainly drainages running into the La Plata River system (i.e., Inland Slope). Dispersal events through headwater capture and sea-level changes explain the congruences evidenced among other fish lineages that exhibit similar diversification patterns in the Central Brazil drainages and in the drainages on the Atlantic coast of South America. The ranking of priority areas established for Cnesterodontini indicates that greater attention should be directed to the regions located in the Central Coastal and Upper Parana areas, mainly in the Atlantic Forest, which represents one of the most threatened biodiversity hotspots on the planet with serious threats to freshwater biodiversity.

Keywords: Atlantic Forest. Endangered fish. Headwater captures. Sea-level changes. Small-sized fish. South America.

BIOGEOGRAFIA EVOLUTIVA DE CNESTERODONTINI (TELEOSTEI: POECILIIDAE): RELACIONAMENTOS DE ÁREA E CLASSIFICAÇÃO DE PRIORIDADE PARA CONSERVAÇÃO

Resumo: Dados de distribuições, filogenéticos, moleculares e paleontológicos podem ser integrados para descobrir padrões biogeográficos exibidos pelos organismos. A biogeografia cladística usa informações sobre as relações filogenéticas entre os táxons e sua distribuição geográfica para especificar uma sequência precisa de fragmentação da área. A Análise de Parcimônia de Brooks (BPA) foi realizada com base nas propostas filogenéticas de Cnesterodontini para hipotetizar as relações históricas entre nove áreas previamente reconhecidas pelo endemismo de peixes de água doce na América do Sul. Além disso, investigamos o cenário de conservação para Cnesterodontini com base em critérios biogeográficos, filogenéticos e de extinção para estabelecer uma classificação de prioridade para as áreas analisadas. Descobrimos que as relações de área para Cnesterodontini estão fortemente ligadas aos padrões históricos de evolução das bacias hidrográficas, revelando cenários bem definidos de formação de barreiras hidrológicas entre bacias hidrográficas costeiras (i.e., Encosta Atlântica) e principalmente drenagens que drenam para o sistema do rio da Prata (i.e., Declive Interior). Eventos de dispersão por meio de captura de cabeceira e mudanças no nível do mar explicam as congruências evidenciadas entre outras linhagens de peixes que exibem padrões de diversificação semelhantes nas drenagens do Brasil Central e nas drenagens da costa atlântica da América do Sul. A classificação de áreas prioritárias estabelecido para Cnesterodontini indica que maior atenção deve ser direcionada às regiões localizadas nas áreas do Litoral Central e Alto Paraná, principalmente na Mata Atlântica, que representa um dos *hotspots* de biodiversidade mais ameaçados do planeta com sérias ameaças à biodiversidade de água doce.

Palavras-chave: América do Sul. Capturas de cabeceiras. Mata Atlântica. Mudanças no nível do mar. Peixes ameaçados. Peixes de pequeno porte.

2.1 Introduction

Integrating information about the biota and its relationship with the geological evolution of the Earth is the primary target of evolutionary biogeography. Distributional, phylogenetic, molecular, and paleontological data may be linked in order to discover biogeographic patterns exhibited by living beings and assess the historical changes that have shaped biotic assembly (Morrone 2009). There is a clear equivalence between systematics (taxa) and biogeography (areas), which makes it possible to raise an analogy between them (Platnick and Nelson 1978; Wiley 1988a; Humphries and Parenti 1999; Morrone 2009). Classifying taxa by their shared characters is an essential requisite to study areas and classify them by their shared taxa (Morrone 2020). Thereby, to specify a precise sequence of area fragmentation, cladistic biogeography uses information on the phylogenetic relationships between the taxa and their geographic distribution to infer past area relationships (Platnick and Nelson 1978; Rosen 1978; Wiley 1988a; Humphries and Parenti 1999; Morrone 2009; Parenti and Ebach 2009), assuming an association between the history of organisms and the history of the planet (Nihei 2016). After the recognition of general patterns of relationship between areas, it is possible to infer the processes (vicariance, dispersal, and extinction) that affected the biotic history of these areas (Nihei 2016). Thus, pattern-based methods assume that specific biogeographic processes of the taxa optimized in the cladogram can be inferred by synapomorphies as vicariance events, parallelisms as dispersal events, and reversals as extinction events (Morrone 2009). This approach has been allowed new findings on the evolutionary biogeography of Neotropical freshwater organisms (e.g., Tumini et al. 2018; Caballero-Viñas et al. 2021), especially the South American fishes (e.g., Albert and Carvalho 2011; Dagosta and de Pinna 2017; Frota et al. 2020a).

South America is home to a significant portion of freshwater fish diversity, with numbers certainly exceeding 740 genera and 5,160 valid species (Reis et al. 2016; Albert et al. 2020). The reasons for such pronounced diversity are associated with historical and ecological factors over geological time (Ribeiro et al. 2016; Albert et al. 2020), which interconnect and add up in different biogeographic patterns based on the ecology, systematics, and evolution of the Neotropical fishes (see Albert et al. 2020). Freshwater fishes are an excellent group to investigate evolutionary processes, as terrestrial boundaries and saltwater represent strong barriers to

dispersal. Therefore, distinct river basins can be considered as "islands" for strictly freshwater organisms (Tedesco et al. 2012; Dias et al. 2014). In this context, the historical biogeography of Neotropical freshwater fishes is commonly associated with the events arising from the geological reconfigurations that shaped the current river basins (Tedesco et al. 2005; Hubert and Renno 2006; Albert and Carvalho 2011; Ribeiro et al. 2018; Albert et al. 2020). For example, freshwater fish distribution in southeastern South America is highly associated with headwater captures, barrier formation, and drainage connection/isolation due to the sea-level changes, which promote isolation or faunal exchanges and, consequently, foster lineage dispersal and speciation (e.g., Ribeiro 2006; Ingenito and Buckup 2007; Costa 2010; Piálek et al. 2012; Thomaz et al. 2015; Lima et al. 2016, 2017, 2021; Aquino and Colli 2017; Ramos-Fregonezi et al. 2017; Tschá et al. 2017; Machado et al. 2018; Morais-Silva et al. 2018; Thomaz and Knowles 2018, 2020; Říčan et al. 2019; Wendt et al. 2019; Frota et al. 2020a).

Cnesterodontini (Poeciliinae) are a group of small-sized livebearers (Lucinda and Reis 2005). Currently, *Cnesterodon* Garman, 1895, *Phallotorynus* Henn, 1916, and *Phalloceros* Eigenmann, 1907 are a well-sustained clade within this tribe (Lucinda 2005, 2008; Lucinda and Reis 2005; Lucinda et al. 2005). In addition to the well-known phylogenetic positioning of the tribe in Poeciliinae (see Lucinda and Reis 2005), species of the three mentioned genera also have taxonomic revisions with phylogenetic relationships based on morphological (Lucinda 2005, 2008; Lucinda et al. 2005, 2006; Aguilera et al. 2009; Lucinda and Graça 2015) and molecular (Thomaz et al. 2019) data. Regarding their geographical distribution, all species are endemic to South America (Lucinda and Reis 2005). *Cnesterodon* comprises 10 valid species (Aguilera et al. 2009; Fricke et al. 2021) distributed in the upper Araguaia, Uruguay, and Parana-Paraguay river basins, and in coastal drainages from São Paulo State to Argentina, as well as in small basins in western Argentina (Lucinda 2005; Lucinda et al. 2006; Aguilera et al. 2009). *Phallotorynus* has six valid species (Lucinda and Graça 2015; Fricke et al. 2021), distributed in the Parana-Paraguay and Uruguay river basins (Chuctaya et al. 2018), coastal drainages from the Paraíba do Sul River basin in the Southeast (Lucinda et al. 2005), and Paraná State in South Brazil (Reis et al. 2020). *Phalloceros* is the most species-rich South American genus of Poeciliidae, with 22 valid species (Lucinda 2008; Thomaz et al. 2019; Fricke et al. 2021) distributed in coastal drainages from

northeastern Brazil to Uruguay, as well as in the Tocantins, São Francisco, Uruguay, and Parana-Paraguay river basins (Lucinda 2008; Thomaz et al. 2019).

The International Union for Conservation of Nature (IUCN) adopts the Red List of categories and criteria establishing rules to categorize a given species at risk of extinction (IUCN 2019). The most recent edition of the Brazilian Red Book adopts these international rules (ICMBio 2018), classifying approximately 10% of the formally described freshwater fish species into one of three threat categories, i.e., CR—Critically Endangered, EN—Endangered, and VU—Vulnerable, with the greatest contribution of the small-sized fishes living in the Atlantic Forest biome (Castro and Polaz 2020). Nowadays, several cnestodontins endemic to this biome are threatened or endangered presenting one species classified as VU, four as EN, and one species as CR (ICMBio 2018), which represents almost 16% of the valid species for the tribe.

Until now, despite interesting discussions and clarifications about the historical diversification scenario (Lucinda and Reis 2005; Lucinda et al. 2005; Lucinda 2008; Lucinda and Graça 2015; Ramos-Fregonezi et al. 2017; Thomaz et al. 2019), there is no methodological framework that has actually been tested to investigate the evolutionary biogeography and conservation assessment of Cnestodontini. Specifically, our study addresses three major questions: (1) Which are the patterns of area relationships recovered based on the distributional and phylogenetic information compiled for Cnestodontini? (2) Is it possible to identify putative vicariance, dispersal, and extinction events associated with the patterns of area relationships and species distribution analyzed? (3) Which areas hold the highest conservation priorities for Cnestodontini based on biogeographical, phylogenetic, and extinction criteria? To this end, we compare hypotheses about the historical relationships among areas previously recognized by endemism and distinct fish assemblages in South America applying a cladistic biogeographic analysis and using Cnestodontini as a model. Besides, the updated distribution of the species of the tribe includes some putative new species which were also analyzed to rank the areas for their conservation.

2.2 Materials and methods

2.2.1 Data collection

Geographical distribution data of the species were mainly retrieved from the literature (Lucinda 2005, 2008; Lucinda et al. 2005; 2006; Aguilera et al. 2009; Lucinda and Graça 2015; Silva et al. 2015; Bertaco et al. 2016; Frota et al. 2016, 2019, 2021; Chuctaya et al. 2018; Souto-Santos et al. 2019; Thomaz et al. 2019; Reis et al. 2020; Mezzaroba et al. 2021). Additionally, the following databases of ichthyological collections were used: Global Biodiversity Information Facility (<http://www.gbif.org/>), FishNet2 (<http://www.fishnet2.net/>), and SpeciesLink (<http://splink.cria.org.br/>). Locality records for individual species were checked and corrected when necessary according to the same literature and Fricke et al. (2021). Records of specimens lacking associated coordinates were georeferenced on Google Earth, and such records were excluded from the distribution map (Figure 2.1) and from biogeographical analysis, in case there were uncertainties about the localities of the collections or incompatibilities with sites described in the literature.

In addition to the 38 valid species of cnesterodontins (Fricke et al. 2021), we include three putative new species of *Cnesterodon*, two from the Rio Grande do Sul (sp. A and sp. B, see Bertaco et al. 2016) and one from the Paraná (sp. C, see Frota et al. 2016; Reis et al. 2020) states, and two species of *Phalloceros* from the São Paulo State (sp. L and sp. R, see Thomaz et al. 2019). A summary of the search for records, filtering, and final geographic distribution of cnesterodontins species is presented in Appendix A. The final database encompassed 524 georeferenced records (Figure 2.1) and is available in Appendix B.

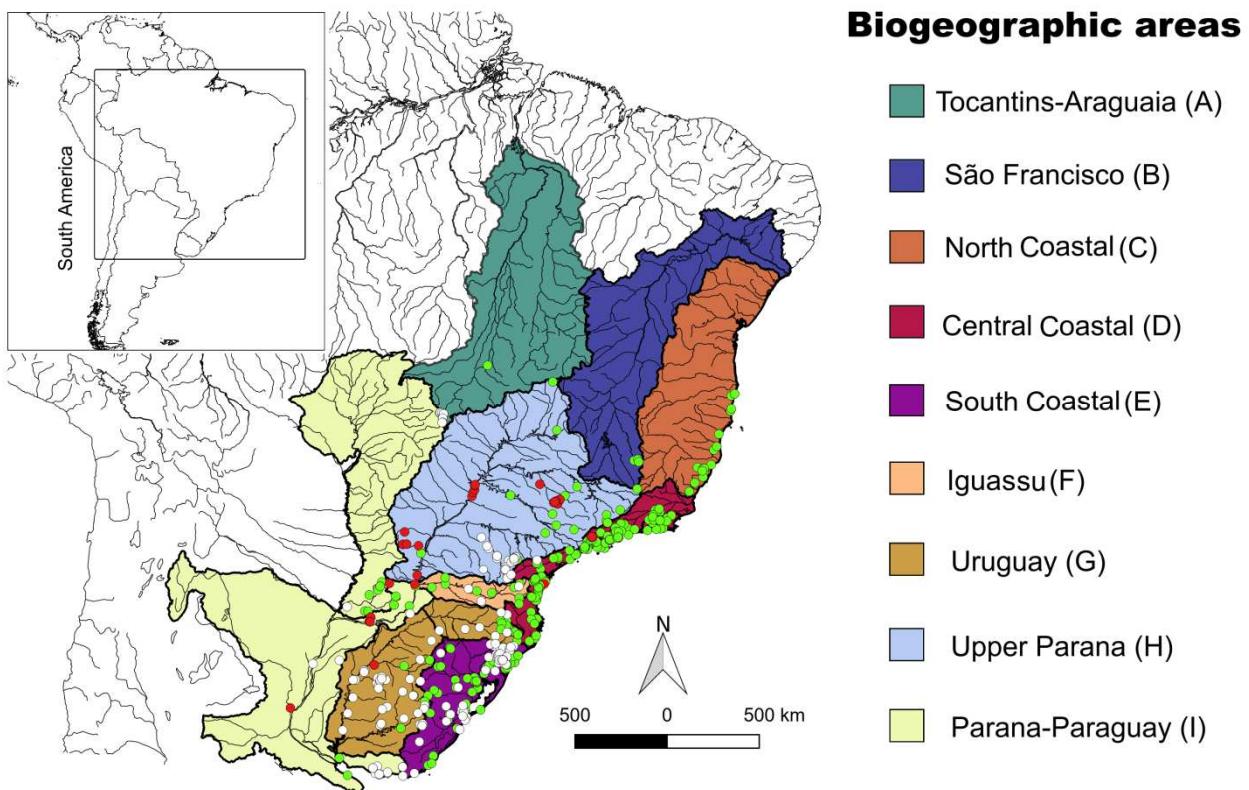


Figure 2.1 Map showing the geographic distribution of Cnesterodontini and the nine biogeographic areas selected in South America overlaid with the ecoregions defined by Abell et al. (2008). Records of *Cnesterodon* = white dots; records of *Phalloceros* = green dots; records of *Phallotrynus* = red dots.

2.2.2 Biogeographic areas delimitation

Biogeographic areas considered in this study are similar to the Freshwater Ecoregions of the World (FEOW) proposed by Abell et al. (2008), who presented a map of global biogeographic regionalization based on distributional data of fish species incorporating their main ecological and evolutionary patterns. In fact, the FEOW have been shown promising operational geographic units in biographical studies of aquatic organisms (e.g., Albert and Carvalho 2011; Tagliacollo et al. 2015; Machado et al. 2018; Tumini et al. 2018; Wendt et al. 2019; Frota et al. 2020a). Based on the current geographical distribution and records of Cnesterodontini (Figure 2.1; Appendix A), we considered it appropriate to use the FEOW adapting the junction of Lower Parana and Paraguay ecoregions (see Lucinda 2005; Lucinda et al. 2005; Aguilera et al. 2009; Lucinda and Graça 2015), and the same adaptations made by Wendt et al. (2019). Thus, we selected the

following nine biogeographic areas (Figure 2.1):

A: Tocantins-Araguaia—corresponds to the Tocantins-Araguaia River basin situated in the ecoregion 324 (Tocantins-Araguaia) of Abell et al. (2008) in Brazil. Cnesterodontins that occur in this area are *Cnesterodon septentrionalis* Rosa & Costa, 1993 and *Phalloceros leticiae* Lucinda, 2008 (Table 2.1, Appendix A).

B: São Francisco—corresponds to the São Francisco River basin situated in the ecoregion 327 (S. Francisco) of Abell et al. (2008) in Brazil. The Cnesterodontini that occurs in this area is *Phalloceros uai* Lucinda, 2008 (Table 2.1, Appendix A).

C: North Coastal—corresponds to the totality of coastal river basins situated in the ecoregion 328 (Northeastern Mata Atlantica) of Abell et al. (2008) in Brazil. Cnesterodontins that occur in this area are *Phalloceros elachistos* Lucinda, 2008, *P. harpagos* Lucinda, 2008, *P. mikrommatus* Lucinda, 2008, and *P. ocellatus* Lucinda, 2008 (Table 2.1, Appendix A).

D: Central Coastal—corresponds to the totality of coastal river basins situated in the ecoregions 329 (Paraíba do Sul), 330 (Ribeira de Iguape), 331 (Southeastern Mata Atlantica), and 352 (Fluminense) of Abell et al. (2008) in Brazil. Cnesterodontins that occur in this area are *Cnesterodon brevirostratus* Rosa & Costa, 1993, *C. iguape* Lucinda, 2005, *Phalloceros alessandri* Lucinda, 2008, *P. anisophallos* Lucinda, 2008, *P. aspilos* Lucinda, 2008, *P. buckupi* Lucinda, 2008, *P. enneaktinos* Lucinda, 2008, *P. harpagos*, *P. leptokeras* Lucinda, 2008, *P. lucenorum* Lucinda, 2008, *P. malabarai* Lucinda, 2008, *P. megapolos* Lucinda, 2008, *P. pello*s Lucinda, 2008, *P. reisi* Lucinda, 2008, *P. spiloura* Lucinda, 2008, *P. titthos* Lucinda, 2008, *P. tupinamba* Lucinda, 2008, *Phallocerosspp. L*, *Phallocerosspp. R*, and *Phallotorynus fasciolatus* Henn, 1916 (Table 2.1, Appendix A).

E: South Coastal—corresponds to the totality of coastal river basins situated in the ecoregions 334 (Laguna dos Patos) and 335 (Tramandai-Mampituba) of Abell et al. (2008) in Brazil and Uruguay. Cnesterodontins that occur in this area are *Cnesterodon brevirostratus*, *C. decemmaculatus* (Jenyns, 1842), *Cnesterodon* sp. A, *Cnesterodon* sp. B, *Phalloceros caudimaculatus* (Hensel, 1868), *P. heptaktinos* Lucinda, 2008, and *P. spiloura* (Table 2.1,

Appendix A).

F: Iguassu—corresponds to the Iguassu River basin situated in the ecoregion 346 (Iguassu) of Abell et al. (2008) in Brazil. Cnesterodontins that occur in this area are *Cnesterodon carnegiei* Haseman, 1911, *C. omorgmatus* Lucinda & Garavello, 2001, *Phalloceros harpagos*, and *P. spiloura* (Table 2.1, Appendix A).

G: Uruguay—corresponds to the Uruguay River basin situated in the ecoregions 332 (Lower Uruguay) and 333 (Upper Uruguay) of Abell et al. (2008) in Brazil and Uruguay. Cnesterodontins that occur in this area are *Cnesterodon brevirostratus*, *C. decemmaculatus*, *C. holopterus* Lucinda, Litz & Recuero, 2006, *Cnesterodon* sp. A, *Phalloceros caudimaculatus*, *P. spiloura*, and *Phallotorynus victoriae* Oliveros, 1983 (Table 2.1, Appendix A).

H: Upper Parana—corresponds to the Upper Parana River basin situated in the ecoregion 344 (Upper Parana) of Abell et al. (2008) in Brazil. Cnesterodontins that occur in this area are *Cnesterodon hypselurus* Lucinda & Garavello, 2001, *Cnesterodon* sp. C, *Phalloceros harpagos*, *P. reisi*, *Phallotorynus fasciolatus*, *P. jucundus* Ihering, 1930, *P. pankalos* Lucinda, Rosa & Reis, 2005, and *P. victoriae* (Table 2.1, Appendix A).

I: Parana-Paraguay—corresponds to the Paraguay and Middle and Lower Parana river basins situated in the ecoregions 343 (Paraguay) and 345 (Lower Parana) of Abell et al. (2008) in Argentina, Brazil, and Paraguay. Cnesterodontins that occur in this area are *Cnesterodon decemmaculatus*, *C. pirai* Aguilera, Mirande & Azpelicueta, 2009, *C. raddai* Meyer & Etzel, 2001, *Phalloceros caudimaculatus*, *P. harpagos*, *Phallotorynus dispilos* Lucinda, Rosa & Reis, 2005, *P. psittakos* Lucinda, Rosa & Reis, 2005, and *P. victoriae* (Table 2.1, Appendix A).

Table 2.1 Data matrix of areas versus species used to ranking the areas. Areas A to I are in accordance with the description in the “Materials and methods” section and Figure 2.1. Species are: 1) *Cnesterodon brevirostratus*, 2) *C. carnegiei*, 3) *C. decemmaculatus*, 4) *C. holopterus*, 5) *C. hypselurus*, 6) *C. iguape*, 7) *C. omorgmatus*, 8) *C. pirai*, 9) *C. raddai*, 10) *C. septentrionalis*, 11) *Cnesterodon* sp. A, 12) *Cnesterodon* sp. B, 13) *Cnesterodon* sp. C, 14) *Phalloceros alessandri*, 15) *P. anisophallos*, 16) *P. aspilos*, 17) *P. buckupi*, 18) *P. caudimaculatus*, 19) *P. elachistos*, 20) *P. enneaktinos*, 21) *P. harpagos*, 22) *P. heptaktinos*, 23) *P. leptokeras*, 24) *P. leticiae*, 25) *P. lucenorum*, 26) *P. malabarbai*, 27) *P. megapolos*, 28) *P. mikrommatus*, 29) *P. ocellatus*, 30) *P. pellos*, 31) *P. reisi*, 32) *P. spiloura*, 33) *P. titthos*, 34) *P. tupinamba*, 35) *P. uai*, 36) *Phalloceros* sp. L, 37) *Phalloceros* sp. R, 38) *Phallotorynus dispilos*, 39) *P. fasciolatus*, 40) *P. jucundus*, 41) *P. pankalos*, 42) *P. psittakos*, and 43) *P. victoriae*. * = species categorized as CR = Critically Endangered, EN = Endangered, VU = Vulnerable, or NT = Near Threatened.

Table 2.2 Data matrix of the areas versus cladogram components used in the Brooks Parsimony Analysis (BPA). Areas A to I are in accordance with the description in the “Materials and methods” section and Figure 2.1, and the components are the same encodings represented in the cladograms in Figure 2.2 for *Phalloceros*, *Phallotorynus*, and *Cnesterodon* clades.

Areas	Components																														
	Phalloceros																	Phallotorynus				Cnesterodon									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
Root	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
A	1	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	1	0	0	0	1	0
B	1	1	1	1	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C	1	1	1	1	1	1	1	0	0	1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
D	1	1	1	1	1	1	1	1	1	1	0	0	1	1	1	1	1	1	1	0	1	1	1	1	1	1	1	0	0	1	1
E	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	1	0	0	0	0	1	1	1	1	1	1	0	1	1	1
F	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	0	0	0	0	1	1	1	1	1	0	0	0	0	0
G	1	1	1	1	1	1	1	1	1	0	0	1	0	0	1	1	0	1	1	0	1	1	1	1	1	1	0	1	0	1	1
H	1	1	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	1	1	1	1	1	1	1	1	1	1	0	0	0	0
I	1	1	1	1	1	1	1	0	0	0	1	1	1	1	0	0	0	1	1	0	1	1	1	1	1	1	0	1	1	0	0

2.2.3 Biogeographic analysis

Brooks Parsimony Analysis (BPA) was employed following its original proposition (Brooks 1981; Brooks et al. 2001), based on the phylogenetic relationships of Cnesterodontini proposed by Lucinda and Reis (2005), who established that *Cnesterodon* is the sister-group of *Phalloceros* + *Phallotrynus* (Figure 2.2a). Following phylogenetic hypotheses based on morphological data available to the tribe, individual cladograms were extracted from Lucinda (2008) for *Phalloceros* (Figure 2.2b), Lucinda and Graça (2015) for *Phallotrynus* (Figure 2.2c), and Aguilera et al. (2009) for *Cnesterodon* (Figure 2.2d). The BPA involved constructing an individual area cladogram for the genera by replacing the species names in the phylogenies with their geographical distribution areas (Figure 2.2b–d). The internal and terminal relevant nodes (components defined as hypothetical ancestors) were numbered in the cladograms (Figure 2.2b–d). Based on them, we constructed a matrix of areas (rows) versus cladogram components (columns) and coded it as “1” if a given component was present in a particular area and as “0” if it was absent (Table 2.2). The data matrix was analyzed using a parsimony algorithm (Appendix C).

A hypothetical ancestral area (root) with a total absence of species was added to the data matrix (Table 2.2; Appendix C) to allow grouping by presence rather than the absence of taxa, which was employed to root the general area cladogram (Crisci et al. 2003). The matrix (Appendix C) was run with a parsimony analysis using TNT (Goloboff et al. 2008) through the WINCLADA interface (Nixon 2002), with the heuristic search option (tree bisection and reconnection, 100 replications) to obtain the most parsimonious trees and the strict consensus cladogram. Finally, applying a historical biogeographic explanation, synapomorphies were interpreted as vicariance events, parallelisms as dispersal events, and reversals as extinction events (Wiley 1988a, b; Brooks 1990; Morrone 2009).

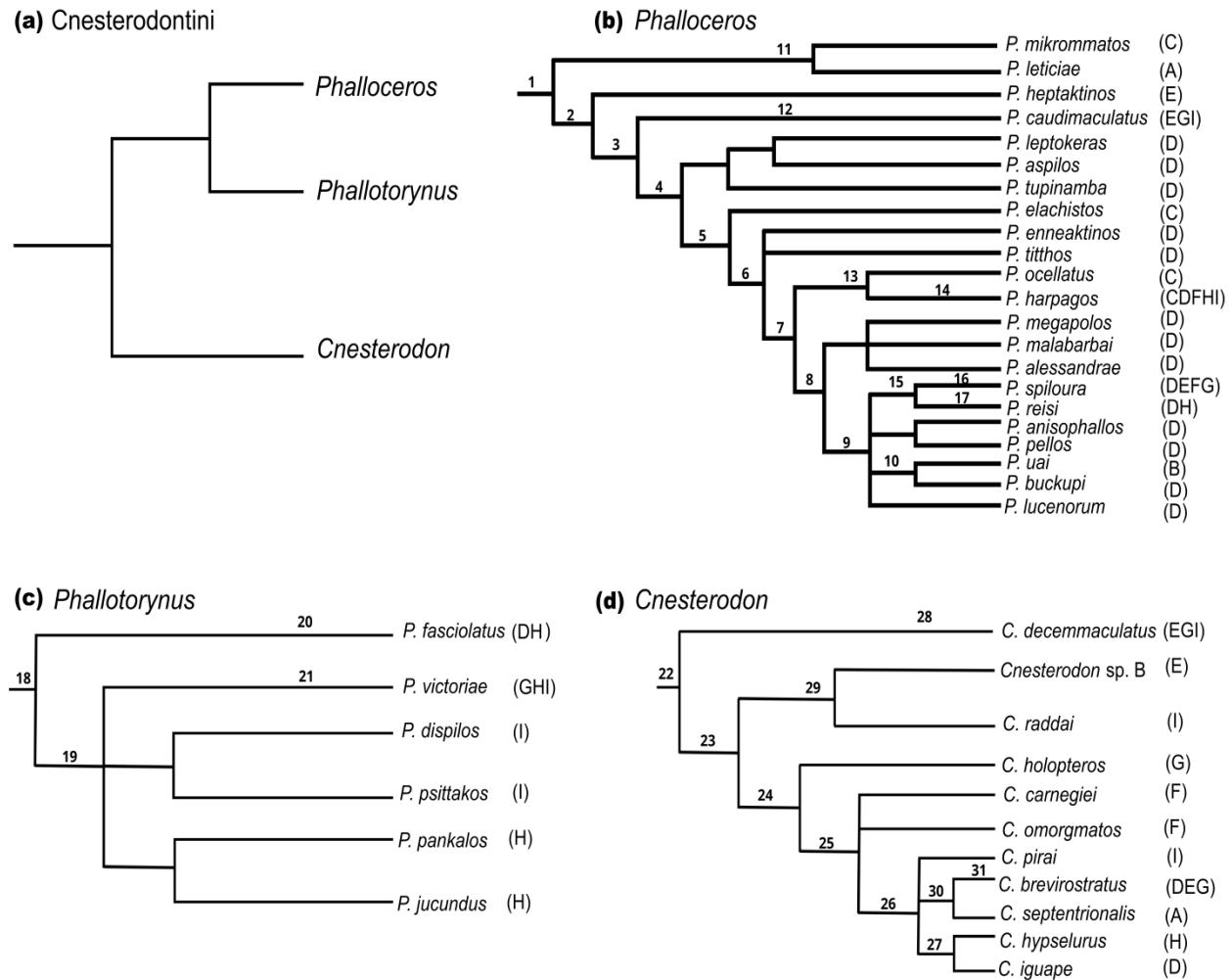


Figure 2.2 Area cladograms derived from the phylogenetic hypotheses for (a) Cnesterodontini (Lucinda and Reis 2005), (b) *Phalloceros* (Lucinda 2008), (c) *Phallotrynus* (Lucinda and Graça 2015), and (d) *Cnesterodon* (Aguilera et al. 2009). Components are numbered from 1 to 31. Areas are represented by the letters A to I according to Figure 2.1 and description in the “Material and methods” section.

2.2.4 Ranking the areas

A sequence of priority areas was proposed to conserve cnesterodontins based on biogeographical, phylogenetic, and extinction parameters and on complementarity, adopting the methodological procedure proposed by Tumini et al. (2019). Firstly, the nine biogeographic areas were ranked in which the area with the greatest species richness was positioned first. Unrepresented taxa in the next area correspond to the biotic complement of the last area(s) positioned in the rank (Vane-Wright et al. 1991) until the maximum biological diversity is

identified in the minimum number of areas (Posadas et al. 2001; Álvarez Mondragón and Morrone 2004) reducing the complement to zero (Morrone 1999). In a second step, another ranking was performed adopting the phylogenetic criterion in which the area with the greatest species richness was also positioned first and the next area was selected following the complementarity and considering the decreasing biodiversity score of each clade, i.e., *Phalloceros* (Figure 2.2b), *Phallotorynus* (Figure 2.2c), and *Cnesterodon* (Figure 2.2d). The biotic complement of a clade comprised the taxa unrepresented in a second clade. For both criteria, we use the data matrix of areas (rows) versus species (columns) constructed and coded as “1” if a given species was present in a particular area and as “0” if it was absent (Table 2.1). In a third step, the extinction criterion was defined based on the species extinction risk to the cnesterodontins (ICMBio 2018) in which the area with the highest number of CR (Critically Endangered), EN (Endangered), VU (Vulnerable), and/or NT (Near Threatened) (IUCN 2019) was positioned first (Table 2.1). Following the complementarity previously described, a new area was chosen considering both the level of threat and the decreasing biodiversity score.

Based on the biogeographical, phylogenetic, and extinction risk analyses, a consensus ranking of priority areas for conservation was defined. Each specific position of the areas in the consensus ranking was first determined by the number of criteria in which each area was ranked (at least two criteria), and in a second step, a score was calculated adopting a value equal to the positioning of each area in each criterion. For example, if a given area was placed second in some criterion, the individual value of that area for that criterion was 2. The final score represents the sum of the individual values assigned for each area to the criteria in which it was ranked. Thus, higher priority was given to areas with lower scores in the consensus ranking.

2.3 Results

2.3.1 Biogeography

The area cladograms obtained from the phylogenetic hypotheses for Cnesterodontini contained 31 components in which 17 were in the *Phalloceros* clade (1 to 17; Figure 2.2b; Table 2.2), four in the *Phallotorynus* clade (18 to 21; Figure 2.2c; Table 2.2), and 10 in the *Cnesterodon*

clade (22 to 31; Figure 2.2d; Table 2.2). Based on the presence-absence matrix of areas versus cladogram components (Table 2.2; Appendix C), six equally most parsimonious trees were obtained with 56 steps, a consistency index of 0.55, and a retention index of 0.58 (Figures S1–S6 in Appendix D). The strict consensus cladogram (Figure 2.3) with 65 steps, a consistency index of 0.47, and a retention index of 0.43 revealed Tocantins-Araguaia as the sister-group to the clade containing the other areas supported by 10 synapomorphies, composed exclusively by components of the *Phalloceros* clade (Figure 2.3; Table 2.3). The clade herein named “Southeastern South America” composed of areas from the La Plata River system (i.e., Upper Parana, Iguassu, Parana-Paraguay, and Uruguay) + Central Coastal and South Coastal was supported by another three synapomorphies by components of the *Phalloceros* and *Phallotorynus* clades (Figure 2.3; Table 2.3). Within this clade, Uruguay + South Coastal composed the clade herein named “Southern South America”, but it was supported only by homoplasies representing dispersal and/or extinction events (Figure 2.3; Table 2.3).

Involving practically all areas of the consensus cladogram, components in *Phalloceros* clade supported synapomorphies (components 1 to 9 and 13 to 16; Figure 2.3; Table 2.3), parallelisms (components 10 to 12 and 17; Figure 2.3; Table 2.3), in addition to reversals (components 8, 9, and 13 to 16; Figure 2.3; Table 2.3). Components in *Phallotorynus* clade supported one synapomorphy (component 18; Figure 2.3; Table 2.3), parallelisms involving Upper Parana, Parana-Paraguay, and Uruguay (components 19 and 21; Figure 2.3; Table 2.3), and Central Coastal and Upper Parana (component 20; Figure 2.3; Table 2.3), in addition to reversals in Iguassu and South Coastal (component 18; Figure 2.3; Table 2.3). Components in *Cnesterodon* clade supported parallelisms involving Southeastern South America and Tocantins-Araguaia (components 22 to 26; Figure 2.3; Table 2.3), Central Coastal and Upper Parana (component 27; Figure 2.3; Table 2.3), Parana-Paraguay and Southern South America (component 28; Figure 2.3; Table 2.3), Parana-Paraguay and South Coastal (component 29; Figure 2.3; Table 2.3), Tocantins-Araguaia, Central Coastal, and Southern South America (component 30; Figure 2.3; Table 2.3), and Central Coastal and Southern South America (component 31; Figure 2.3; Table 2.3), in addition to one reversal in Iguassu (component 26; Figure 2.3; Table 2.3).

Table 2.3 Summary of the Brooks Parsimony Analysis (BPA) results highlighting all possibilities of the components in the strict consensus cladogram to represent vicariance (synapomorphy), dispersal (parallelism), and/or extinction (reversal) events in the delimited biogeographic areas. Asterisks (*) indicate extinctions in the areas that contain them.

Component	Ancestor	Event	Areas
1	<i>Phalloceros</i>	Vicariance	All areas
2	<i>Phalloceros</i> except <i>P. mikrommatus</i> and <i>P. leticiae</i>	Vicariance	All areas except Tocantins-Araguaia
3	<i>Phalloceros</i> except <i>P. mikrommatus</i> , <i>P. leticiae</i> , and <i>P. heptaktinos</i>	Vicariance	All areas except Tocantins-Araguaia
4	<i>Phalloceros</i> except <i>P. mikrommatus</i> , <i>P. leticiae</i> , <i>P. heptaktinos</i> , and <i>P. caudimaculatus</i>	Vicariance	All areas except Tocantins-Araguaia
5	<i>Phalloceros</i> except <i>P. mikrommatus</i> , <i>P. leticiae</i> , <i>P. heptaktinos</i> , <i>P. caudimaculatus</i> , <i>P. leptokeras</i> , <i>P. aspilos</i> , and <i>P. tupinamba</i>	Vicariance	All areas except Tocantins-Araguaia
6	<i>Phalloceros</i> except <i>P. mikrommatus</i> , <i>P. leticiae</i> , <i>P. heptaktinos</i> , <i>P. caudimaculatus</i> , <i>P. leptokeras</i> , <i>P. aspilos</i> , <i>P. tupinamba</i> , and <i>P. elachistos</i>	Vicariance	All areas except Tocantins-Araguaia
7	<i>Phalloceros</i> except <i>P. mikrommatus</i> , <i>P. leticiae</i> , <i>P. heptaktinos</i> , <i>P. caudimaculatus</i> , <i>P. leptokeras</i> , <i>P. aspilos</i> , <i>P. tupinamba</i> , <i>P. elachistos</i> , <i>P. enneaktinos</i> , and <i>P. titthos</i>	Vicariance	All areas except Tocantins-Araguaia
8	<i>Phalloceros megapolos</i> , <i>P. malabarbai</i> , <i>P. alessandrae</i> , <i>P. spiloura</i> , <i>P. reisi</i> , <i>P. anisophallos</i> , <i>P. pellos</i> , <i>P. uai</i> , <i>P. buckupi</i> , and <i>P. lucenorum</i>	Vicariance; extinction*	All areas except Tocantins-Araguaia; North Coastal*; Parana-Paraguay*
9	<i>Phalloceros spiloura</i> , <i>P. reisi</i> , <i>P. anisophallos</i> , <i>P. pellos</i> , <i>P. uai</i> , <i>P. buckupi</i> , and <i>P. lucenorum</i>	Vicariance; extinction*	All areas except Tocantins-Araguaia; North Coastal*; Parana-Paraguay*

10	<i>Phalloceros uai</i> and <i>P. buckupi</i>	Dispersal	São Francisco; Central Coastal
11	<i>Phalloceros mikrommatus</i> and <i>P. leticiae</i>	Dispersal	Tocantins-Araguaia; North Coastal
12	<i>Phalloceros caudimaculatus</i>	Dispersal	Parana-Paraguay; Southern South America
13	<i>Phalloceros ocellatus</i> and <i>P. harpagos</i>	Vicariance; extinction*	All areas except Tocantins-Araguaia; São Francisco*; Southern South America*
14	<i>Phalloceros harpagos</i>	Vicariance; extinction*	All areas except Tocantins-Araguaia; São Francisco*; Southern South America*
15	<i>Phalloceros spiloura</i> and <i>P. reisi</i>	Vicariance; extinction*	Southeastern South America; Parana-Paraguay*
16	<i>Phalloceros spiloura</i>	Vicariance; extinction*	Southeastern South America; Upper Parana*; Parana-Paraguay*
17	<i>Phalloceros reisi</i>	Dispersal	Upper Parana; Central Coastal
18	<i>Phallotrynus</i>	Vicariance; extinction*	Southeastern South America; Iguassu*; South Coastal*
19	<i>Phallotrynus</i> except <i>P. fasciolatus</i>	Dispersal	Upper Parana; Parana-Paraguay; Uruguay
20	<i>Phallotrynus fasciolatus</i>	Dispersal	Upper Parana; Central Coastal
21	<i>Phallotrynus victoriae</i>	Dispersal	Upper Parana; Parana-Paraguay; Uruguay

22	<i>Cnesterodon</i>	Dispersal	Tocantins-Araguaia; Southeastern South America
23	<i>Cnesterodon</i> except <i>C. decemmaculatus</i>	Dispersal	Tocantins-Araguaia; Southeastern South America
24	<i>Cnesterodon</i> except <i>C. decemmaculatus</i> , <i>Cnesterodon</i> sp. B, and <i>C. raddai</i>	Dispersal	Tocantins-Araguaia; Southeastern South America
25	<i>Cnesterodon</i> except <i>C. decemmaculatus</i> , <i>Cnesterodon</i> sp. B, <i>C. raddai</i> , and <i>C. holopterus</i>	Dispersal	Tocantins-Araguaia; Southeastern South America
26	<i>Cnesterodon pirai</i> , <i>C. brevirostratus</i> , <i>C. septentrionalis</i> , <i>C. hypselurus</i> , and <i>C. iguape</i>	Dispersal; extinction*	Tocantins-Araguaia; Southeastern South America; Iguassu*
27	<i>Cnesterodon hypselurus</i> and <i>C. iguape</i>	Dispersal	Upper Parana; Central Coastal
28	<i>Cnesterodon decemmaculatus</i>	Dispersal	Parana-Paraguay; Southern South America
29	<i>Cnesterodon</i> sp. B and <i>C. raddai</i>	Dispersal	Parana-Paraguay; South Coastal
30	<i>Cnesterodon brevirostratus</i> and <i>C. septentrionalis</i>	Dispersal	Tocantins-Araguaia; Central Coastal; Southern South America
31	<i>Cnesterodon brevirostratus</i>	Dispersal	Central-Coastal; Southern South America

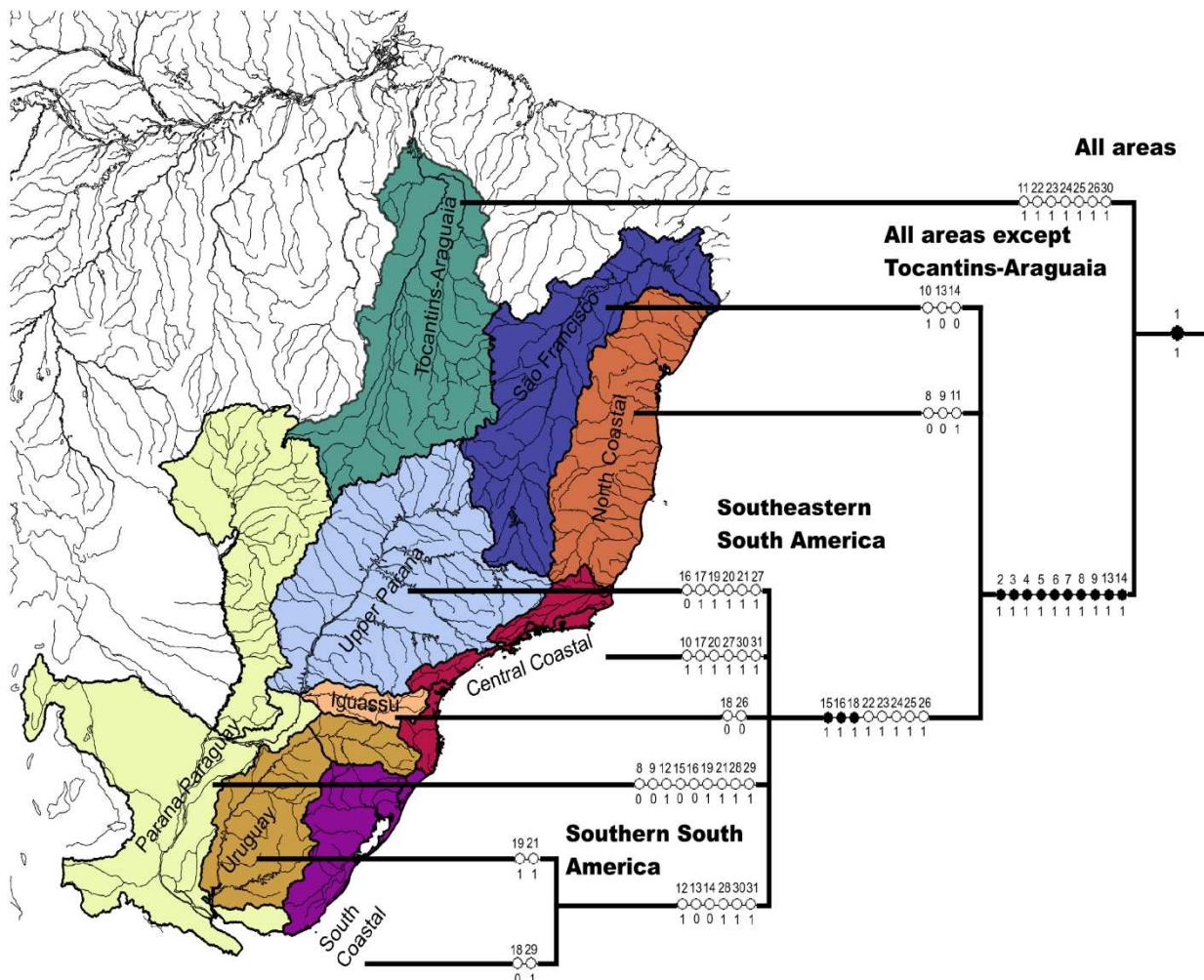


Figure 2.3 Strict consensus cladogram evidencing the results of Brooks Parsimony Analysis (BPA) and the historical relationships among the delimited areas using Cnesterodontini. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

2.3.2 Ranking the areas

The decreasing ranking of priority areas for the conservation of cnesterodontins defined by the biogeographical criterion involved all areas with the following priority (Table 2.4): Central Coastal (highest richness, 20 species), Parana-Paraguay (seven species), Upper Parana (four species), North Coastal and South Coastal in the same position (three species in each area), Tocantins-Araguaia and Iguassu in the same position (two species in each area), and São Francisco and Uruguay in the same position (one species in each area).

For the phylogenetic criterion, the decreasing ranking of priority areas also involved all areas with the following priority (Table 2.4): Central Coastal (highest richness comprising 17 species in the *Phalloceros* clade, two species in the *Cnesterodon* clade, and one species in the *Phallotorynus* clade), Parana-Paraguay (one species in the *Phalloceros* clade and three species in each the *Cnesterodon* and *Phallotorynus* clades), Upper Parana (two species in each the *Cnesterodon* and *Phallotorynus* clades), South Coastal (two species in the *Cnesterodon* clade and one species in the *Phalloceros* clade), Tocantins-Araguaia (one species in each the *Cnesterodon* and *Phalloceros* clades), Iguassu and Uruguay in the same position (two species in the *Cnesterodon* clade for each area), and São Francisco (one species in the *Phalloceros* clade).

Regarding the species extinction risk, one species was categorized as CR (*C. iguape*; Table 2.1, Appendix A), four as EN (*C. hypselurus*, *C. omorgmatus*, *P. fasciolatus*, and *P. jucundus*; Table 2.1, Appendix A), one as VU (*C. carnegiei*; Table 2.1, Appendix A), and three as NT (*C. septentrionalis*, *P. ocellatus*, and *P. victoriae*; Table 2.1, Appendix A). The decreasing ranking of priority areas defined by the extinction criterion involved five areas with the following priority (Table 2.4): Upper Parana (highest number, four species), Iguassu (two species), Central Coastal (one species categorized as CR), and North Coastal and Tocantins-Araguaia in the same position (one species categorized as NT in each area). Hence, the consensus sequence of areas for the cnesterodontins conservation was integrated into all areas adopting the number of criteria and score with the following priority: Central Coastal (first place), Upper Parana (second place), Iguassu, North Coastal and Tocantins-Araguaia in the same position (third place) followed by Parana-Paraguay, South Coastal, Uruguay, and São Francisco (Table 2.4).

Table 2.4 Biogeographic areas ranked by biogeographical, phylogenetic, and extinction criteria (each with complementarity) together with the consensus ranking for the conservation of Chesterodontini.

Criteria and complementarities						Consensus ranking	Number of criteria	Score
Biogeographical	Complement	Phylogenetic	Complement	Extinction	Complement			
1st) Central Coastal	<i>C. brevirostratus</i> , <i>C. iguape</i> , <i>P. alessandrae</i> , <i>P. anisophallos</i> , <i>P. aspilos</i> , <i>P. buckupi</i> , <i>P. enneaktinos</i> , <i>P. harpagos</i> , <i>P. leptokeras</i> , <i>P. lucenorum</i> , <i>P. malabarbai</i> , <i>P. megapolos</i> , <i>P. pellos</i> , <i>P. reisi</i> , <i>P. spiloura</i> , <i>P. titthos</i> , <i>P. tupinamba</i> , <i>Phalloceros</i> spp., <i>Phalloceros</i> sp. R, and <i>P. fasciolatus</i>	1st) Central Coastal	<i>Phalloceros</i> (17 species), <i>Cnesterodon</i> (two species), and <i>Phallotorynus</i> (one species)	1st) Upper Parana	<i>C. hypselurus</i> (EN), <i>P. fasciolatus</i> (EN), <i>P. jucundus</i> (EN), and <i>P. victoriae</i> (NT)	1st) Central Coastal	3	1+1+3 = 5
2nd) Parana-Paraguay	<i>C. decemmaculatus</i> , <i>C. pirai</i> , <i>C. raddai</i> , <i>P. caudimaculatus</i> , <i>P. dispilos</i> , <i>P. psittakos</i> and <i>P. victoriae</i>	2nd) Parana-Paraguay	<i>Phalloceros</i> (one species), <i>Cnesterodon</i> (three species), and <i>Phallotorynus</i> (three species)	2nd) Iguassu	<i>C. carnegiei</i> (VU) and <i>C. omorgmatus</i> (EN)	2nd) Upper Parana	3	3+3+1 = 7

3rd)Upper Parana	<i>C. hypselurus</i> , <i>Cnesterodon</i> sp.C, <i>P. jucundus</i> , and <i>P. pankalos</i>	3rd) Upper Parana	<i>Cnesterodon</i> (two species) and <i>Phallotorynus</i> (two species)	3rd) Central Coastal	<i>C. iguape</i> (CR)	3rd) Iguassu– North Coastal – Tocantins-Araguaia	3	5+7+2 = 14 –4+6+4 = 14 –5+5+4 = 14
4th)North Coastal–South Coastal	<i>P. elachistos</i> , <i>P. mikrommatus</i> , and <i>P. ocellatus</i> – <i>Cnesterodon</i> sp. A, <i>Cnesterodon</i> sp. B, and <i>P. heptaktinos</i>	4th) South Coastal	<i>Cnesterodon</i> (two species) and <i>Phalloceros</i> (one species)	4th) North Coastal–Tocantins- Araguaia	<i>P. ocellatus</i> (NT)– <i>C. septentrionalis</i> (NT)	4th) Parana-Paraguay	2	2+2+0 = 4
5th) Tocantins-Araguaia –Iguassu	<i>C. septentrionalis</i> and <i>P. leticiae</i> – <i>C. carnegiei</i> and <i>C. omorgmatus</i>	5th) Tocantins-Araguaia	<i>Cnesterodon</i> (one species) and <i>Phalloceros</i> (one species)			5th) South Coastal	2	4+4 = 8
6th) São Francisco–Uruguay	<i>P. uai</i> – <i>C. holopterus</i>	6th) North Coastal	<i>Phalloceros</i> (three species)			6th) Uruguay	2	6+7 = 13
		7th) Iguaçu–Uruguay	<i>Cnesterodon</i> (two species)			7th) São Francisco	2	6+8 = 14
		8th) São Francisco	<i>Phalloceros</i> (one species)					

2.4 Discussion

2.4.1 Area relationships and implications for the biogeography of Cnesterodontini

We found that the components in *Phalloceros*, *Cnesterodon*, and *Phallotorynus* clades represented, respectively, 54.8, 32.3, and 12.9% of all hypothetical ancestors established in the internal and terminal relevant nodes derived from the phylogenetic proposals for these genera. Although there is greater species richness in *Phalloceros*, which could naturally lead to more potential ancestors for BPA, this genus exhibited several dispersal events, allowing many of its co-distributed species are not sister taxa, which corroborates interesting cases of secondary sympatry (see Lucinda 2008; Thomaz et al. 2019). In *Cnesterodon*, the homoplasy tendencies evidenced by the ancestors of the genus, especially parallelisms, support several dispersal episodes that occasioned sister taxa living in different areas (see Lucinda 2005; Lucinda et al. 2006; Aguilera et al. 2009; Ramos-Fregonezi et al. 2017). In *Phallotorynus*, adding to the lower species richness, a smaller number of hypothetical ancestors can be also explained by sister taxa inhabiting the same area, which corroborates a scenario with putative vicariance events between the delimited areas (see Lucinda et al. 2005; Lucinda and Graça 2015).

Our results point to the basal separation of the Tocantins-Araguaia in relation to the other areas in the consensus cladogram. For this area, a scenario supported only by dispersal events of ancestors in the *Phalloceros* and *Cnesterodon* clades was evidenced. Although Poeciliinae are distributed throughout the Americas, with some genera of Poeciliini (sister-group of Cnesterodontini; see Lucinda and Reis 2005) being typically Amazonian, cnesterodontins are distributed in southeastern South America (Lucinda and Reis 2005), which strengthens the fact that the presence in Amazonian drainages (i.e., Tocantins-Araguaia) is a consequence of dispersal events in Central Brazil (Hubert and Renno 2006; Albert and Carvalho 2011; Aquino and Colli 2017; Dagosta and de Pinna 2017; Machado et al. 2018; Ribeiro et al. 2018). Dispersal events were also evident in the São Francisco and North Coastal, corroborating that fish assemblages from the areas situated in the southeastern South America clade recently colonized the neighboring Upper Tocantins-Araguaia and São Francisco river basins probably due to headwater capture (e.g., Aquino and Colli 2017; Machado et al. 2018) and the North Coastal due

to sea-level changes in coastal palaeodrainages (e.g., Ribeiro 2006; Thomaz et al. 2015; Lima et al. 2016, 2021; Tschá et al. 2017; Thomaz and Knowles 2018).

The boundaries of the geological formations representing watershed dividers between coastal river basins (i.e., Atlantic Slope) and mainly drainages running into the La Plata River system (i.e., Inland Slope) correspond to interesting patterns of the geographic distribution of freshwater fishes with great effects in biogeographic (e.g., Ribeiro 2006; Ingenito and Buckup 2007; Costa 2010; Piálek et al. 2012; Frota et al. 2016, 2019, 2020b; Wendt et al. 2019) and phylogeographic studies (e.g., Ramos-Fregonezi et al. 2017; Morais-Silva et al. 2018). These regions are directly linked to strong implications on the cnestodontins evolutionary diversification due to several episodes of headwater captures and sea-level changes in the Atlantic coast in Southeastern South America clade.

The areas of the Southeastern South America clade were supported by synapomorphies and parallelisms reinforcing that the distribution of fishes in headwaters from the La Plata River system, especially the Upper Parana, Iguassu, and Uruguay, is linked to several vicariance and dispersal episodes between the Central Coastal and South Coastal (e.g., Ribeiro 2006; Ingenito and Buckup 2007; Costa 2010; Loureiro et al. 2011; Frota et al. 2019, 2020a, b; Wendt et al. 2019). Corroborating these findings, some watershed dividers such as high mountains (e.g., Serra da Mantiqueira and Ponta Grossa Arch) are often seen as effective dispersal barriers for fish between coastal and La Plata River system drainages (Ribeiro 2006; Ingenito and Buckup 2007; Frota et al. 2016, 2019, 2020b). The Serra da Mantiqueira (watershed divider between the Upper Parana and the Paraíba do Sul River basin in the Central Coastal) and the Ponta Grossa Arch (watershed divider between the Upper Parana, Iguassu River basin, and Ribeira de Iguapec River basin in Central Coastal) represent barriers to fish populations (Ribeiro 2006; Ingenito and Buckup 2007; Frota et al. 2019, 2020b), however, some species are shared between the La Plata River system (including the Upper Parana, Iguassu, and Uruguay) with neighboring coastal drainages, constituting a characteristic pattern recognized by Ribeiro (2006) as “Pattern C”. This pattern represents recent vicariant events between the crystalline plateau rivers (i.e., La Plata River system) and adjacent coastal drainages, but allows the fish species sharing and the

formation of hybrid zones by recent headwater captures (Ribeiro 2006; Frota et al. 2016, 2019, 2020b; Morais-Silva et al. 2018). Specifically, the areas of the southern South America clade (i.e., Uruguay and South Coastal) were supported by homoplasies representing dispersal and/or extinction events. Again, dispersal in this clade can be explained by the same "Pattern C" for the entire southeastern South America clade since headwater captures are closely related to the greater proximity of fish fauna between the Uruguay River basin and drainages from the South Coastal (Ribeiro 2006; Costa 2010; Loureiro et al. 2011; Frota et al. 2020a).

Discrepancies between patterns of genetic structure and palaeodrainage connectivity may indicate alternative dispersal processes in the coastal river basins (Thomaz et al. 2015; Lima et al. 2017; Thomaz and Knowles 2018, 2020), probably differing by the temporal discordance in common dispersal routes and/or ecological specialization (Thomaz and Knowles 2020), which may be closely related to the strong incompatibilities of sexual characteristics among sympatric taxa in cnestodontins (Thomaz et al. 2019). It is also noteworthy that the water divide between the Amazonian, Parana-Paraguay (including herein the Upper Parana), São Francisco, and coastal river basins is old and dated to the Late Cretaceous. Therefore, erosive and tectonic headwater captures, marine incursions, and climatic changes satisfactorily complement the interpretation of the biogeographic patterns shown by fishes in this region (Tedesco et al. 2005; Hubert and Renno 2006; Ribeiro 2006; Ribeiro et al. 2018).

Extinction events (i.e., reversals) involving cnestodontins were substantial in La Plata River system, i.e., Parana-Paraguay (components 8, 9, 15, and 16), Iguassu (components 18 and 26), and Upper Parana (component 16), North Coastal (components 8 and 9), São Francisco (components 13 and 14), and Southern South America clade in South Coastal (component 18). Overall, extinction of aquatic biodiversity mainly in the La Plata River system and coastal river basins has certainly occurred in an exacerbated manner due to several prolonged marine incursions that drastically reduced freshwater environments (Albert and Carvalho 2011). Climatic changes over geological time have also resulted in a substantial contraction of tropical climates to lower latitudes, further reducing the amount of habitat available and promoting harsh climatic conditions for Neotropical fish (Tedesco et al. 2005; Albert and Carvalho 2011).

2.4.2 Conservation of Cnesterodontini

Overall, our findings highlight that Central Coastal and Upper Parana should be considered the most priority areas for the conservation of cnesterodontins diversity, encompassing species richness, phylogenetic diversity, as well as several of their endangered species. Both areas are dominated by the phytophysiognomies of the Atlantic Forest or Cerrado biomes, which concentrate the largest number of threatened fishes species, respectively presenting 35 and 23% of the total endangered species in Brazil (Castro and Polaz 2020). In the case of small-sized fishes inhabiting streams like cnesterodontins, the main impacts are primarily due to the suppression or reduction of original vegetation cover by agriculture, forestry, mining, and urban expansion (Casatti et al. 2012; Castro and Polaz 2020), especially in the Atlantic Forest which is the biome that concentrates the greatest diversity of Cnesterodontini while being severely devastated (ICMBio 2018; Castro and Polaz 2020). In the Atlantic Forest, only 11.7% of the vegetation remains in an extremely fragmented landscape, where most fragments are smaller than 50 ha (Ribeiro et al. 2009). Even so, the Atlantic Forest continues to be deforested at impressive rates of 13,053 ha in one year (SOS Mata Atlântica 2021). Therefore, a pessimistic scenario is the most likely future for the diversity of cnesterodontins if effective conservation and restoration measures are not extended to the biome (Lemes et al. 2014; Zwiener et al. 2017), mainly considering its freshwater biodiversity (Azevedo-Santos et al. 2019).

The consensus ranking established corroborates the insertion of the most priority areas in biodiversity hotspots, i.e. Atlantic Forest and Cerrado (Myers et al. 2000), which harbor a high concentration of endemic species and high rates of habitat loss (Myers 1988). Additionally, it is expected that the occurrence of the largest numbers of endangered cnesterodontins is really in the Atlantic Forest, since fish species that inhabit more modestly sized aquatic environments have almost invariably restricted geographic distributions (Albert et al. 2020; Castro 2021; Tagliacollo et al. 2021; Appendix A) and are generally dependent on the existence of riparian vegetation for their food, shelter, and breeding sites (ICMBio 2018; Castro and Polaz 2020; Castro 2021). This context has been emphasized in potential locations (see IUCN 2019) in the conservation status assessments of Brazilian freshwater fish species with populations in highly degraded and fragmented landscapes like the Atlantic Forest (ICMBio 2018).

In addition to the aforementioned impacts that also extend to other biomes and watersheds in South America, cnesterodontins are under constant threat from non-native species, mainly due to the release of aquarium fish (Magalhães and Jacobi 2017). For example, in the Iguassu River basin (see Mezzaroba et al. 2021) and watersheds from the São Francisco, Upper Parana, Central, and North Coastal basins (see Magalhães and Jacobi 2013), endangered species can decline to the increasing percentages of non-native ornamental fish species (Magalhães and Jacobi 2013; Reis et al. 2020; Mezzaroba et al. 2021). More worrying due to their phylogenetic proximity, some poeciliids—species of *Poecilia* Bloch & Schneider, 1801 and *Xiphophorus* Heckel, 1848—are highly appreciated in the ornamental trade, causing serious negative impacts such as changes in the structure of the native fish assemblages, biotic homogenization (Magalhães and Jacobi 2017), and they are linked to reduction in zooplankton, damselfly/dragonfly populations, and eggs and larval stages of several amphibian species (Stockwell and Henkanaththegedara 2011). Native and non-native poeciliids can respond similarly to variations in environmental conditions (Araújo et al. 2009) hence inter-specific competition for shelter, food, and parturition sites could arise between non-native poeciliids and cnesterodontins, representing dangerous threats to the latter (Magalhães and Jacobi 2017; Ganassin et al. 2020).

In conclusion, our findings point that the priority ranking of areas established for the conservation of cnesterodontins in South America is a useful approach to integrate biogeographical, phylogenetic, and conservation status criteria, which were maximized by the implementation of complementarity (Tumini et al. 2019). Herein we have established a ranking with extensive biogeographic areas that can support high species richness and phylogenetic diversity including populations of endangered/threatened and undescribed species. Our results may enrich efforts to effectively manage and conserve several other small-sized freshwater fishes (see Tagliacollo et al. 2021), mainly in streams of the Brazilian coastal river basins and Upper Parana, Iguassu, and Tocantins-Araguaia ecoregions, where the endangered or threatened species of the tribe studied are located. For example, the findings may be useful to identify a greater number of Key Biodiversity Areas, which must support viable populations of organisms that are considered key parts for the maintenance of local and/or regional biodiversity (Eken et al. 2004). Some of these areas are being selected as a priority for small-size freshwater fishes, including

some populations of Cnesterodontini (see Frota et al. 2021). Although there are records of threatened cnesterodontins in some protected areas (ICMBio 2018), a scenario of legal unprotection for some of these species that are rare and geographically restricted is evident (e.g., Frota et al. 2021), as well as political and climate uncertainties about the maintenance (e.g., Alves et al. 2019) and effectiveness (e.g., Lemes et al. 2014; Zwiener et al. 2017; Azevedo-Santos et al. 2019; Tagliacollo et al. 2021) of these protected areas in the future. We hope that our analysis helps establish more effective directions in the conservation and delimitation of new protected areas harboring high richness, phylogenetic diversity, and endemism for other threatened populations of small-sized freshwater fishes, especially in the Atlantic Forest biome and considering the deleterious effects of non-native poeciliids.

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3 EVOLUTIONARY BIOGEOGRAPHY OF THE FRESHWATER FISH FAMILY ANABLEPIDAE (TELEOSTEI: CYPRINODONTIFORMES), A MARINE-DERIVED NEOTROPICAL LINEAGE

Abstract: Cladistic biogeography is an evolutionary biogeographic approach that infers area relationships by comparing area cladograms derived from different phylogenetic hypotheses. The South American freshwater ichthyofauna is enriched by an extraordinary number of marine-derived lineages, presenting its own phylogenetic and biogeographic patterns. Here, we performed a Brooks Parsimony Analysis (BPA) with the latest phylogenetic proposals for Anablepidae to compare hypotheses about the historical relationships among areas previously recognized based on fish species from the Neotropical region. We found that the area relationships for Anablepidae are in accordance with the pattern evidenced for other

marine-derived lineages. The general area cladogram obtained shows a three-area relationship pattern, where freshwater is the sister-group of Pacific + Atlantic marine areas. Within the freshwater clade, Southern Brazil + Uruguay River basin and Northwestern Argentina +Midwestern Argentina form two clades. Vicariance, dispersal, and extinction events related to Miocene and Quaternary marine transgressions and ancient connections between the Iguaçu and Upper Uruguay river basins supported the historical relationships among areas proposed here. Our results may be applied to patterns shown by other marine-derived lineages, as well as other freshwater organisms not necessarily having marine origins.

Keywords: Area cladogram. Components. Historical biogeography. Miocene. Neotropical region. Quaternary

BIOGEOGRAFIA EVOLUTIVA DA FAMÍLIA DE PEIXES DE ÁGUA DOCE ANABLEPIDAE (TELEOSTEI: CYPRINODONTIFORMES), UMA LINHAGEM NEOTROPICAL DE ORIGEM MARINHA

Resumo: A biogeografia cladística é uma abordagem biogeográfica evolutiva que infere relações entre áreas comparando cladogramas de áreas derivados de diferentes hipóteses filogenéticas. A ictiofauna de água doce da América do Sul é enriquecida por um número extraordinário de linhagens de origem marinha, apresentando seus próprios padrões filogenéticos e biogeográficos. Aqui, realizamos uma Análise de Parcimônia de Brooks (BPA) com as últimas propostas filogenéticas para Anablepidae para comparar hipóteses sobre as relações históricas entre áreas previamente reconhecidas com base em espécies de peixes da região Neotropical. Descobrimos

que as relações de área para Anablepididae estão de acordo com o padrão evidenciado para outras linhagens de origem marinha. O cladograma geral da área obtido mostra um padrão de relacionamento de três áreas, onde a água doce é o grupo-irmão das áreas marinhas do Pacífico e do Atlântico. Dentro do clado de água doce, o sul do Brasil + a bacia do rio Uruguai e o noroeste da Argentina + o centro-oeste da Argentina formam dois clados. Eventos de vicariância, dispersão e extinção relacionados às transgressões marinhas do Mioceno e Quaternário, e conexões antigas entre as bacias dos rios Iguaçu e Alto Uruguai apoiaram as relações históricas entre as áreas aqui propostas. Nossos resultados podem ser aplicados aos padrões mostrados por outras linhagens de origem marinha, bem como por outros organismos de água doce que não têm necessariamente origem marinha.

Palavras-chave: Biogeografia histórica. Cladograma de área. Componentes. Mioceno. Quaternário. Região Neotropical.

3.1 Introduction

Cladistic biogeography is based on the premise that there is a clear correspondence between the phylogenetic relationships of the taxa with their distribution patterns and geological history (Nihei 2016). This evolutionary biogeographic approach aims to infer past area relationships (Platnick and Nelson 1978; Rosen 1978; Wiley 1988a; Morrone 2009; Parenti and Ebach 2009), assuming an association between the history of organisms and the history of the planet. This fact suggests that general patterns may be recognized by comparing area cladograms derived from different phylogenetic hypotheses with taxa existing in a given area (Morrone 2005; Parenti and

Ebach 2009).

Cladistic biogeographic methods are divided into event-based and pattern-based methods (Van Veller and Brooks 2001). Event-based methods assume explicit models for the processes that would have affected the history of one or more taxa, specifying the types of events (vicariance, dispersal, and/or extinction) that would have generated the geographic distributions (Sanmartín 2007). On the other hand, pattern-based methods seek to recognize general patterns of relationship between areas and only then attempt to infer the processes (vicariance, dispersal, and/or extinction) that would have commonly affected the history of taxa in these areas (Nihei 2016). In this case, if a historical interpretation is being applied, the specific biogeographic processes of the taxa optimized in the cladogram can be inferred by synapomorphies as vicariance events, parallelisms as dispersal events, and reversals as extinction events (Morrone 2009).

With around 5,160 species, the South American ichthyofauna is very rich (Reis et al. 2016), including an extraordinary number of marine-derived lineages, namely those composed of species endemic to continental freshwater that evolved from clades ancestrally distributed in marine and estuarine environments (Bloom and Lovejoy 2017). Biotic assembly of marine-derived lineages results from shared phylogenetic and biogeographic patterns among these taxa (Lovejoy et al. 2006; Bloom and Lovejoy 2011, 2017). The most recent discussion on the origins of marine-derived lineages of South American freshwater taxa was based on an ancestral character reconstruction, without invoking any mechanisms (dispersal or vicariance) of divergence (Bloom and Lovejoy 2017). A three-area relationship pattern – freshwater lineages sister to Pacific/Atlantic taxon pairs – suggests that their biotic assembly may be attributed to certain paleogeographic events rather than opportunistic invasions of freshwater environments (Bloom and Lovejoy 2011, 2017). There is still, however, a shortage of data on the freshwater area relationships based on marine-derived lineages.

Anablepidae, currently with 19 living species, are endemic to the Neotropical region and consist of three living genera: *Oxyzygonectes* Fowler, 1916 (monotypic), *Anableps* Scopoli, 1777 (three species) and *Jenynsia* Günther, 1866 (15 species), commonly known as white-eye,

four-eyed fishes, and one-sided livebearers, respectively (Parenti 1981). Fossils are also known for this family, with three distinct monotypic genera reported from the Miocene: *Sanjuanableps calingasta* Bogan, Contreras, Agnolin, Tomassini & Peralta, 2018 from Western Argentina (Bogan et al. 2018), and *Sachajenynsia pacha* Sferco, Herbst, Aguilera & Mirande, 2017 and *Tucumanableps cionei* Sferco, Herbst, Aguilera & Mirande, 2017 from Northwestern Argentina (Sferco et al. 2018).

According to Amorim and Costa (2018), Anablepididae appeared in the Oligocene (29.6 Ma), with the freshwater lineage emerging in the Miocene (12.5 Ma) and showing high diversification in the Early Pliocene (4.6 Ma). The last phylogenetic hypotheses for members of Anablepididae (Amorim 2018; Amorim and Costa 2018, 2019; Bogan et al. 2018; Sferco et al. 2018; Aguilera et al. 2019) point to the known three-area relationship pattern of marine-derived lineages. In fact, *Jenynsia* species represent a freshwater lineage with marine ancestors (Amorim and Costa 2018, 2019) and with species that have returned to brackish (Amorim 2018; Amorim and Costa 2018, 2019) and marine (Calviño and Alonso 2016) environments.

Recently, the evolutionary biogeography of this family has been improved with the application of event-based methods (Amorim and Costa 2018, 2019), as well as the identification of areas of endemism (Frota et al. 2019). The diversification scenario of Anablepididae has not been tested in the context of area relationships (pattern-based method). Especially applied to marine-derived lineages, here we compare hypotheses about the historical relationships among areas of endemism previously recognized for fish species in the Neotropical region. Our study addresses two major questions: (1) what are the patterns of area relationships recovered based on the distributional and phylogenetic information compiled for Anablepididae? (2) Is it possible to identify putative vicariance, dispersal and extinction events associated with the patterns of area relationships and species distribution analyzed? To this end, we performed a cladistic biogeographic analysis based on the more recent phylogenies and distributional records of fossils and living species of Anablepididae.

3.2 Materials and methods

3.2.1 Areas and records

Abell et al. (2008) presented a map of global biogeographic regionalization of freshwater ecoregions, based on distributional data of freshwater fish species and incorporating their main ecological and evolutionary patterns. Based on the current geographical distribution and records of Anablepidae species, we considered appropriate to use some biogeographical units of Abell et al. (2008), adapted to the areas of endemism identified by Frota et al. (2019) and the main conclusions of Amorim and Costa (2019) for *Jenynsia* species. Each of the areas analyzed has at least two species (living or fossil). All species are endemic to one or two areas, with the exception of *Jenynsia lineata* (Jenyns, 1842) which is widespread and probably represents a species complex (Amorim 2018). Thus, we selected the following eight areas (Figure 3.1):

A: Pacific – corresponds to the Pacific coast of Central America. In this area, there are two living species: *Oxyzygonectes dovii* (Günther, 1866) and *Anableps doweii* Gill, 1861.

B: Northern South America – corresponds to the Atlantic coast of northern South America defined as an area of endemism by Frota et al. (2019). In this area, there are two living species: *Anableps anableps* (Linnaeus, 1758) and *A. microlepis* Müller & Troschel, 1844.

C: Northwestern Argentina – corresponds to the limits of ecoregions 339 (Mar Chiquita – Salinas Grandes), 342 (Chaco), and 345 (Lower Parana) of Abell et al. (2008) in Northwestern Argentina. This area was defined as an area of endemism by Frota et al. (2019) and presents the occurrence of two fossil species (*Sachajenynsia pacha* and *Tucumanableps cionei*) and seven living species: *Jenynsia alternimaculata* (Fowler, 1940), *J. lineata*, *J. luxata* Aguilera, Mirande, Calviño & Lobo, 2013, *J. maculata* Regan, 1906, *J. obscura* (Weyenbergh, 1877), *J. sulfurica* Aguilera, Terán, Mirande, Alonso, Rometsch, Meyer & Torres-Dowdall, 2019, and *J. tucumana* Aguilera & Mirande, 2005.

D: Midwestern Argentina – corresponds to the limits of ecoregions 339 (Mar Chiquita – Salinas Grandes) and 340 (Cuyan – Desaguadero) of Abell et al. (2008) in Midwestern Argentina. This area presents the occurrence of one fossil species (*Sanjuanableps calingasta*) and two living

species: *Jenynsia lineata* and *J. obscura*.

E: Southern Brazil – corresponds to the limits of ecoregions 331 (Southeastern Mata Atlantica), 334 (Laguna dos Patos), and 335 (Tramandai – Mampituba) of Abell et al. (2008) in Southern Brazil. This area was defined as an area of endemism by Frota et al. (2019) and presents the occurrence of six living species: *Jenynsia eirmostigma* Ghedotti & Weitzman, 1995, *J. lineata*, *J. onca* Lucinda, Reis & Quevedo, 2002, *J. sanctaecatarinae* Ghedotti & Weitzman, 1996, *J. unitaenia* Ghedotti & Weitzman, 1995, and *J. weitzmani* Ghedotti, Meisner & Lucinda, 2001.

F: Uruguay River basin – corresponds to the entire Uruguay River basin located in Brazil and Uruguay comprising ecoregions 332 (Lower Uruguay) and 333 (Upper Uruguay) of Abell et al. (2008). In this area, there are two living species: *Jenynsia eirmostigma* and *J. onca*.

G: Iguaçu River basin – corresponds to the entire Iguaçu River basin in Brazil situated in ecoregion 346 (Iguassu) of Abell et al. (2008). In this area, there are two living species: *Jenynsia diphyes* Lucinda, Ghedotti & da Graça, 2006 and *J. eigenmanni* (Haseman, 1911).

H: Southeastern Brazil – corresponds to the coastal regions (brackish environments) within the limits of the ecoregions 329 (Paraíba do Sul) and 352 (Fluminense) of Abell et al. (2008) in Southeastern Brazil. In this area, there are two living species: *Jenynsia darwini* Amorim, 2018 and *J. lineata*.

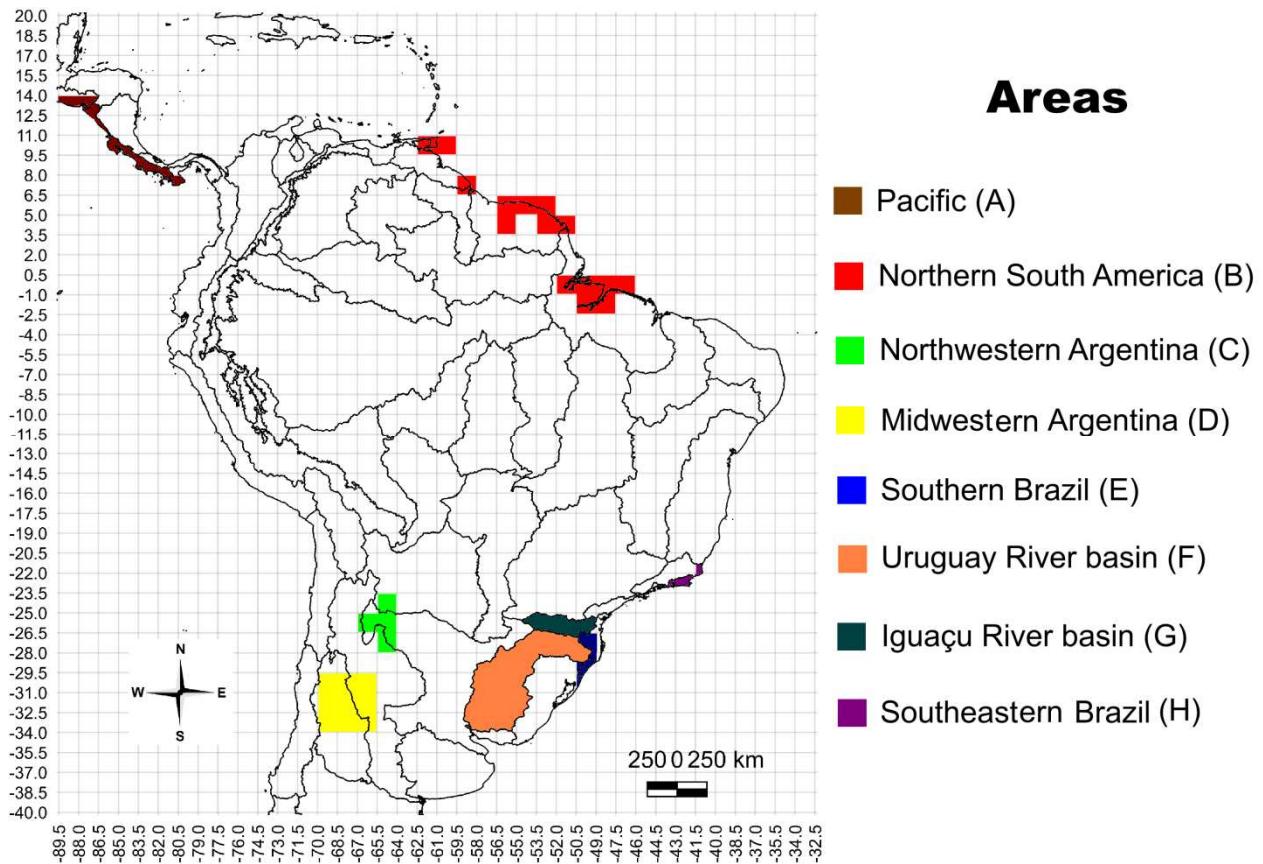


Figure 3.1 Map showing the eight areas selected in the Neotropical region overlaid with the ecoregions defined by Abell et al. (2008). These areas were selected based on Amorim and Costa (2019) and Frota et al. (2019). For details see material and methods section.

3.2.2 Biogeographic analysis

To establish the historical relationships between the selected areas and identify the associated events, we run a Brooks Parsimony Analysis (BPA). Using this procedure, an area cladogram was established to show the general pattern of area relationships (Brooks et al. 2001). BPA was originally formulated by Brooks (1981) to ascertain the historical parasite-host relationships. The idea of the method is that a species of parasites can be associated with their hosts as a result of two distinct events: (1) the ancestor of the parasite species was associated with the ancestor of its host, resulting in association by descent, or (2) the parasite species evolved with one host, subsequently passing to another, resulting in an association by colonization (Brooks 1988). This analytical method was introduced in historical biogeography by Wiley (1988a, b) who disseminated its use for studies of the taxon-area association. BPA uses data from phylogenetic

hypotheses in a parsimony analysis to reconstruct either biogeographic relationships of areas or co-evolutionary relationships among species inhabiting the same area. The distribution areas are considered analogous to the hosts and the taxa that occupy these areas are considered analogous to the parasite species. Associations by descent are understood as a direct result of vicariance events responsible for cladogenesis, while associations by colonization represent dispersal events between areas (Wiley 1988a, b; Brooks 1990).

Although the use of only one group represents a shortcoming, the aim of our study is to compare the area relationships with previously established patterns for other marine-derived lineages. Thus, biogeographic analyses based on the distribution of South American monophyletic groups represent operative tests directed to corroborate or refute area relationship hypotheses (de Carvalho et al. 2013; da Silva and Noll 2015). BPA was employed following its original proposition (Brooks 1981; Brooks et al. 2001), based on the more recent phylogenetic relationships of Anablepidae proposed by Aguilera et al. (2019; Figure 3.2a) and Amorim and Costa (2019; Figure 3.2b). The primary BPA involved constructing an individual area cladogram for the taxa by replacing the species names in the phylogeny with their geographical distribution areas (Figure 3.2). The internal and terminal relevant nodes were numbered in both cladograms (Figure 3.2a,b) for later representation in the data matrices that were analyzed using a parsimony algorithm. Based on them, we made the presence-absence matrices of areas (rows) vs cladogram components (columns) evidenced in Table 3.1.

A hypothetical ancestral area (root) with a total absence of species was added to the data matrices (Table 3.1a,b) to allow grouping by presence rather than the absence of taxa, which was employed to root the general area cladogram (Crisci et al. 2003). The matrices were run with a parsimony analysis using TNT (Goloboff et al. 2008) through the WINCLADA interface (Nixon 2002), with the heuristic search option (tree bisection and reconnection, 100 replications) to obtain the most parsimonious trees and the strict consensus cladograms. Finally, synapomorphies were interpreted as vicariance events, parallelisms as dispersal events, and reversals as extinction events.

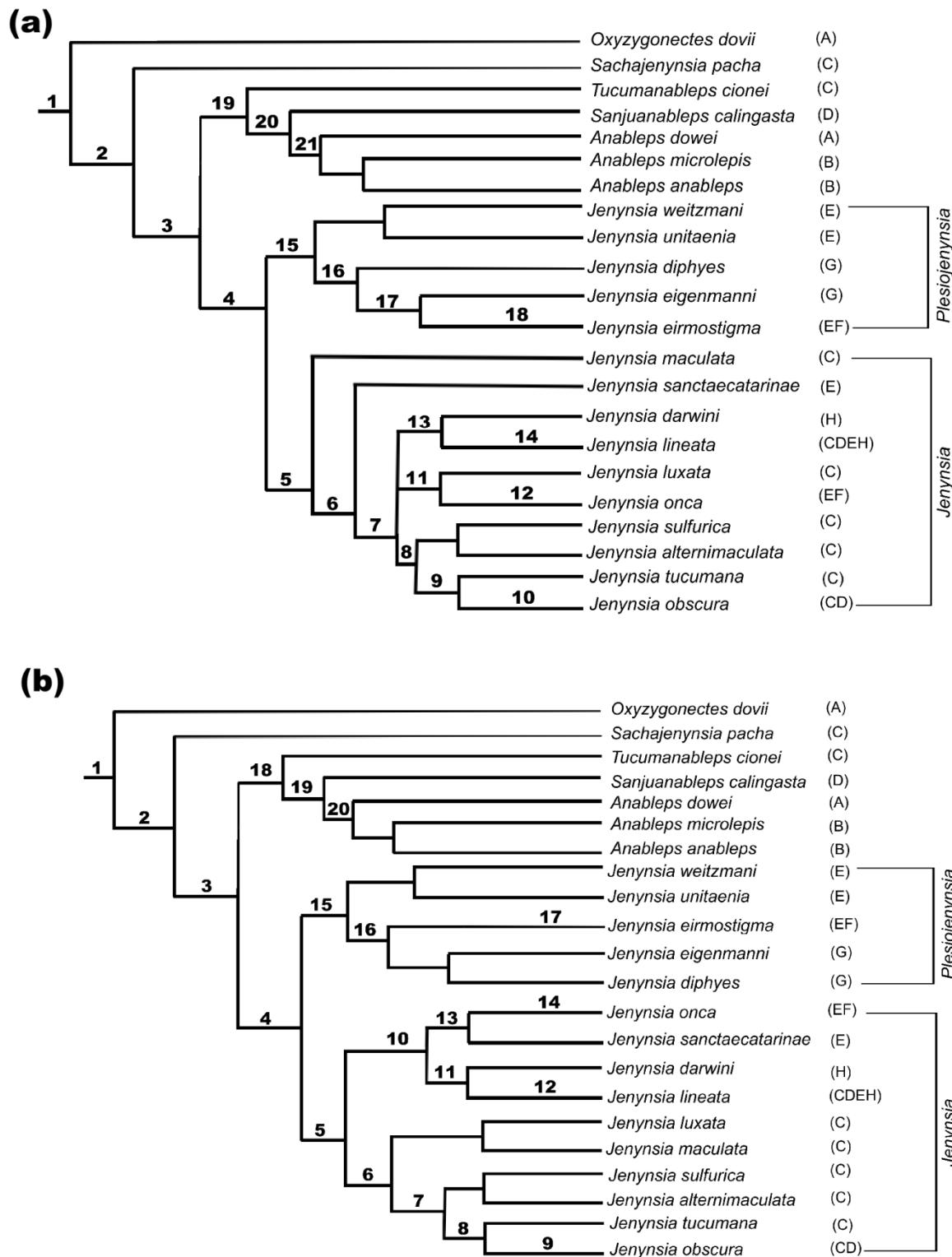


Figure 3.2 Area cladogram obtained based on the phylogenetic trees published by (a) Aguilera et al. (2019) and (b) Amorim and Costa (2019). Components are numbered from 1 to 21 and from 1 to 20, respectively. Areas are represented by the letters A to H according to Figure 3.1 and description in the material and methods section.

Table 3.1 Data matrices of the areas vs cladogram components used in the Brooks Parsimony Analysis (BPA): **(a)** phylogenetic tree of Aguilera et al. (2019); **(b)** phylogenetic tree of Amorim and Costa (2019). Areas A to H are in accordance with the description in materials and methods and Figure 3.1.

(a)

Areas	Components																				
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
Root	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
A	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
B	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1
C	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	0	0	0	1	0	0
D	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	0	0	0	1	1	0
E	1	1	1	1	1	1	1	0	0	0	1	1	1	1	1	1	1	1	0	0	0
F	1	1	1	1	1	1	1	0	0	0	1	1	0	0	1	1	1	1	0	0	0
G	1	1	1	1	0	0	0	0	0	0	0	0	0	0	1	1	1	0	0	0	0
H	1	1	1	1	1	1	1	0	0	0	0	0	1	1	0	0	0	0	0	0	0

(b)

3.3 Results

The area cladograms obtained from the phylogenetic trees of Aguilera et al. (2019) and Amorim and Costa (2019) had 21 and 20 components, respectively (Figure 3.2). Based on the presence-absence matrices of areas vs components cladogram (Table 3.1), four equally most parsimonious trees were obtained (two for each analysis). Notwithstanding both consensus cladograms showed the same relationships, we have chosen the one from Aguilera et al. (2019) for presenting a greater number of synapomorphies supporting the clades.

For the phylogenetic tree of Aguilera et al. (2019), BPA resulted in two most parsimonious general area cladograms (Figure 3.3a, b) with 29 steps, a consistency index of 0.72, and a retention index of 0.77. Both cladograms evidenced the same basal synapomorphies for all areas (components 1, 2, and 3; Table 3.2) and the monophyly between Pacific and northern South America, supported by one synapomorphy (component 21; Table 3.2). The parallelisms in this clade are also the same (components 19 and 20; Table 3.2). The strict consensus cladogram (Figure 3.3c), with 34 steps, a consistency index of 0.61, and a retention index of 0.63 shows the three-area relationship pattern: freshwater is the sister-group of Pacific + Atlantic.

Differences between both most parsimonious cladograms are for the area relationships in the freshwater clade. This clade was supported exclusively by synapomorphies represented by the subgenus *Jenynsia* components (components 4, 5, 6, 7, 13, and 14; Table 3.2) in the strict consensus cladogram with reversals in the Iguaçu and Uruguay river basins (Figure 3.3c; Table 3.2). However, the differences in this clade are due especially to the subgenus *Plesiojenynsia* components (components 15, 16, and 17; Table 3.2). These components are decisive for the positioning of the Iguaçu River basin and Southeastern Brazil.

In the first hypothesis (Figure 3.3a), the Iguaçu River basin is the sister-group to the other freshwater areas. These latter areas are supported by five synapomorphies (components 5, 6, 7, 13, and 14), all are *Jenynsia* subgenus components (Table 3.2). In this hypothesis, the subgenus *Plesiojenynsia* components (15, 16, and 17) are parallelisms between the Iguaçu River basin and the clade composed by Southern Brazil + Uruguay River basin (Figure 3.3a; Table 3.2). In the second hypothesis, the Iguaçu River basin is situated in a clade with areas restricted to the

southernmost part of South America, supported as the sister-group of the clade composed by Southern Brazil + Uruguay River basin (Figure3.3b; Table 3.2). In this hypothesis, the interpretations are opposite since the synapomorphies are subgenus *Plesiojenynsia* components, and the parallelisms are subgenus *Jenynsia* components (Figure3.3b). For this last hypothesis, three reversals are necessary for the Iguaçu River basin (components 5, 6, and 7; Figure 3.3b; Table 3.2).

The positioning of Southeastern Brazil is also crucial to the establishment of historical relationships among areas in the freshwater clade. If components 5, 6, 7, 13 and 14 are really synapomorphies of the clade with all freshwater areas, except Iguaçu River basin, then Southeastern Brazil is the sister-group of a clade formed by two monophyletic groups (Southern Brazil + Uruguay River basin and Northwestern Argentina + Midwestern Argentina), which is supported by one synapomorphy (component 11; Figure3.3a; Table 3.2). For this scenario, in addition to the parallelisms already mentioned in the first hypothesis for the Iguaçu River basin, two reversals (components 13 and 14) are necessary for the Uruguay River basin and one reversal (component 11) for Midwestern Argentina (Figure3.3a; Table 3.2). However, if the subgenus *Plesiojenynsia* components (15, 16, and 17) are really synapomorphies, Southeastern Brazil is the sister-group to Northwestern Argentina + Midwestern Argentina with two parallelisms (components 13 and 14) also present in Southern Brazil (Figure3.3b; Table 3.2).

Southern Brazil and Uruguay River basin form a monophyletic clade in the strict consensus cladogram (Figure3.3c) supported by two synapomorphies (component 12 in subgenus *Jenynsia* clade, and component 18 in subgenus *Plesiojenynsia* clade; Table 3.2). Northwestern Argentina and Midwestern Argentina form another clade in the strict consensus cladogram (Figure3.3c) supported by three synapomorphies (components 8, 9, and 10 in subgenus *Jenynsia* clade; Table 3.2). Lastly, the areas in Argentina have the parallelism of component 19 that is also present in the Pacific + northern South America (Atlantic) clade (Figure3.3c; Table 3.2).

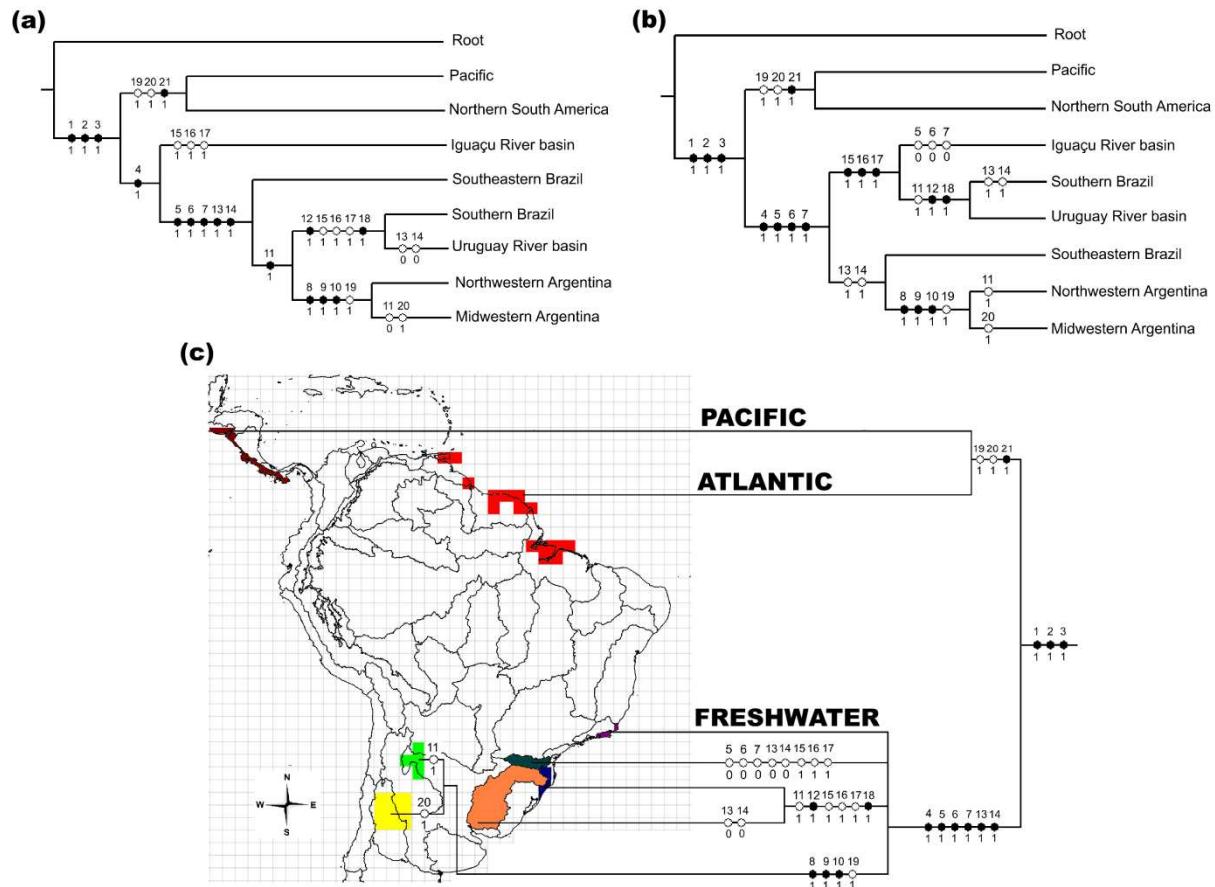


Figure 3.3 Cladograms showing the results of Brooks Parsimony Analysis and the historical relationships among the selected areas using *Anablepidae* as a case study. The two most parsimonious general area cladograms are represented by the letters **(a)** and **(b)**. Note that the positions of the Iguazu River basin and Southeastern Brazil are decisive for biogeographic interpretations. The strict consensus cladogram is represented by the letter **(c)** and highlights the three-area relationship pattern between Pacific, Atlantic, and freshwater, and the monophyly of Southern Brazil + Uruguay River basin, and Northwestern Argentina + Midwestern Argentina. In the cladograms, the black and white circles are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

Table 3.2 Summary of the Brooks Parsimony Analysis (BPA) results highlighting all possibilities of the components in the phylogenetic tree published by Aguilera et al. (2019) to represent vicariance (synapomorphy), dispersal (parallelism), and/or extinction (reversal) events in the selected biogeographic areas. Cladogram (a), Cladogram (b), and Consensus represent Figure 3.3a, Figure 3.3b, and Figure 3.3c, respectively. Asterisks (*) indicate extinctions in the areas that contain them.

Components	Ancestor	Cladogram (a)		Cladogram (b)		Consensus	
		Event	Areas	Event	Areas	Event	Areas
1	Anablepidae	Vicariance	All areas	Vicariance	All areas	Vicariance	All areas
2	<i>Sachajenynsia</i> , <i>Tucumanableps</i> , <i>Sanjuanableps</i> , <i>Anableps</i> , <i>Plesiojenynsia</i> and <i>Jenynsia</i>	Vicariance	All areas	Vicariance	All areas	Vicariance	All areas
3	<i>Tucumanableps</i> , <i>Sanjuanableps</i> , <i>Anableps</i> , <i>Plesiojenynsia</i> and <i>Jenynsia</i>	Vicariance	All areas	Vicariance	All areas	Vicariance	All areas
4	<i>Plesiojenynsia</i> and	Vicariance	Freshwater	Vicariance	Freshwater	Vicariance	Freshwater

	<i>Jenynsia</i>						
5	<i>Jenynsia</i>	Vicariance	Freshwater, except Iguaçu River basin	Vicariance; Extinction*	Freshwater; Iguaçu River basin*	Vicariance; Extinction*	Freshwater; Iguaçu River basin*
6	<i>Jenynsia</i>	Vicariance	Freshwater, except Iguaçu River basin	Vicariance; Extinction*	Freshwater; Iguaçu River basin*	Vicariance; Extinction*	Freshwater; Iguaçu River basin*
7	<i>Jenynsia</i>	Vicariance	Freshwater, except Iguaçu River basin	Vicariance; Extinction*	Freshwater; Iguaçu River basin*	Vicariance; Extinction*	Freshwater; Iguaçu River basin*
8	<i>Jenynsia</i>	Vicariance	Northwestern and Midwestern Argentina	Vicariance	Northwestern and Midwestern Argentina	Vicariance	Northwestern and Midwestern Argentina
9	<i>Jenynsia</i>	Vicariance	Northwestern and Midwestern Argentina	Vicariance	Northwestern and Midwestern Argentina	Vicariance	Northwestern and Midwestern Argentina
10	<i>Jenynsia</i>	Vicariance	Northwestern and Midwestern Argentina	Vicariance	Northwestern and Midwestern Argentina	Vicariance	Northwestern and Midwestern Argentina

11	<i>Jenynsia</i>	Vicariance; Extinction*	Southern Brazil, Uruguay River basin, Northwestern and Midwestern* Argentina	Dispersal	Southern Brazil, Uruguay River basin and Northwestern Argentina	Dispersal	Southern Brazil, Uruguay River basin and Northwestern Argentina
12	<i>Jenynsia</i>	Vicariance	Southern Brazil and Uruguay River basin	Vicariance	Southern Brazil and Uruguay River basin	Vicariance	Southern Brazil and Uruguay River basin
13	<i>Jenynsia</i>	Vicariance; Extinction*	Freshwater, except Iguaçu River basin; Uruguay River basin*	Dispersal	Southern and Southeastern Brazil and Northwestern and Midwestern Argentina	Vicariance; Extinction*	Freshwater; Uruguay and Iguaçu River basins*
14	<i>Jenynsia</i>	Vicariance; Extinction*	Freshwater, except Iguaçu River basin; Uruguay River basin*	Dispersal	Southern and Southeastern Brazil and Northwestern and Midwestern Argentina	Vicariance; Extinction*	Freshwater; Uruguay and Iguaçu River basins*

15	<i>Plesiojenynsia</i>	Dispersal	Uruguay and Iguaçu River basins and Southern Brazil	Vicariance	Uruguay and Iguaçu River basins and Southern Brazil	Dispersal	Uruguay and Iguaçu River basins and Southern Brazil
16	<i>Plesiojenynsia</i>	Dispersal	Uruguay and Iguaçu River basins and Southern Brazil	Vicariance	Uruguay and Iguaçu River basins and Southern Brazil	Dispersal	Uruguay and Iguaçu River basins and Southern Brazil
17	<i>Plesiojenynsia</i>	Dispersal	Uruguay and Iguaçu River basins and Southern Brazil	Vicariance	Uruguay and Iguaçu River basins and Southern Brazil	Dispersal	Uruguay and Iguaçu River basins and Southern Brazil
18	<i>Plesiojenynsia</i>	Vicariance	Uruguay River basin and Southern Brazil	Vicariance	Uruguay River basin and Southern Brazil	Vicariance	Uruguay River basin and Southern Brazil
19	<i>Tucumanableps,</i> <i>Sanjuanableps</i> and <i>Anableps</i>	Dispersal	Pacific, Northern South America, Northwestern and Midwestern Argentina	Dispersal	Pacific, Northern South America, Northwestern and Midwestern Argentina	Dispersal	Pacific, Northern South America, Northwestern and Midwestern Argentina

20	<i>Sanjuanableps</i> and <i>Anableps</i>	Dispersal	Pacific, Northern South America and Midwestern Argentina	Dispersal	Pacific, Northern South America and Midwestern Argentina	Dispersal	Pacific, Northern South America and Midwestern Argentina
21	<i>Anableps</i>	Vicariance	Pacific and Northern South America	Vicariance	Pacific and Northern South America	Vicariance	Pacific and Northern South America

3.4 Discussion

We found that the area relationships for Anablepidae are in accordance with the three-area relationship pattern of other marine-derived lineages. The basal synapomorphies (vicariance events) found for the area relationships proposed here, as well as other lineages with marine ancestors (Bloom and Lovejoy 2017), show that the ancestor of a marine-derived lineage could have been distributed along the Pacific and Atlantic coasts (Lovejoy et al. 2006; Bloom and Lovejoy 2011, 2017). This scenario is especially conclusive to the ancestral reconstruction of Anablepidae (see Amorim and Costa 2018). Our results point that diversification of many marine-derived lineages should be related to the main geological vicariance events in South America, such as the rise of Panamanian Isthmus and Miocene marine transgressions (Lovejoy et al. 2006; Bloom and Lovejoy 2011).

Ocean-level oscillations that have shaped marine transgressions and regressions across continental lands are among the major vicariance events that might have provided allopatric speciation in the South American ichthyofauna (Hubert and Renno 2006). These events can explain our basal synapomorphies (vicariances) for all areas and for the freshwater clade (subgenera *Jenynsia* and *Plesiojenynsia*). The capture of a Pacific marine fauna by the orogeny of the Andes (Brooks et al. 1981; Domning 1982; Grabert 1984) or the isolation of a marine fauna via incursions from the Caribbean into the upper Amazon (Nuttall 1990; Webb 1995; Lovejoy 1997; Lovejoy et al. 1998, 2006) are vicariance hypotheses proposed for marine-derived lineages origins (Bloom and Lovejoy 2011, 2017). This is the case of Anablepidae, which currently show confined populations close to these sources, including the Paraná River mouth, the Amazon River mouth, and the Caribbean (Amorim and Costa 2018).

The Miocene marine incursions in megazones of the Pebas lake/wetland system (Bloom and Lovejoy 2011; Cooke et al. 2012) were the most recent events of marine ichthyofauna transitions to freshwater, with strong evidence of the evolution of lineages that entered in South America (Bloom and Lovejoy 2017). This scenario occurred with the ancestor of the subgenera *Jenynsia* and *Plesiojenynsia* (Amorim and Costa 2018). The megazone of Pebas Lake consisted of various environments, including lakes, swamps, deltas, and estuaries (Hoorn et al. 2010), which are

environments that promote marine lineage diversification (Bloom and Lovejoy 2017). The absence of Anablepidae from the Amazon River basin may be linked to the fact that the current shape and basin size of the Amazon River postdate the marine incursions that flooded the megazones of the wetland system (Figueiredo et al. 2009). Thus, those wetland areas of transition between marine and freshwater environments ceased to exist in the Amazon (Bloom and Lovejoy 2017).

Our results corroborate the proposal that the most recent common ancestor of the subgenera *Jenynsia* and *Plesiojenynsia* must have invaded the Paranean Sea with the diversification related to the regression of that sea (Amorim and Costa 2018, 2019). However, the fossil records of Anablepidae reported for Northwestern and Midwestern Argentina represent taxa that are not sister to the *Jenynsia* and *Plesiojenynsia* lineages (see phylogenies in Bogan et al. 2018; Sferco et al. 2018; Aguilera et al. 2019). This fact, together with the parallelisms of components 19 and 20, could represent dispersals of ancestors not linked to the Paranean Sea (Bogan et al. 2018). The capture of a Pacific marine fauna by the orogeny of the Andes could be a plausible explanation in this case.

In the Middle Miocene (15-11 Ma), successive Atlantic marine transgressions occupied a wide region of current Southern Brazil, extending through Southern Bolivia and through a large area in northern Argentina and Uruguay (Hulka et al. 2006). These marine transgressions caused alternating periods of isolation and connection, primarily between the areas Southern Brazil and Uruguay River basin, which favored both biotic interchange and isolation (Costa 2010). Similarly, two periods with different oceanic levels occurred in Southern South America, the Laguna Paiva Transgression and the Paraná Formation Transgression, which flooded the entire set of lands of the Pampa and Chaco plains, as well as Northwestern Argentina, at different times in the Miocene (Brea and Zucol 2011). Thus, the ancestors of the subgenera *Jenynsia* and *Plesiojenynsia* likely experienced vicariance during a period of marine regression in which the *Jenynsia* ancestor was restricted to the vicinity of Northwestern and Midwestern Argentina and coastal plains to Southeastern Brazil. Meanwhile, the *Plesiojenynsia* ancestor was restricted to uplands on the boundaries of Southern Brazil and the Uruguay and Iguaçu river basins (Amorim and

Costa2019).

Within the freshwater clade, the area diversification scenario is related to ancient connections between the Iguaçu and Upper Uruguay river basins, sea level variation along the Pliocene in South American Atlantic coast, and the decrease of the Paranean Sea along the Pliocene and Pleistocene (Amorim and Costa 2019). For this clade, the results corroborate other studies that have shown the relevance of ocean-level oscillations in the demographic history and distributional patterns of the ichthyofauna of coastal drainage basins (Ribeiro 2006; Pereira et al. 2013; Roxo et al. 2014; Bruno et al. 2015; Thomaz et al. 2015; Tschá et al. 2017a, b). Although the precise ages of events associated with ocean-level oscillations remain poorly established (Angulo et al. 2006), at least three main cycles of marine regressions and transgressions occurred during the Quaternary (Martin et al. 1996; Woodburne 2010), reaching the Southern Brazil and Uruguay River basin boundaries. These area connections in Southern Brazil were fundamental for the diversification of Anablepidae species that inhabit freshwater and brackish environments (Amorim and Costa 2019). Moreover, in the freshwater clade, Southern Brazil + Uruguay River basin and Northwestern Argentina +Midwestern Argentina form two clades. This result is consistent with other studies that established relationships between the ecoregions of Abell et al. (2008) using other freshwater organisms (e.g. Tumini et al. 2018). Thus, our findings can also be tested outside the context of the marine-derived fish lineages.

Extinction events (reversals) occurred in all the area cladograms found. We are not convinced that the components of the subgenus *Plesiojenynsia* can represent synapomorphies or parallelisms. However, it would be more prudent to establish that the *Plesiojenynsia* ancestor was restricted to uplands on the boundaries of the areas Southern Brazil and Uruguay River basin, and reached the Iguaçu River basin by an ancient connection between these areas (Amorim and Costa 2019). Thus, the subgenus *Plesiojenynsia* components might represent dispersal events to the uplands, and the extinctions in that scenario would be for components 13 and 14 in the Uruguay River basin (Figure3.3a). These latter components correspond to the ancestors of species that returned to the brackish environment (Amorim and Costa 2018), which would justify their extinctions in the Uruguay River basin. Therefore, we are more likely to establish that the

components of the subgenus *Jenynsia* never reached the Iguaçu River basin, which would be necessary for the second area relationship hypothesis (Figure 3.3b).

In conclusion, vicariance, dispersal, and extinction events were evidenced by the cladistic biogeography of the family Anablepidae using a pattern-based method. Overall, all these events allowed that several Anablepidae species are endemic to particular areas, especially in northern South America, Northwestern Argentina, and Southern Brazil (Frota et al. 2019). From the Miocene to the present day, the paleogeological and paleogeographic evolution of South America combined with well-resolved phylogenetic hypotheses from groups of fish explain many questions about the biogeography of marine-derived lineages (Bloom and Lovejoy 2017). The historical relationships among areas here evidenced are congruent with the main geological events that shaped the biogeography of Anablepidae and could be applied to the patterns shown by other marine-derived lineages.

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4 SPATIAL DISTRIBUTION PATTERNS AND PREDICTORS OF FISH BETA-DIVERSITY IN A LARGE DAM-FREE TRIBUTARY FROM A NEOTROPICAL FLOODPLAIN

Abstract: Understanding how and why fish species composition varies between locations through beta-diversity has received increased interest recently. The physical structure, extension, and environmental characteristics along the river basins influence the dispersal of organisms, which ultimately affects metacommunity dynamics and biodiversity patterns. Here, we aimed to identify major breaks delimiting fish assemblages in a large dam-free river basin on its main channel, which is almost pristine in hydrology, and to evaluate the influence of geo-climatic variables in the observed beta-diversity patterns. We used the statistical techniques Species Composition Interpolation (SCI) and Generalized Dissimilarity Modeling (GDM) to analyze beta-diversity patterns based on georeferenced fish records from the Ivaí River basin. Ichthyofauna units were delimited estimating the association between geo-climatic predictors and beta-diversity. We identified three major breaks delimiting fish assemblages in the Ivaí River basin (i.e., the upper, middle, and lower sections). The fish communities were very distinct at the upper and lower sections, while the middle section was recovered as less differentiated. Turnover was the main component contributing to total beta-diversity and dissimilarity in fish species composition was moderately associated with geo-climatic variables. Fish communities from the Ivaí River basin must be associated with niche-based processes and we hypothesize that dispersal processes can be equally significant at the basin scale. The investigations may produce insights on strategies for conservation and restoration of degraded rivers, contributing to the long-term maintenance of fish communities and to the delimitation of new strategic areas for the effective conservation of freshwater biodiversity.

Keywords: Dispersal limitation. Ivaí River basin. Mass effect. Patch dynamics. Species sorting.

PADRÕES DE DISTRIBUIÇÃO ESPACIAL E PREDITORES DA DIVERSIDADE BETA DE PEIXES EM UM GRANDE TRIBUTÁRIO LIVRE DE BARRAGENS DE UMA PLANÍCIE DE INUNDAÇÃO NEOTROPICAL

Resumo: Compreender como e por que a composição das espécies de peixes varia entre os locais por meio da diversidade beta tem recebido um interesse crescente recentemente. A estrutura física, extensão e características ambientais ao longo das bacias hidrográficas influenciam a dispersão dos organismos, o que afeta a dinâmica da metacomunidade e os padrões de biodiversidade. Aqui, objetivamos identificar quebras principais delimitando assembléias de peixes em uma grande bacia hidrográfica livre de barragens em seu canal principal, cuja apresenta hidrologia quase intocada, e avaliar a influência de variáveis geoclimáticas nos padrões de diversidade beta observados. Utilizamos as técnicas estatísticas de Interpolação de Composição de Espécies e Modelagem de Dissimilaridade Generalizada para analisar padrões de diversidade beta baseados em registros de peixes georreferenciados da bacia do rio Ivaí. Unidades ictiofaunísticas foram delimitadas estimando a associação entre preditores geoclimáticos e a diversidade beta. Identificamos três quebras principais que delimitam as assembléias de peixes na bacia do rio Ivaí (ou seja, a parte superior, média e inferior da bacia). As comunidades de peixes foram muito distintas nas seções superior e inferior, enquanto a seção intermediária foi recuperada como menos diferenciada. A substituição de espécies foi o principal componente que contribuiu para a diversidade beta total e a dissimilaridade na composição das espécies de peixes foi moderadamente associada às variáveis geoclimáticas. As comunidades de peixes da bacia do rio Ivaí devem estar associadas a processos baseados em nicho e hipotetizamos que os processos baseados em dispersão podem ser igualmente significativos na escala da bacia. As investigações podem produzir interessantes perspectivas sobre estratégias de conservação e restauração em rios já degradados, contribuindo para a manutenção de longo prazo das comunidades de peixes e para a delimitação de novas áreas estratégicas para a efetiva conservação da biodiversidade de água doce.

Palavras-chave: Bacia do rio Ivaí. Classificação de espécies. Dinâmica de manchas. Efeito de massa. Limitação por dispersão.

4.1 Introduction

For ecologists and biogeographers, how and why species composition varies among sites are some of the most frequent questions. Answers to these questions may point to the mechanisms structuring biological communities (Kraft et al., 2011; Qian & Ricklefs, 2007; Soininen et al., 2007). In this context, beta-diversity is an essential metric to understand regional biodiversity patterns, either testing ecological theories (Baselga, 2010), contributing to conservation planning, or guiding practical management decisions (Socolar et al., 2016). Beta-diversity patterns may be a result of predominant species replacement (i.e., turnover) or species loss and gain (i.e. nestedness) across regions (Baselga, 2010). The percentage of turnover and nestedness components contributing to total beta-diversity may be used as criteria for conservation strategies. For example, a large number of protected areas may be necessary to conserve regional biodiversity if species turnover is the main component, whereas one large protected area comprising a high species richness may be sufficient if nestedness stands out (Baselga, 2010; Socolar et al., 2016; Wright & Reeves, 1992).

Four classical models of metacommunity dynamics have been proposed (species sorting, mass effect, patch dynamics, and neutral model), each defined by the relative influences of dispersal, environmental filtering, habitat selection, habitat disturbance, biotic interactions, and stochastic factors (see Leibold et al., 2004; Tonkin et al., 2018). Environmental filters (i.e., physical and chemical conditions and biological interactions) and dispersal processes are commonly associated with a predominance of turnover in biotic communities (e.g., Soininen et al., 2018; Zbinden & Matthews, 2017), while geographical distance and recolonization-after-extinction events are usually correlated with a predominance of the nestedness component (Baselga, 2010; Dobrovolski et al., 2012; Si et al., 2016; Soininen et al., 2018). Regarding the distribution of aquatic organisms, both niche- and dispersal-based processes are commonly evoked (Heino, Melo, Siqueira, et al., 2015; Tonkin et al., 2018; Zbinden & Matthews, 2017). Although niche-based processes have historically been recognized as the major drivers (e.g., López-Delgado et al., 2020), dispersal also plays a relevant role structuring some fish assemblages (e.g., Dala-Corte et al., 2017; Dias et al., 2014), or both processes are equally important (e.g., Borges et al., 2020; Carvalho et al., 2021; Cetra et al.,

2017).

The drainage basin spatial configuration and the river network branching determine the physical diversity of channel and riverine conditions (Benda et al., 2004). In this context, both niche- and dispersal-based processes may act in a synergic way determining synchrony in branched river networks (Larsen et al., 2021). The hierarchical branching system of river drainage basins produces highly fragmented “islands”, under the influence of unidirectional water flow and the temporal variation in hydrology and connectivity (Altermatt, 2013; Grant et al., 2007). Thus, freshwater organisms are always constrained to some degree by the branching structure of rivers (Tonkin et al., 2018). For instance, network location affects fish assemblages depending on the geographic position, i.e., headwaters versus mainstems (Brown & Swan, 2010; Vitorino Júnior et al., 2016). Particularly in headwater streams, fish assemblages are structured by environmental filtering and tend to be compositionally distinct (Zbinden & Matthews, 2017).

On large scales, geo-climatic variables can be effective surrogates for local environmental variables and can be good predictors of freshwater fish distribution (Frederico et al., 2014; McGarvey et al., 2018). Local characteristics (e.g., Barbosa et al., 2019; Cetra et al., 2017; Zbinden & Matthews, 2017) and current and past climate conditions (e.g., Dias et al., 2014; Leprieur et al., 2011) affect fish communities, while dispersal in response to environmental changes depends on both hydrological connectivity (Carrara et al., 2012; Carvajal-Quintero et al., 2019) and species dispersability (Radinger & Wolter, 2014). Basin shape and branching geometry are also controlled by factors such as climatic and geology (Seybold et al., 2017; Yi et al., 2018) suggesting that riverine metapopulation dynamics can depend on a larger-scale biogeographical process (Larsen et al., 2021). At the basin scale, dispersal can limit the ability of species to track their preferred environmental conditions (i.e., patch dynamics), override local habitat control (i.e., mass effect), or species are assumed to be best sorted according to their preferred environmental conditions in an intermediate rate of dispersal (i.e., species sorting) (Heino, Melo, & Bini, 2015; Leibold et al., 2004; Tonkin et al., 2018; Winegardner et al., 2012). In this sense, patch dynamics and mass effect can be considered special cases of species sorting with the predominance of limiting and homogenizing effects of dispersal, respectively (Heino, Melo, Siqueira, et al., 2015;

Winegardner et al., 2012).

In the upper Paraná ecoregion, both environmental filters and dispersal limitation are good predictors of fish communities, which harbour heterogeneous fish assemblages throughout its headwater streams, presenting high values of total beta-diversity and remarkable endemism in its distinct sub-basins (Borges et al., 2020). Particularly, fish beta-diversity patterns in the upper Paraná River floodplain—the only dam-free section of the Paraná River in Brazil (Affonso et al., 2015)—are correlated with environmental heterogeneity also across its dam-free tributaries (Bailly et al., 2021; Peláez et al., 2017; Peláez & Pavanelli, 2019). Representing one of these tributaries, the Ivaí River basin keeps its connectivity and hydrological processes almost entirely intact (Affonso et al., 2015; Azevedo et al., 2016), although habitat degradation and human-caused impacts on environmental variables have been reported in some sites (Garcia et al., 2021). Thereby, this river basin can be an excellent model to test hypotheses regarding distributional patterns along fluvial gradients. Considering the large geographic extent of the Ivaí River basin and the wide variation in climate and geology across that region, different levels of environmental heterogeneity and connectivity among headwaters and mainstem regions are expected (Altermatt, 2013; Tonkin et al., 2018), especially throughout its upper, middle and lower sections (Leli et al., 2017).

Here, we use a recent list of georeferenced fish records to visualize beta-diversity patterns, identify major breaks delimiting fish assemblages in the Ivaí River basin, and evaluate the influence of geo-climatic variables on fish species turnover. Specifically, we address the following issues: (1) fish species composition varies along the Ivaí River basin and distinct sets of species are associated with the upper, middle, and lower sections of that basin. Considering that sections of that basin are distinct in elevation, geomorphology, topography, vegetation, and climate with rapids and falls more frequent in the upper and middle sections (Leli et al., 2017), we expect that the ichthyofauna will differ significantly along the basin due to different environmental and dispersal conditions associated; (2) turnover is the principal component of the total fish beta-diversity in the Ivaí River basin. Considering the elevational gradient from the upper to the lower sections of the basin as a proxy to distinct environmental (i.e., habitat

conditions) and physical conditions (i.e, river network), we expect that a gradual species replacement will be more relevant than nestedness driving beta-diversity patterns throughout that basin; (3) variation in the fish species composition across the Ivaí River basin can be explained by geo-climatic predictors. The Ivaí River basin is associated with environmental gradients (Leli et al., 2017), and we expect that geo-climatic variables to be good predictors of variation in fish species composition throughout that basin by both niche- and dispersal-based processes.

4.2 Materials and methods

4.2.1 Study area

The Ivaí River basin is located in the Southern region of Brazil and is entirely inserted in Paraná State (Figure 4.1). From its formation at the confluence of the dos Patos and São João rivers in the Serra da Esperança (Second Plateau; about 800 m elev.), the Ivaí River runs 671 km until it flows into the Paraná River, municipality of Icaraíma (230 m elev.). Counting from its origin at the dos Patos River (1,160 m elev.), its channel reaches 798 km in total length and has an average flow of 702.9 m³.s⁻¹ and a draining area of 36,587 km² (Leli et al., 2017).

With a predominance of narrow rocky valleys and rapids across its upper and middle courses, the Ivaí River is one of the largest dam-free tributaries of the upper Paraná River floodplain (East margin), and stands out by its largely intact hydrologic dynamics. Besides affording refuge to the ichthyofauna of that floodplain (Affonso et al., 2015), the Ivaí River basin also harbors rare and endemic fish species, several of which occur outside the limits of the conservation units (see Frota, Pacifico, & Graça, 2021). Actually, there is a huge gap for effective legal protection of the Ivaí River basin's aquatic biota. Three relevant conservation units overlap at least partially with the Ivaí Riverbasin (SEMA, 2013): (1) the Serra da Esperança Environmental Protection Area in its upper section, (2) the Perobas Biological Reserve in its middle section, and (3) the Ilhas e Várzeas do Rio Paraná Environmental Protection Area in its lower section (Figure 4.1b).

Efforts to increase the sampling of the fish fauna from the Ivaí River basin (Reis, Frota, Deprá, et al., 2020) resulted in the discovery of several new endemic taxa (Dias & Zawadzki,

2021; Graça & Pavanelli, 2008; Reis, Frota, Fabrin, & Graça, 2020; Roxo et al., 2014; Tencatt et al., 2014; Zawadzki et al., 2016). An updated checklist including 108 native and 24 non-native species (Reis, Frota, Deprá, et al., 2020), as well as distribution patterns of rare and restricted species in some mountain streams (Frota, Pacifico, & Graça, 2021), and growth parameters for endemic species (Frota, Ganassin, & Graça, 2021) have been recently described. In Paraná State, all remaining sub-ecoregions of the upper Parana ecoregion have a lower rate of endemism if compared to the Ivaí River basin. In this state, 21 fish species are considered endemic of the upper Parana ecoregion, of which 11 (52.4%) are endemic from the Ivaí River basin (Reis, Frota, Deprá, et al., 2020).

The Ivaí River basin overlaps with the Second and Third Paranaense Plateaus ($22^{\circ}56'17''$ – $25^{\circ}35'27''S$ and $50^{\circ}44'17''$ – $53^{\circ}41'43''W$) and runs through a wide range of landscapes that are distinct in lithology (Leli et al., 2017; Meurer et al., 2010; Souza et al., 2018). It includes five large ecoregions (each divided into two sub-ecoregions) distinct in geomorphology, pedology, vegetation, topography, climate, and precipitation (Meurer et al., 2010), providing a reasonable basis for the delimitation of its upper, middle, and lower sections by Leli et al. (2017). In this context, the fluvial system of this basin is heterogeneous and composed of complex geo-climatic gradients (Meurer et al., 2010). A synthesis of physical and climatic characteristics of the upper, middle, and lower Ivaí River basin is provided in Table 4.1.

Table 4.1 Synthesis of physical and climatic characteristics of the three sections of the Ivaí River basin.

Basin section	Length (km)	Area (km²)	Relief (m)	Relief characteristics	Vegetation	Land use (%)	Climate	Annual rainfall (mm)
upper (From headwater to Alonso River mouth)	370	12,926	1,200 – 500	Very sculpted relief with falls and rapids in the main and tributary rivers	Mixed Ombrophilous with Araucaria Forest	Natural landscape	Subtropical	2,000
middle (From Alonso River to the Porto Paraíso do Norte gauging station)	230	15,218	500 – 300	Tabular relief with <i>mesetas</i> , and <i>demi-orange</i> mountains and hills. River flow with rapids and minor falls	Seasonal semideciduous Forest	30–40% (agriculture and reforestation)	Subtropical	1,700
lower (From Porto Paraíso do Norte to its mouth in the Paraná River)	200	8,380	300 – 230	Gentle hills, tranquil flow with very scarce rapids in main channel and tributaries.	Seasonal semideciduous Forest	60–70% (agriculture and pasture)	Tropical	1,400

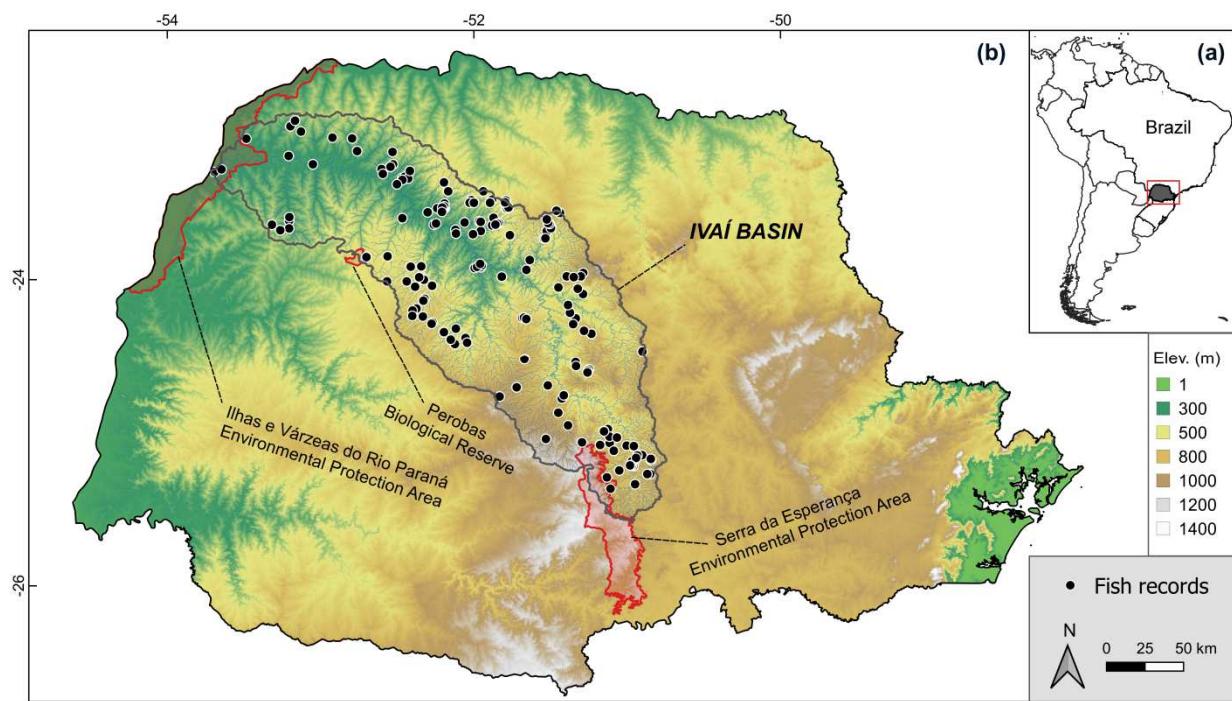


Figure 4.1 Maps showing the study area. (a) South America highlighting the Paraná State in Southern Brazil; (b) hypsometric map of the Paraná State evidencing the Ivaí River basin, the georeferenced fish records, and the relevant conservation units found in the basin.

4.2.2 Database preparation

The fish distributional data from the Ivaí River basin used in this study consists of a compilation of georeferenced records (Figure 4.1b) from the following ichthyological collections: Coleção de Peixes da Universidade Federal do Rio Grande do Sul, Porto Alegre (UFRGS); Coleção de Peixes do Laboratório de Ictiologia de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto (LIRP); Coleção Ictiológica do NUPÉLIA, Maringá (NUP); Museu de Ciências e Tecnologia da PUCRS, Porto Alegre (MCP); Museu de História Natural Capão da Imbuia, Curitiba (MHNCI); Museu de Zoologia da Universidade Estadual de Londrina, Londrina (MZUEL); and Museu de Zoologia da Universidade de São Paulo, São Paulo (MZUSP). These collections are available at SpeciesLink (<http://splink.cria.org.br>), FishNet2 (<http://fishnet2.net>), and SiBBr (<https://collectory.sibbr.gov.br/collectory/public/show/dr186>) databases.

Complementary data were obtained in journal articles from Thomson Reuter (ISI Web of Knowledge, apps.isiknowledge.com), Elsevier-ScienceDirect (<http://www.sciencedirect.com>),

and Scielo (<http://www.scielo.org>) databases, using the search terms “fish OR ichthyo OR checklist AND Ivaí River” as the Topic field, including all years until December 2020. The search was then refined according to the following areas: Environmental Sciences, Ecology, Zoology, Freshwater Biology, Biodiversity, Conservation and Fisheries, and water sources. We also included records from manuscripts published in the journal *Check List: Journal of Species Lists and Distributions*, which is not indexed in the databases mentioned above.

Coordinates of all localities associated with fish records were checked. Records lacking coordinates were georeferenced on Google Earth® or were excluded in cases of uncertainty. The fish identification of all specimens was based on Frota et al. (2016), Ota et al. (2018), and Reis, Frota, Deprá et al. (2020). Only records of the native species recently listed for the Ivaí River basin (Reis, Frota, Deprá, et al., 2020) were used. The final database comprised 2,606 georeferenced records and is available on request to the corresponding author.

4.2.3 Species Composition Interpolation

Species Composition Interpolation (SCI) is a statistical technique that relies on the interpolation of compositional species turnover using values of Non-metric Multidimensional Scaling (NMDS) from a beta-diversity index matrix (Oliveira et al., 2017, 2019). Initially, fish records from the Ivaí River basin were sampled using a grid of 0.4° hexagons. Only cells with a minimum of five species were considered in the analyses. Then, a Jaccard dissimilarity matrix was generated and converted into linear values using three NMDS axes, and these values were interpolated on a map using a Bayesian method (Oliveira et al., 2017). The interpolation operation relies on the assumption that dissimilarity values are spatially auto-correlated as values of non-sampled sites are predicted to be intermediate to values of nearby sampled sites, in proportion to the distance among them. The assumption of spatial auto-correlation of NMDS values was tested using the Global Moran' I test. For visualization purposes, the three NMDS axes are assigned to the red, green, and blue (RGB) bands of a color image. Regions recovered with a very distant color are predicted to be highly dissimilar in species composition, while areas with a similar color are predicted to be similar in species composition. We analyzed separately

the beta-diversity (total Jaccard), as well as the nestedness and turnover components. Finally, three discrete regions were delimited using the Clustering for Large Applications (CLARA) classification, resulting in three ichthyofaunistic units (upper, middle, and lower sections). All these analyses were run in Dinamica Ego platform (<http://www.dinamicaego.com>), using the tool “SCI” available from the toolkit BioDinamica 2.2 (Oliveira et al., 2019).

4.2.4 Total beta-diversity analysis

Based on absence and presence records of each fish species in the upper, middle, and lower sections of the Ivaí River basin (as delimited using SCI; Appendix E), we used the Baselga (2010) method to calculate beta-diversity and partition it into its turnover and nestedness components. We employed the Jaccard index and extracted the dissimilarity values from the dissimilarity matrices (β_{jac} , β_{jtu} , and β_{jne}) to perform pairwise comparisons between sections of the basin (upper vs middle, upper vs lower, and middle vs lower sections). All analyses were implemented in R (R Core Team, 2020) using the “beta.pair” function available from the betapart package (Baselga & Orme, 2012).

4.2.5 Geo-climatic data

In the absence of complete instream data, climate covariates may be used to model freshwater fish distribution in large-scale analyses (Frederico et al., 2014; McGarvey et al., 2018). Current time (1970-2000) climate data for 19 climatic variables were downloaded from WorldClim (Fick & Hijmans, 2017; <https://www.worldclim.org/>). Air temperature was employed as a substitute for water temperature, as these two variables are usually highly positively correlated in studies on regional and large scales (Buisson et al., 2008). Twelve predictors related to soil and lithology downloaded from SoilGrids (Hengl et al., 2017; <https://soilgrids.org/>) were added to the model as a proxy for geomorphological units of the Ivaí River basin. Other five variables related to the physical environment (elevation, drainage density, height above the nearest drainage and slope) obtained from INPE website (<http://www.dsr.inpe.br/>) were also

included, as well as geographic distance. The elevation layer used in this study, generated from the Shuttle Radar Topographic Mission, was also obtained from WorldClim homepage. Generalized Dissimilarity Modeling (GDM; Ferrier et al., 2007) was performed using raster layers downscaled to the spatial resolution of 30 arc-seconds.

4.2.6 Generalized Dissimilarity Modeling

To evaluate the association between geo-climatic predictors and the beta-diversity patterns, we performed Generalized Dissimilarity Modeling (Ferrier et al., 2007). This modeling method uses maximum-likelihood and I-splines to transform predictor variables and find the best-supported function between geo-climatic variation and species turnover (Ferrier et al., 2007). The inter-site distances are converted to accommodate curvilinear relationships between compositional dissimilarity and geo-climatic variation (Ferrier et al., 2007). A subset of geo-climatic variables was selected using the step-wise backward elimination method of Williams et al. (2012). Backward elimination procedures for variables selection were recommended by Ferrier et al. (2007) and are commonly used in GDM analyses (e.g., Coccia et al., 2021; Guerin et al., 2021; Saiter et al., 2016; Williamson et al., 2019). The model was originally built with 35 geo-climatic variables. Then, predictors that explained less than 0.1% of the model were progressively removed, until the 35 predictors were reduced to 13 (BIO3, isothermality; BIO5, maximum temperature of warmest month; BIO6, minimum temperature of coldest month; BIO7, annual temperature range; BIO13, precipitation of wettest month; BIO15, precipitation seasonality; BIO16, precipitation of wettest quarter; BIO17, precipitation of driest quarter; sand content; silt content; height above nearest drainage, elevation, and geographic distance). GDM can handle higher multicollinearity among predictors than most commonly used regression models (Glassman et al., 2017; Heino et al., 2019; see also Pacifico et al., 2021). Still, to verify whether our results could have been affected by multicollinearity, we calculated Pearson correlation coefficients among the subset of 13 geo-climatic variables used in this study. Pearson coefficients were higher than 0.7 between four pairs of predictors: BIO5 and BIO6; BIO13 and BIO16; elevation and BIO17; sand content and silt content. The joint removal of the less

significant highly correlated predictors (BIO5, BIO16, BIO17, and sand content) had little effect on the explanation power of GDM, which was reduced by 0.16%. In the GDM analysis, we kept the initial subset of 13 variables. A grid of 0.4° hexagons was used to sample fish distribution records from the Ivaí River basin, considering only cells with at least five species. The relative importance of each predictor was determined by summing the coefficients of the I-splines from GDM. Analyses were run through the Dinamica Ego platform (<http://www.dinamicaego.com>), using the tool “GDM” available from the toolkit BioDinamica 2.2 (Oliveira et al., 2019).

4.3 Results

4.3.1 Species Composition Interpolation

The NMDS ordination provided a representation of the observed dissimilarity among sampled sites (Stress = 0.14). Plots of the NMDS ordination of the total beta-diversity, as well as the nestedness and turnover components, are presented in Figure 4.2. Elevated Global Moran’s I statistic values showed that the assumption of spatial auto-correlation required for the Spline interpolation of NMDS values was successfully achieved for all three axes (axis 1, Red: 0.9953; axis 2, Green: 0.9943, axis 3, Blue: 0.9935).

In surface maps, the NMDS axis 1 indicated that the ichthyofauna of the middle Ivaí River basin is less differentiated and is largely shared with the other sections of this basin (Figure 4.3a). In turn, the NMDS axes 2 (green) and 3 (blue) indicated that the upper and lower Ivaí River basin are distinct in fish species composition (Figure 4.3b and Figure 4.3c, respectively). Overall, the three NMDS axes revealed three visible ichthyofauna regions (Figure 4.3d). These regions are referred to as the upper Ivaí River basin (axis 3, green), middle Ivaí River basin (axis 1, red), and lower Ivaí River basin (axis 2, blue), and were successfully classified in three discrete ichthyofauna units using CLARA clustering (Figure 4.3e). The turnover map (Figure 4.3f) was more highly correlated with the total beta-diversity map (Figure 4.3d) than the nestedness map (Figure 4.3g) for the NMDS axes 2 and 3. In turn, the turnover map (Figure 4.3f) was strongly negatively correlated with the total beta-diversity map (Figure 4.3d) for the NMDS axis 1.

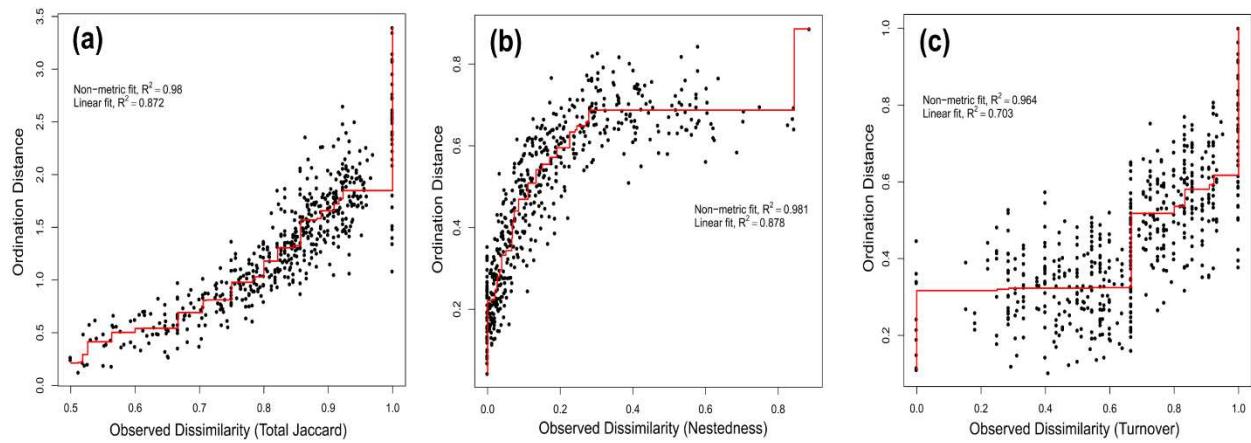


Figure 4.2 Plots from the NMDS analysis of fish species composition in the Ivaí River basin. (a) Relationship between total observed dissimilarity and NMDS ordination distance; (b) relationship between the turnover partition and ordination distance; (c) relationship between the nestedness partition and ordination distance.

4.3.2 Total beta-diversity

The final database of fish records from the Ivaí basin comprised six orders, 22 families, and 108 native species (11 endemics; Appendix E). In relation to the three discrete ichthyofauna units delimited using CLARA clustering (Figure 4.3e), 65 species were recorded at the upper section of the basin, 89 for the middle section, and 60 for the lower section. The middle section shared 56 and 50 species with the upper and lower sections, respectively. In addition, the upper and lower sections shared 31 species (Appendix E). Overall, the beta-diversity values indicate that turnover predominates in the basin ($\beta_{jtu} = 0.475$; Table 4.2). In pairwise comparisons of both turnover and nestedness partitions, the dissimilarity values confirmed that the middle section of the Ivaí River basin is more similar in species composition to the upper and lower sections (Table 4.2). In contrast, turnover predominates between the upper and lower sections, indicating a higher species replacement between these regions ($\beta_{jtu} = 0.652$; Table 4.2).

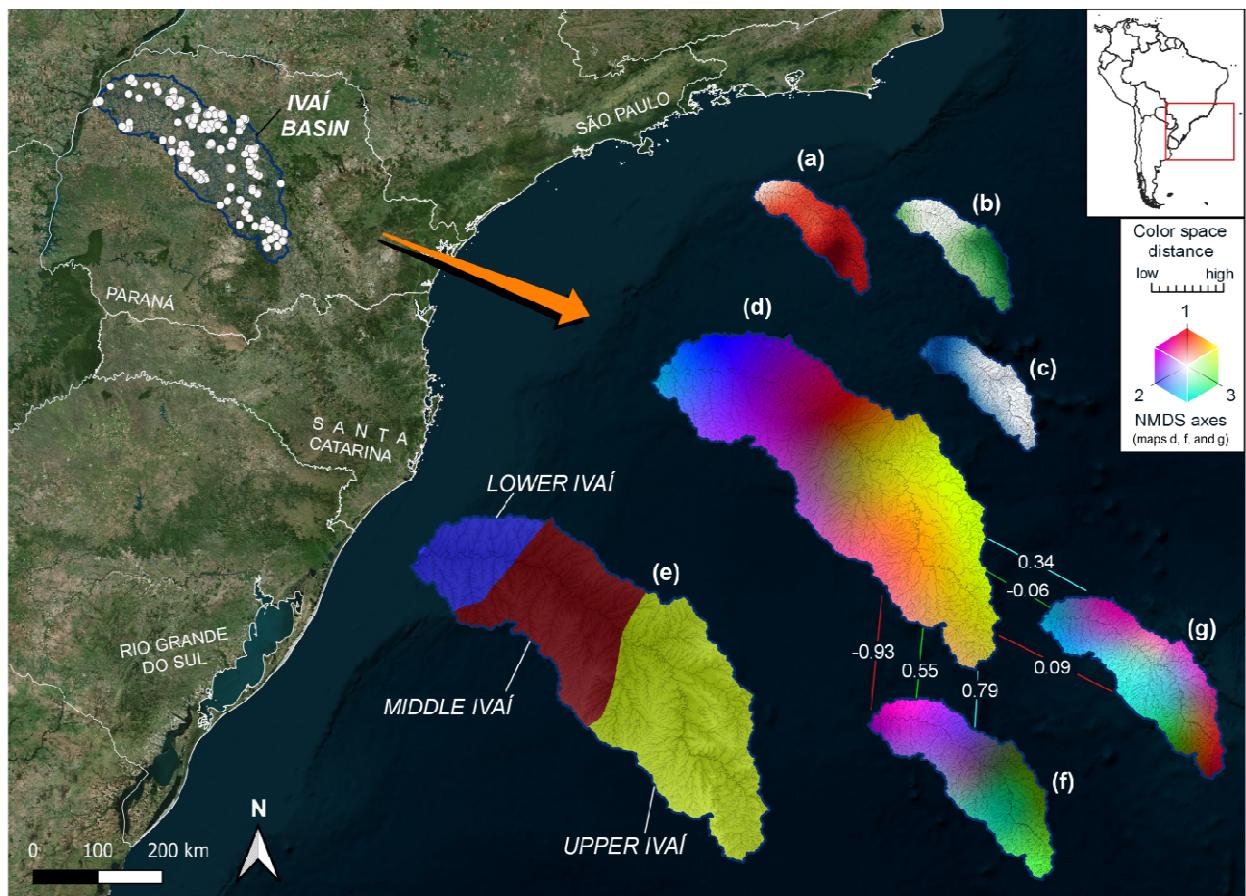


Figure 4.3 Spatial variation in fish species composition in the Ivaí River basin revealed by surface maps based on the interpolation of NMDS linear values. (a) NMDS axis 1 (red); (b) NMDS axis 2 (green); (c) NMDS axis 3 (blue); (d) total beta-diversity pattern revealed by the sum of the three NMDS axes; (e) discrete ichthyofaunistic units classified using Clustering for Large Applications (CLARA) algorithm; (f) map based only on the turnover component of beta-diversity; (g) map based only on the nestedness component of beta-diversity. Colour lines indicate the correlation values among each of the components and the total beta-diversity for each of the NMDS axes.

4.3.3 Generalized Dissimilarity Modeling

Geo-climatic variables explained 19.07% of the compositional turnover of fish communities in the Ivaí River basin. In descending order, the most significant predictors were isothermality (relative importance = 0.551), silt content (0.376), elevation (0.193), precipitation of wettest month (0.143), sand content (0.120), temperature annual Range (0.105), precipitation of driest quarter (0.085), precipitation of wettest quarter (0.071), minimum temperature of coldest month (0.062), geographic distance (0.028), maximum temperature of warmest month (0.024), height

above nearest drainage (0.007), and precipitation seasonality (0.003). Plots of the observed dissimilarity in fish species composition against the predicted ecological distance, the dissimilarity predicted by GDM against the observed dissimilarity, as well as the I-splines fitted to the individual predictors are presented in Figure 4.4. In the surface map (Figure 4.5), the upper (red axis) and lower (green axis) regions of the Ivaí River basin were recovered as distinct fish communities (Figure 4.5). The community from the lower section, however, expands towards the middle Ivaí River basin through major rivers as the Ivaí, Mourão, Keller, Corumbataí, and Alonso rivers (Figure 4.5). Lastly, in the middle section, discontinuous patches of another distinct fish community were identified (Figure 4.5).

Table 4.2 Beta-diversity values based on the Jaccard index for the total basin and pairwise comparisons between fish assemblages that occur in the upper, middle, and lower sections of the Ivaí River basin. Abbreviations: β_{jtu} , Jaccard dissimilarity; β_{jtu} , turnover component; β_{jne} , nestedness component.

Sampled area(s)	β_{jtu}	β_{jne}	β_{jac}
Upper versus middle	0.243	0.185	0.428
Upper versus lower	0.652	0.018	0.670
Middle versus lower	0.286	0.209	0.495
Total basin	0.475	0.117	0.592

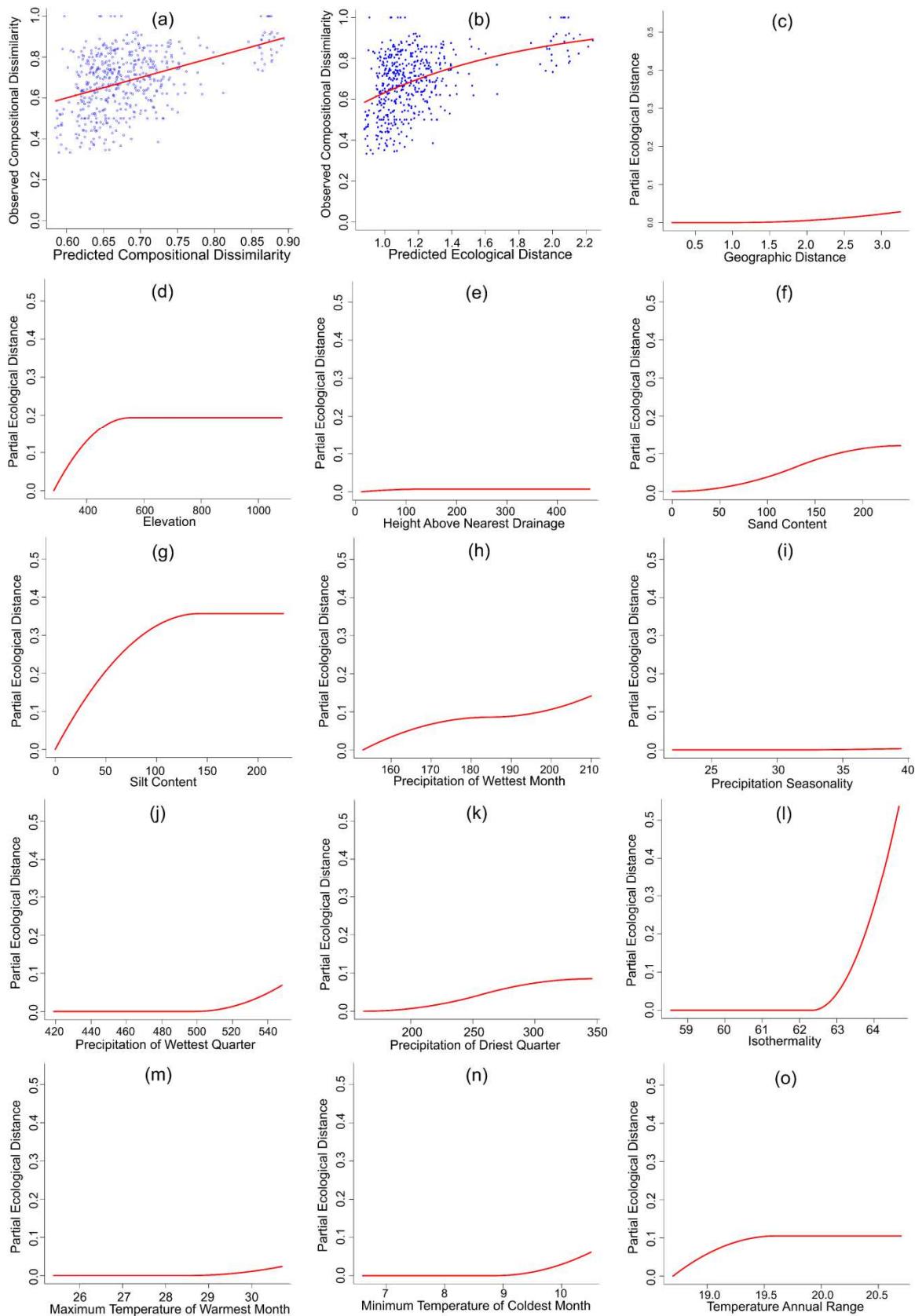


Figure 4.4 (a) Plot of the relationship between observed dissimilarity of fish species composition between each site pair and the linear predictor of the regression equation from

GDM; (b) plot of the observed dissimilarity in fish species composition against the dissimilarity predicted by GDM; (c-o) fitted I-splines from GDM for predictors associated with variation in fish species composition. The shape of each function demonstrates how the rate of compositional turnover associated with the predictor varies across the environmental gradient, while the peak expresses the total amount of compositional turnover associated with that predictor.

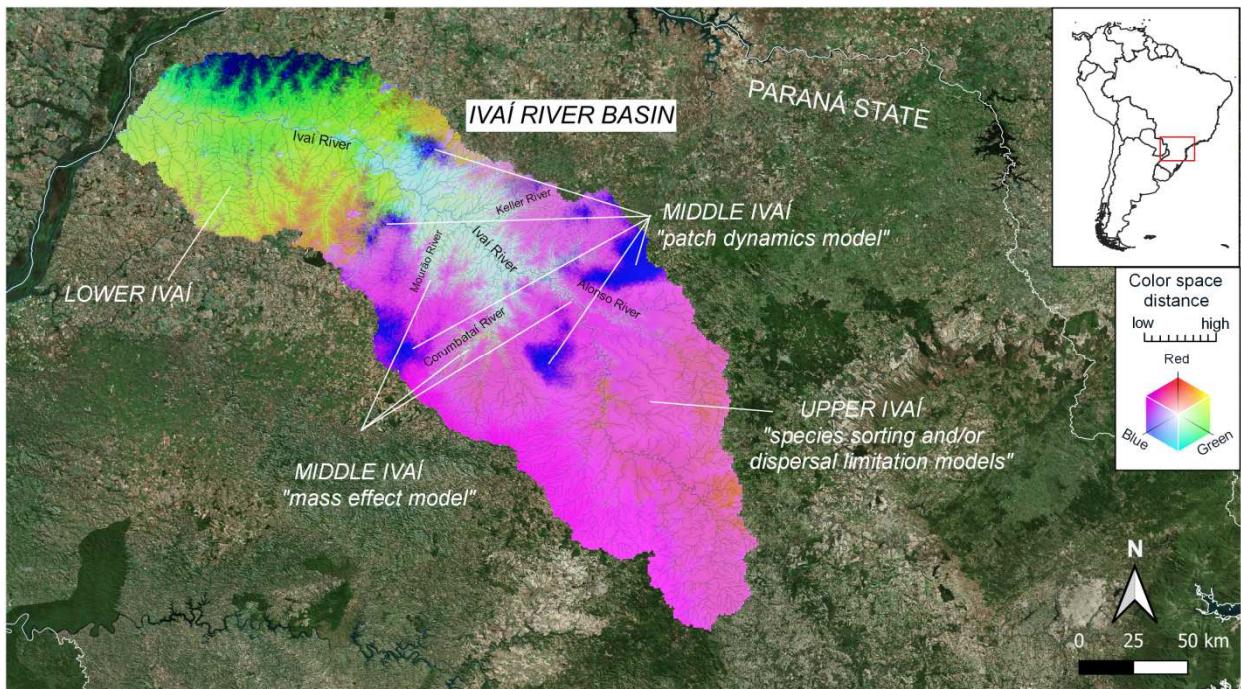


Figure 4.5 Surface map from GDM based on fish records and 13 geo-climatic predictors from the Ivaí River basin. Hypothetical metacommunity models that predominate in each section of the Ivaí River basin are indicated by white lines.

4.4 Discussion

We found moderate support for the three issues addressed in this study: (1) fish assemblages varied along the Ivaí River basin, with very distinct communities at the upper and lower sections of the basin and a less differentiated community at the middle section, as it was unveiled by both SCI and GDM; (2) turnover was the main component contributing to total beta-diversity as revealed by the higher correlations between the turnover map and the total beta-diversity map for two of the three NMDS axes from SCI; and (3) dissimilarity in fish species composition was associated with geo-climatic predictors across the Ivaí River basin, although only 19.07% of the

variability was explained by GDM.

Major breaks delimiting fish assemblages in the Ivaí River resemble the limits of the geological units (Leli et al., 2017). Besides, geo-climatic variables as isothermality, elevation, and soil types were correlated with the fish species replacement along the basin. However, the remaining residual variation from GDM was about 81%. In freshwater fish ecology, it is quite common to find substantial unexplained variation when analyzing species composition associated with environmental variables (e.g., Barbosa et al., 2019; Cetra et al., 2017; Sály et al., 2011; Zbinden & Matthews, 2017). Although it is usually challenging to explain the residual variation, it is possible to infer some underlying factors (Zbinden & Matthews, 2017). In this study, a possible explanation and/or limitation is the absence of more refined predictors like hydrological variables (i.e., collected in the water body itself) and the number of predictors used in GDM. The variables included in this study could represent only a small fraction of the ecological factors structuring regional fish assemblages (Poff, 1997). However, we suspect that a combination of biotic interactions, dispersal dynamics, and unmeasured environmental factors is the most intuitive explanation. Likewise, Carvalho et al. (2021) suggested that turnover is the main component explaining the beta-diversity of fish assemblages along fluvial gradients (i.e., from the headwaters to the mainstem channel) and it is correlated with both geo-climatic variables and species dispersal limitation in headwaters (Altermatt & Fronhofer, 2018).

Recent studies conducted in the Neotropical region revealed that taxonomic turnover provides a good explanation of beta-diversity patterns for freshwater fishes (e.g., Carvalho et al., 2021; Peláez & Pavanelli, 2019; Vitorino Júnior et al., 2016; Zbinden & Matthews, 2017). At a river basin level, consistently high beta-diversity is influenced by species turnover (López-Delgado et al., 2020; Zbinden & Matthews, 2017), while the importance of environmental filters is scale-dependent (Henriques-Silva et al., 2013). In fact, turnover towards headwater habitats is a consistent trend for different lateral sections of a given river basin (Carvalho et al., 2021; Zbinden & Matthews, 2017). Mainstem channel regions are usually environmentally stable, presenting higher habitat heterogeneity than headwater regions, and linking fish assemblages throughout the fluvial gradient (Jackson et al., 2001). Considering the

entire Ivaí River basin as the study area, distinct diversity patterns across headwaters (i.e., lateral sections) and drainage basin (i.e., longitudinal gradient) (Cetra et al., 2017) could potentially have masked associations with geo-climatic variables (Heino, Melo, & Bini, 2015). In this context, as both headwaters and mainstem regions were included in the study area, we expect that regional processes like dispersal tend to predominate at this scale (Heino, Melo, Siqueira, et al., 2015).

The upper Ivaí River ichthyofaunistic region is probably linked to two distinct scenarios related to niche- and dispersal-based processes. Headwater localities, from this section, presumably receive few migrants and local assemblages are expected to be strongly influenced by local environmental conditions and species sorting (Carvalho et al., 2021), as they are relatively isolated within the river network (López-Delgado et al., 2019). Given the great variation in elevation (see Figure 4.1b), very sculpted relief, among other distinct geo-climatic aspects (Leli et al., 2017), headwaters from this section are expected to be climatically heterogeneous (Richardson, 2019) along with the highly ramified river system (Altermatt, 2013; Altermatt & Fronhofer, 2018). Therefore, fish species composition in the upper Ivaí River basin is probably driven by species sorting and/or dispersal limitation. In the first scenario, fish assemblages may have been filtered by the environment into suitable locations, while dispersal rates may have allowed individual species to track their preferred environmental conditions (Heino, Melo, & Bini, 2015; Heino, Melo, Siqueira, et al., 2015; Leibold et al., 2004). On the other hand, inadequate dispersal capacity could have negatively affected species ability to reach very distant suitable sites (Heino, Melo, & Bini, 2015; Tonkin et al., 2018). Consequently, high rates of turnover among the upper Ivaí and the other sections of the basin were expected (Carvalho et al., 2021). Besides, isolated fish populations in headwaters are expected to be highly structured genetically (Thomaz et al., 2016). Over time, this process may have limited fish species distribution and caused local speciation (Carvajal-Quintero et al., 2019) in mountains streams from the upper Ivaí River basin (Frota, Pacifico, & Graça, 2021).

The middle Ivaí River basin was the least differentiated ichthyofaunistic unit and presented similar values of turnover and nestedness with the upper and lower sections. This result can be

explained in part by the patch dynamics model, according to which habitat patches are capable of maintaining populations with limited dispersal capacity (Leibold et al., 2004). In this context, habitat patches are assumed to be distinct in their geo-climatic conditions, allowing species establishment and interchange with other sections of the basin (i.e., species arriving from headwaters and mainstem channels) (Altermatt & Fronhofer, 2018). Since the middle Ivaí River basin is considerably homogeneous in climate and geography, where the mainstem and tributaries are highly connected, local communities should also respond to mass effect (Brown & Swan, 2010; Heino, Melo, Siqueira, et al., 2015; Henriques-Silva et al., 2019; López-Delgado et al., 2019; Vitorino Júnior et al., 2016). This interpretation is corroborated by the fact that large tributaries from the middle Ivaí River basin (e.g., Alonso, Corumbataí, Mourão, and Keller rivers) present comparatively lower water discharge (Leli et al., 2017), probably favoring species dispersal towards sites environmentally similar with the lower Ivaí River basin. Thus, we suspect that the middle Ivaí River basin acts as an ecological barrier (environmental filters and dispersal limitation) for a subset of fish species, corroborating the hypotheses made by Deprá et al. (2018). Simultaneously, interchange of individuals between mainstem channel and sites close to the river mouth is expected (see López-Delgado et al., 2019), as well as the presence of taxa typical from headwaters in specific habitat patches.

The distinctiveness of the fish communities from the lower Ivaí River was evidenced by both SCI and GDM analyses. This result is likely related to the fact that the Ivaí River develops a floodplain of about 150 km long toward its lowest limit, where it exhibits a sinuous and embedded meandering channel (Leli et al., 2017). As an alluvial river section, the lower Ivaí River basin is functionally associated with the upper Paraná River floodplain in the confluence of the Paraná and Ivaí rivers. That floodplain is currently affected by flood events caused by both rivers (Morais et al., 2016). The Ivaí River differs from the Paraná River by the predominance of suspended load consisting mostly of silt and clay (Morais et al., 2016), which is likely associated with limnological parameters (i.e., environmental heterogeneity) as turbidity and electrical conductivity (Peláez & Pavanelli, 2019). Therefore, we suspect that the variation in fish species turnover in the lower Ivaí River basin is related to environmental variation among sampling sites along the Paraná River and in its main dam-free tributaries as the Ivaí River (Peláez & Pavanelli,

2019).

4.5 Future perspectives and conclusions

Studies on unperturbed systems like the Ivaí River basin may improve our understanding of fish community dynamics and may provide useful insights for the conservation and restoration of highly degraded rivers (López-Delgado et al., 2019). Thus, the evaluation of the drivers of fish species distribution is necessary due to the complexity of niche- and dispersal-based processes and could result in new conservation priorities for aquatic bodies in different river basins (Borges et al., 2020; López-Delgado et al., 2019). Fish beta-diversity patterns in the Ivaí River basin were characterized by a predominance of species turnover, with communities varying consistently along its sections. Fish distribution in this basin is moderately explained by niche-based models and we hypothesize that dispersal-based processes can be equally important at the basin scale, with complementary effects to the geo-climatic drivers.

Frota, Pacifico & Graça (2021) reported populations of rare, restricted, and endemic fish species occurring in unprotected of the Ivaí River Basin, mainly along its upper and middle sections. Our findings reinforce that new guidelines to protect the ichthyofauna of the Ivaí River basin are necessary (Affonso et al., 2015; Azevedo et al., 2016; Bailly et al., 2021). Additional conservation units are desirable to afford protection to the regional biodiversity of this basin; these conservation units should include sites strategically located in the upper, middle, and lower sections. The upper Ivaí River basin has proved to be an important cradle of new fish species (e.g., Dias & Zawadzki, 2021; Graça & Pavanelli, 2008; Reis, Frota, Fabrin, & Graça, 2020; Roxo et al., 2014; Tencatt et al., 2014; Zawadzki et al., 2016). Besides, this region is apparently of historical significance for the evolutionary diversification of several fish lineages. Some species from the upper Ivaí River basin probably have adapted to “ideal” local environmental conditions, whereas others are narrowly distributed as a result of dispersal limitation. In contrast, the lower Ivaí basin harbors fish communities that are distinct both taxonomically and functionally. Our results support the interpretation that the lower Ivaí River basin is an important maintainer of the regional fish diversity from the upper Paraná River floodplain. This scenario is probably

associated with its high regional environmental heterogeneity (Peláez et al., 2017; Peláez & Pavanelli, 2019). The lower Ivaí River basin is also a strategic area for the conservation of fish diversity and fish stocks as it is used as spawning grounds for migratory fishes inhabiting the Paraná River (Affonso et al., 2015; Antonio et al., 2007; Bailly et al., 2021; Silva et al., 2017). The middle Ivaí River could be considered a relevant area for fish conservation for its nested beta-diversity. In fact, it could include strategic patches harboring species shared with other sections of the basin. Overall, the few and small conservation units existing in the Ivaí River basin (Figure 4.1) seem to be very little effect in protecting its freshwater biodiversity (Azevedo-Santos et al., 2019).

Maintaining the ecological integrity and connectivity of dam-free river basins is crucial for minimizing the expected trend of biotic homogenization (Marques et al., 2018), especially across floodplains that depend on high flow pulses to increase the spatial variability and recruitment of fish assemblages (Cruz et al., 2020; Espínola et al., 2020). Pristine river basins can contribute significantly to the conservation of fish diversity in areas already impacted by dams (Marques et al., 2018; Silva et al., 2017; Silva et al., 2019; Vasconcelos et al., 2021) or by several other human activities (Garcia et al., 2021). We encourage the expansion of public and social initiatives such as the 'Pró Ivaí/Piquiri movement'. This initiative has been supporting conservation actions in the Ivaí and Piquiri river basins and warning public opinion about the negative impacts of the construction of dams (Affonso et al., 2015; Azevedo et al., 2016). Raising public awareness and pursuing legal and administrative measures are crucial in developing countries like Brazil, where several dams are planned and their negative impacts on the environment, people, and economy are often underestimated (Azevedo et al., 2016). Bringing science and public policy closer together towards sustainable development is possible and necessary (Affonso et al., 2015; Azevedo et al., 2016; Azevedo-Santos et al., 2017). In this context, we strongly recommend that conservation measures to preserve fish communities in the Ivaí River basin must take into consideration habitat heterogeneity, environmental gradients, hydrological conditions, and habitat connectivity.

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5 CONCLUDING REMARKS

The results obtained can provide important theoretical support to historical and ecological perspectives involving the biogeography of Neotropical freshwater fishes. Essentially, the findings are promising to support the relationship between Earth's evolution and aquatic life, in the case of fish populations, between the processes that shaped current drainage networks and the patterns of evolutionary diversification evidenced by different lineages. The evolutionary history in Cnesterodontini corroborated well-defined scenarios of the formation of hydrological barriers

between coastal river basins and drainages running into the La Plata River system in South America, establishing that dispersal events through headwater capture and sea-level changes explain the congruences evidenced among other fish lineages that exhibit similar diversification patterns in the Central Brazil drainages and in the drainages on the Atlantic coast. The evolutionary history in Anablepidae corroborated the pattern evidenced for other marine-derived lineages, allowing more appropriate conclusions about the diversification of these lineages in the Neotropical region. Vicariance, dispersal, and extinction events related to Miocene and Quaternary marine transgressions and ancient connections between river basins in southeastern South America were fundamental for the historical interpretation of the evolution of the family. In ecological aspects, the results can provide new theoretical and methodological frameworks by proposing that the distribution of ichthyofauna in the Ivaí River basin is linked to the main mechanisms and patterns inherent in metacommunities of aquatic organisms, with mutual influences of niche- and dispersal-based processes. Finally, the new findings support effective management for the conservation of freshwater fish diversity, whether considering species already at serious risk of extinction or river basins that are of fundamental importance for the maintenance of biodiversity and ecosystem functionality in continental aquatic environments.

APPENDIX LIST

APPENDIX A - Summary of the geographic distribution data for cnestodontins species based on data from ichthyological collections (databases: GBIF, FishNet2, and SpeciesLink) and specialized literature. Filtering refers to the deletion or correction of uncertain records obtained by databases using the literature cited. Ecoregions are identical to Abell et al. (2008) and biogeographic areas are detailed in the “Materials and methods” section and Figure 2.1. Conservation status is in accordance with ICMBio (2018).

Species	Distribution	Records and filtering	Ecoregion(s)	Biogeographic area(s)	Conservation status
<i>Cnesterodon brevirostratus</i> Rosa & Costa, 1993	Southern Brazil: Upper Pelotas and Canoas River drainages (Uruguay River basin), Jacuí River basin, and headwater creeks from the Maquiné and Itajaí-Açu River basins (Lucinda 2005; Lucinda et al. 2006)	GBIF (doi: 10.15468/dl.qkfav3); FishNet2; SpeciesLink; Lucinda (2005); Lucinda et al. (2006); Bertaco et al. (2016)	Laguna dos Patos, Lower Uruguay, Sotheastern Mata Atlantica, Tramandai-Mampituba, and Upper Uruguay	Central Coastal, South Coastal, and Uruguay	LC
<i>Cnesterodon carnegiei</i> Haseman, 1911	Southern Brazil: Upper Iguassu River basin (Lucinda 2005)	GBIF (doi: 10.15468/dl.uzrcjx); FishNet2; SpeciesLink; Lucinda (2005); Mezzaroba et al. (2021)	Iguassu	Iguassu	VU
<i>Cnesterodon decemmaculatus</i>	Argentina, Southern Brazil,	GBIF (doi:	Laguna dos Patos, Lower Parana-Paraguay,		LC

(Jenyns, 1842)	and Uruguay: Lower Uruguay River basin, Laguna dos Patos system, Negro and Salado River basins, and small coastal drainages of Uruguay and Argentina (Lucinda 2005)	10.15468/dl.nx7g25); FishNet2; SpeciesLink; Lucinda (2005); Lucinda et al. (2006); Bertaco et al. (2016)	Parana, and Lower Uruguay	South Coastal, and Uruguay	
<i>Cnesterodon holopterus</i> Lucinda, Litz & Recuero, 2006	Uruguay: Cuarein River drainage, Uruguay River basin (Lucinda et al. 2006)	GBIF (doi: 10.15468/dl.smgcqe); SpeciesLink; Lucinda et al. (2006)	Lower Uruguay	Uruguay	Not available
<i>Cnesterodon hypselurus</i> Lucinda & Garavello, 2001	Southern Brazil: Paranapanema River basin in Paraná State (Lucinda 2005; Silva et al. 2015)	GBIF (doi: 10.15468/dl.bp2mgx); FishNet2; SpeciesLink; Lucinda (2005); Silva et al. (2015); Frota et al. (2021)	Upper Parana	Upper Parana	EN
<i>Cnesterodon iguape</i> Lucinda, 2005	Southeastern and Southern Brazil: headwater creeks from the Ribeira de Iguape River basin (Lucinda 2005; Frota et al. 2019)	SpeciesLink; Lucinda (2005); Frota et al. (2019); Frota et al. (2021)	Ribeira de Iguape	Central Coastal	CR
<i>Cnesterodon</i>	Southern Brazil: Das Torres	GBIF (doi: 10.15468/dl.frhn5s);	Iguassu	Iguassu	EN

<i>omorgmatus</i>	Lucinda & Garavello, 2001	River drainage, Iguassu River basin (Lucinda 2005)	FishNet2; SpeciesLink; Lucinda (2005); Mezzaroba et al. (2021)			
<i>Cnesterodon pirai</i>	Aguilera, Mirande & Azpelicueta, 2009	Argentina: Almeida Creek, Parana River basin (Aguilera et al. 2009)	FishNet2; SpeciesLink; Aguilera et al. (2009)	Lower Parana	Parana-Paraguay	Not available
<i>Cnesterodon raddai</i>	Meyer & Etzel, 2001	Argentina and Paraguay: Paraguay and Lower Parana River basins (Lucinda 2005)	GBIF (doi: 10.15468/dl.m2kvvq); Lucinda (2005)	Lower Parana and Paraguay	Parana-Paraguay	Not available
<i>Cnesterodon septentrionalis</i>	Rosa & Costa, 1993	Midwestern Brazil: Upper Araguaia River basin (Lucinda 2005)	GBIF (doi: 10.15468/dl.6gbd7e); SpeciesLink; Lucinda (2005)	Tocantins-Araguaia	Tocantins-Araguaia	NT
<i>Cnesterodon</i> sp. A		Southern Brazil: Uruguay River basin and Laguna dos Patos system (Bertaco et al. 2016)	Bertaco et al. (2016)	Laguna dos Patos and Lower Uruguay	South Coastal and Uruguay	Not available
<i>Cnesterodon</i> sp. B		Southern Brazil: Laguna dos Patos system and Tramandaí River basin (Bertaco et al. 2016)	Bertaco et al. (2016)	Laguna dos Patos and Tramandai-Mampituba	South Coastal	Not available
<i>Cnesterodon</i> sp. C		Southern Brazil: Ivaí River	SpeciesLink; Frota et al. (2016);	Upper Parana	Upper Parana	Not available

	basin (Frota et al. 2016; Reis et al. 2020)	Reis et al. (2020)			
<i>Phalloceroslessandrae</i> Lucinda, 2008	Southern Brazil: tributaries of Paranaguá Bay (Lucinda 2008; Thomaz et al. 2019)	GBIF (doi:10.15468/dl.czbxek); SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Sotheastern Mata Atlantica	Central Coastal	LC
<i>Phallocerosanisophallos</i> Lucinda, 2008	Southeastern Brazil: small coastal drainages from Rio de Janeiro State (Souto-Santos et al. 2019)	GBIF (doi: 10.15468/dl.342cxc); FishNet2; SpeciesLink; Lucinda (2008); Souto-Santos et al. (2019); Thomaz et al. (2019)	Sotheastern Mata Atlantica	Central Coastal	LC
<i>Phalloceros aspilos</i> Lucinda, 2008	Southeastern Brazil: Parati-Mirim River basin (Lucinda 2008)	GBIF (doi: 10.15468/dl.rey8pw); SpeciesLink; Lucinda (2008); Souto-Santos et al. (2019); Thomaz et al. (2019)	Sotheastern Mata Atlantica/Fluminense	Central Coastal	LC
<i>Phalloceros buckupi</i> Lucinda, 2008	Southern Brazil: tributaries of Paranaguá and Babitonga Bays (Lucinda 2008; Thomaz et al. 2019)	GBIF (doi:10.15468/dl.2f6fe7); SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Sotheastern Mata Atlantica	Central Coastal	DD
<i>Phalloceros caudimaculatus</i> (Hensel,	Argentina, Southern Brazil, and Uruguay: Laguna dos Patos system, Lower Uruguay	GBIF (doi: 10.15468/dl.gsafnu); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Laguna dos Patos, Lower Parana, Lower Uruguay, and Patos system, Lower Uruguay	Parana-Paraguay, South Coastal, and	LC

1868)	River basin, Tramandaí and Manpituba River basins, and small coastal drainages of Uruguay and Argentina (Lucinda 2008)	(2008); Thomaz et al. (2019) and Tramandai-Mampituba		Uruguay	
<i>Phalloceros elachistos</i> Lucinda, 2008	Southeastern Brazil: Doce, Santa Maria da Vitória, Jucu, and Timbuí River basins, and others coastal drainages of Espírito Santo State (Lucinda 2008)	GBIF (doi: 10.15468/dl.3dw72c); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Northeastern Mata Atlantica	North Coastal	LC
<i>Phalloceros enneaktinos</i> Lucinda, 2008	Southeastern Brazil: small coastal drainages from Rio de Janeiro State (Lucinda 2008; Souto-Santos et al. 2019)	GBIF (doi: 10.15468/dl.rehsp8); SpeciesLink; Lucinda (2008); Souto-Santos et al. (2019); Thomaz et al. (2019)	Sotheastern Mata Atlantica/Fluminense	Central Coastal	LC
<i>Phalloceros harpagos</i> Lucinda, 2008	Argentina, Brazil, and Paraguay: Paraná and Paraguay River basins, and coastal drainages from Itaboapaná (Espírito Santo	GBIF (doi: 10.15468/dl.zgkq4d); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019); Mezzaroba et al. (2021); Frota et	Fluminense, Iguassu, Lower Parana, Northeastern Mata Atlantica, Paraguay, Paraiba do Sul, Ribeira de	Central Coastal, Iguassu, North Coastal, Parana-Paraguay,	LC

	State) to Araranguá (Santa Catarina State) River basins (Lucinda 2008)	al. (2021)	Iguape, Sotheastern Mata Atlantica, and Upper Parana		
<i>Phalloceros heptaktinos</i> Lucinda, 2008	Southern Brazil: Dos Ratos Creek, Jacuí River basin (Lucinda 2008)	SpeciesLink; Lucinda (2008); Bertaco et al. (2016); Thomaz et al. (2019)	Laguna dos Patos	South Coastal	DD
<i>Phalloceros leptokeras</i> Lucinda, 2008	Southeastern Brazil: Paraíba do Sul River basin (Lucinda 2008)	GBIF (doi: 10.15468/dl.ka26rc); SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Paraiba do Sul	Central Coastal	LC
<i>Phalloceros leticiae</i> Lucinda, 2008	Midwestern Brazil: Upper Araguaia River basin (Lucinda 2008)	GBIF (doi: 10.15468/dl.8rqvah); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Tocantins-Araguaia	Tocantins-Araguaia	DD
<i>Phalloceros lucenorum</i> Lucinda, 2008	Southeastern Brazil: Juquiá River drainage, Ribeira de Iguape River basin in São Paulo State (Lucinda 2008)	GBIF (doi: 10.15468/dl.n4kt5d); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Ribeira de Iguape	Central Coastal	LC
<i>Phalloceros malabarbai</i> Lucinda, 2008	Southern Brazil: small creeks near Itapoá in Santa Catarina State (Lucinda 2008)	SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Sotheastern Mata Atlantica	Central Coastal	LC
<i>Phalloceros</i>	Southern Brazil: São João and	GBIF (doi: 10.15468/dl.4jkqy5); Sotheastern Mata Atlantica	Central Coastal	Central Coastal	LC

<i>megapolos</i> Lucinda, 2008	Cubatão River basins and small adjacent drainages (Lucinda2008)	FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)			
<i>Phalloceros mikrommatus</i> Lucinda, 2008	Northeastern Brazil: João de Tiba River basin in Bahia State (Lucinda 2008)	Lucinda (2008); Thomaz et al. (2019)	Northeastern Mata Atlantica	North Coastal	LC
<i>Phalloceros ocellatus</i> Lucinda, 2008	Northeastern and Southeastern Brazil: coastal drainages from Bahia and Espírito Santo States (Lucinda 2008)	GBIF (doi: 10.15468/dl.xc85s2); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Northeastern Mata Atlantica	North Coastal	NT
<i>Phalloceros pellos</i> Lucinda, 2008	Southern Brazil: tributaries of Paranaguá Bay (Lucinda 2008; Thomaz et al. 2019)	GBIF (doi:10.15468/dl.h275t6); SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Sotheastern Mata Atlantica	Central Coastal	LC
<i>Phalloceros reisi</i> Lucinda, 2008	Southeastern Brazil: headwater creeks from the Tietê, Paraíba do Sul and Ribeira de Iguape River basins in São Paulo State (Lucinda 2008)	GBIF (doi: 10.15468/dl.a84fpz); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Paraíba do Sul, Ribeira de Iguape, and Upper Parana	Central Coastal and Upper Parana	LC
<i>Phalloceros spiloura</i> Lucinda, Uruguay, Jacuí, Tramandaí,	Southern Brazil: Iguassu, Laguna dos Patos,	GBIF (doi:10.15468/dl.xpb7dv); SpeciesLink; Lucinda (2008);	Central Coastal, Sotheastern Mata	Iguassu, South	LC

2008	and other coastal River basins (Lucinda 2008; Thomaz et al. 2019)	Bertaco et al. (2016); Thomaz et al. (2019); Mezzaroba et al. (2021)	Atlantica, Tramandai-Mampituba, and Upper Uruguay	Coastal, and Uruguay	
<i>Phalloceros titthos</i> Lucinda, 2008	Southern Brazil: tributaries of Guaratuba and Paranaguá Bays (Lucinda 2008; Thomaz et al. 2019)	GBIF (doi: 10.15468/dl.bu8q62); FishNet2; SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	Sotheastern Mata Atlantica	Central Coastal	LC
<i>Phalloceros tupinamba</i> Lucinda, 2008	Southeastern Brazil: Itamambuca, Macacu and Japuíba River basins (Lucinda 2008; Souto-Santos et al. 2019)	GBIF (doi: 10.15468/dl.2knppz); FishNet2; SpeciesLink; Lucinda (2008); Souto-Santos et al. (2019); Thomaz et al. (2019)	Sotheastern Mata Atlantica/Fluminense	Central Coastal	LC
<i>Phalloceros uai</i> Lucinda, 2008	Southeastern Brazil: Das Velhas River drainage, São Francisco River basin (Lucinda 2008)	GBIF (doi: 10.15468/dl.pqqx3q); SpeciesLink; Lucinda (2008); Thomaz et al. (2019)	S. Francisco	São Francisco	LC
<i>Phalloceros</i> sp. L	Southeastern Brazil: coastal rivers near Ubatuba in São Paulo State (Thomaz et al. 2019)	Thomaz et al. (2019)	Sotheastern Mata Atlantica/Fluminense	Central Coastal	Not available

<i>Phallocerosspp.</i> R	Southeastern Brazil: coastal rivers near Ubatuba in São Paulo State (Thomaz et al. 2019)	Thomaz et al. (2019)	Sotheastern Mata Atlantica/Fluminense	Central Coastal	Not available
<i>Phallotrynus dispilos</i> Lucinda, Rosa & Reis, 2005	Paraguay: middle portions of Parana River basin (Lucinda et al. 2005)	GBIF (doi: 10.15468/dl.8u8bvg); FishNet2; SpeciesLink; Lucinda et al. (2005)	Lower Parana	Parana-Paraguay	Not available
<i>Phallotrynus fasciolatus</i> Henn, 1916	Southern and Southeastern Brazil: Tietê and Paraíba do Sul River basins and coastal rivers in Paraná State (Lucinda et al. 2005; Reis et al. 2020)	GBIF (doi: 10.15468/dl.8vgu4j); FishNet2; SpeciesLink; Lucinda et al. (2005)	Paraiba do Sul, Upper Parana, and Sotheastern Mata Atlantica	Central Coastal and Upper Parana	EN
<i>Phallotrynus jucundus</i> Ihering, 1930	Southeastern Brazil: Pardo River drainage, Grande River basin (Lucinda et al. 2005)	GBIF (doi: 10.15468/dl.xqeeec); SpeciesLink; Lucinda et al. (2005)	Upper Parana	Upper Parana	EN
<i>Phallotrynus pankalos</i> Lucinda, Rosa & Reis, 2005	Midwestern Brazil: creeks from the Iguaçemi River basin (Lucinda & Graça 2015)	GBIF (doi: 10.15468/dl.7ss6me); SpeciesLink; Lucinda et al. (2005); Lucinda & Graça (2015)	Upper Parana	Upper Parana	LC

<i>Phallotrynus psittakos</i> Lucinda, Rosa & Reis, 2005	Paraguay: lower portions of Paraguay River basin (Lucinda et al. 2005)	GBIF (doi: 10.15468/dl.m9qn62); FishNet2; SpeciesLink; Lucinda et al. (2005)	Lower Parana	Parana-Paraguay	Not available
<i>Phallotrynus victoriae</i> Oliveros, 1983	Argentina, Brazil, and Paraguay: Parana, Paraguay and Uruguay River basins (Chuctaya et al. 2018)	GBIF (doi: 10.15468/dl.fvfwt); FishNet2; SpeciesLink; Lucinda et al. (2005); Chuctaya et al. (2018)	Lower Parana, Lower Uruguay, and Upper Parana	Parana-Paraguay, Upper Parana, and Uruguay	NT

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APPENDIX B - Final database with the georeferenced records of cnesterodontins.

Species, Latitude, Longitude	
<i>P. alessandrae</i> , -25.428048, -48.76025	<i>P. caudimaculatus</i> , -29.913088, -51.238855
<i>P. alessandrae</i> , -25.309243, -48.742279	<i>P. caudimaculatus</i> , -30.106503, -51.711402
<i>P. alessandrae</i> , -25.370083, -48.776056	<i>P. caudimaculatus</i> , -32.177094, -52.992275
<i>P. alessandrae</i> , -25.309167, -48.744722	<i>P. caudimaculatus</i> , -29.355643, -50.899212
<i>P. anisophallos</i> , -23.04158, -44.69267	<i>P. caudimaculatus</i> , -30.146466, -52.05749
<i>P. anisophallos</i> , -23.077205, -44.69774	<i>P. caudimaculatus</i> , -31.697451, -52.157471
<i>P. anisophallos</i> , -23.089062, -44.726007	<i>P. caudimaculatus</i> , -29.57851, -53.486604
<i>P. anisophallos</i> , -22.913694, -43.898252	<i>P. caudimaculatus</i> , -30.583153, -52.608709
<i>P. anisophallos</i> , -23.185298, -44.200666	<i>P. caudimaculatus</i> , -29.587813, -51.101224
<i>P. anisophallos</i> , -23.033333, -44.816667	<i>P. caudimaculatus</i> , -31.886838, -52.927551
<i>P. anisophallos</i> , -23.000833, -44.551333	<i>P. caudimaculatus</i> , -29.260087, -53.893243
<i>P. anisophallos</i> , -23.041389, -44.692778	<i>P. caudimaculatus</i> , -31.671581, -51.407589
<i>P. aspilos</i> , -23.329294, -44.738813	<i>P. caudimaculatus</i> , -29.563645, -53.34977
<i>P. aspilos</i> , -23.341133, -44.745828	<i>P. caudimaculatus</i> , -29.092778, -50.625001
<i>P. aspilos</i> , -23.297576, -44.710174	<i>P. caudimaculatus</i> , -31.377222, -52.126944
<i>P. aspilos</i> , -23.2975, -44.71	<i>P. caudimaculatus</i> , -30.62389, -51.545834
<i>P. buckupi</i> , -25.541682, -48.689569	<i>P. caudimaculatus</i> , -30.810804, -51.823153
<i>P. buckupi</i> , -25.640755, -48.533678	<i>P. caudimaculatus</i> , -30.860117, -53.496519
<i>P. buckupi</i> , -26.382754, -48.725827	<i>P. caudimaculatus</i> , -30.720956, -53.681388
<i>P. buckupi</i> , -25.726389, -48.593611	<i>P. caudimaculatus</i> , -30.985071, -53.972204
<i>P. buckupi</i> , -26.382778, -48.725833	<i>P. caudimaculatus</i> , -29.57711, -51.027579
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	<i>P. caudimaculatus</i> , -30.962861, -54.660701

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<i>P. megapolos</i> , -26.220528, -48.746028	<i>P. reisi</i> , -23.70613, -46.298026
<i>P. megapolos</i> , -26.176611, -48.954083	<i>P. reisi</i> , -23.773487, -46.313924
<i>P. megapolos</i> , -26.197111, -48.922222	<i>P. reisi</i> , -23.528632, -45.858884
<i>P. mikrommatos</i> , -16.388371, -39.181826	<i>P. reisi</i> , -23.524031, -45.802525
<i>P. mikrommatos</i> , -16.288056, -39.024722	<i>P. reisi</i> , -23.526943, -45.764464
<i>P. mikrommatos</i> , -16.288056, -39.024722	<i>P. reisi</i> , -23.77174, -46.33286
<i>P. ocellatus</i> , -17.17221, -39.223557	<i>P. reisi</i> , -23.451708, -46.053347
<i>P. ocellatus</i> , -17.060034, -39.184143	<i>P. reisi</i> , -23.842714, -46.327974
<i>P. ocellatus</i> , -19.064016, -40.03509	<i>P. reisi</i> , -23.856408, -46.348333
<i>P. ocellatus</i> , -18.8975, -39.898056	<i>P. reisi</i> , -23.418611, -45.979444
<i>P. ocellatus</i> , -19.780028, -40.1655	<i>P. reisi</i> , -23.856944, -46.348889
<i>P. ocellatus</i> , -18.296389, -39.794444	<i>P. reisi</i> , -23.766389, -46.334722
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<i>P. pellos</i> , -25.209721, -48.434122	<i>P. spiloura</i> , -28.704526, -52.873996
<i>P. pellos</i> , -25.512622, -48.311535	<i>P. spiloura</i> , -27.691216, -50.052568

<i>P. spiloura</i> , -27.69057, -50.164159	<i>P. titthos</i> , -26.382778, -48.725833
<i>P. spiloura</i> , -27.564599, -50.166484	<i>P. tupinamba</i> , -22.485782, -42.659406
<i>P. spiloura</i> , -27.353709, -50.134323	<i>P. tupinamba</i> , -23.42805, -45.128761
<i>P. spiloura</i> , -27.895231, -49.747062	<i>P. tupinamba</i> , -23.417667, -45.113677
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<i>P. spiloura</i> , -27.591183, -50.13189	<i>P. tupinamba</i> , -23.412663, -45.114401
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<i>P. spiloura</i> , -29.365538, -49.749542	<i>P. tupinamba</i> , -23.427778, -45.128611
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<i>P. spiloura</i> , -29.079293, -49.819691	<i>P. uai</i> , -19.522103, -43.745452
<i>P. spiloura</i> , -26.934096, -48.953907	<i>P. uai</i> , -19.64, -43.698056
<i>P. spiloura</i> , -26.934096, -48.953907	<i>Phalloceros</i> sp. L., -23.35, -44.87
<i>P. spiloura</i> , -26.197124, -48.922071	<i>Phalloceros</i> sp. R., -23.3125, -44.880556
<i>P. spiloura</i> , -26.533333, -49.533333	<i>Phalloceros</i> sp. R., -23.352222, -44.853333
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<i>P. spiloura</i> , -27.4925, -48.786389	<i>C. holopterus</i> , -30.6333333333, -56.3833333333
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<i>P. spiloura</i> , -27.279833, -48.939083	<i>C. brevirostratus</i> , -28.641111, -50.573611
<i>P. spiloura</i> , -27.495444, -48.7825	<i>C. brevirostratus</i> , -28.321389, -53.801667
<i>P. spiloura</i> , -30.298389, -51.690278	<i>C. brevirostratus</i> , -28.924722, -53.358333
<i>P. titthos</i> , -25.921277, -48.586985	<i>C. brevirostratus</i> , -29.223611, -50.250278
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<i>P. titthos</i> , -26.205, -48.661167	<i>C. brevirostratus</i> , -27.916667, -50.083333
<i>P. titthos</i> , -25.963167, -48.604333	<i>C. brevirostratus</i> , -28.733333, -50.033333

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C. hypselurus, -23.7233009338379, -51.0971984863281

<i>C. hypselurus</i> , -23.309700012207, -51.3692016601562	<i>P. fasciolatus</i> , -23.309251, -45.973352
<i>C. iguape</i> , -25.1161111, -49.9161111	<i>P. fasciolatus</i> , -23.278201, -45.978309
<i>C. iguape</i> , -24.411667, -48.656944	<i>P. jucundus</i> , -20.7511, -51.6783
<i>C. omorgmatus</i> , -25.8, -52.0	<i>P. jucundus</i> , -21.47, -47.55
<i>C. pirai</i> , -27.006667, -54.847778	<i>P. jucundus</i> , -21.5, -47.5666667
<i>C. raddai</i> , -26.673333, -57.92	<i>P. jucundus</i> , -21.5, -47.5666667
<i>C. raddai</i> , -27.37, -56.72	<i>P. jucundus</i> , -21.6827778, -47.6572222
<i>C. septentrionalis</i> , -17.305555, -53.272778	<i>P. jucundus</i> , -21.4699993133545, -47.5499992370605
<i>C. septentrionalis</i> , -17.333611, -53.248055	<i>P. jucundus</i> , -21.4699993133545, -47.5499992370605
<i>C. septentrionalis</i> , -17.27389, -53.296944	<i>P. jucundus</i> , -21.55056, -47.70028
<i>C. septentrionalis</i> , -17.401388, -53.225555	<i>P. jucundus</i> , -21.55056, -47.70028
<i>C. septentrionalis</i> , -17.418888, -53.233334	<i>P. jucundus</i> , -21.68139, -47.65667
<i>C. septentrionalis</i> , -17.431944, -53.242779	<i>P. jucundus</i> , -20.72944, -48.52139
<i>C. septentrionalis</i> , -17.519722, -53.259167	<i>P. jucundus</i> , -21.4666671753, -47.5499992371
<i>C. septentrionalis</i> , -17.27, -53.414444	<i>P. jucundus</i> , -21.4666671753, -47.5499992371
<i>C. septentrionalis</i> , -17.25, -53.383335	<i>P. jucundus</i> , -21.549999, -47.75
<i>C. septentrionalis</i> , -17.25, -53.383335	<i>P. jucundus</i> , -21.616699, -47.849998
<i>C. septentrionalis</i> , -17.273889, -53.296944	<i>P. jucundus</i> , -21.583333333333, -47.783333333333
<i>C. septentrionalis</i> , -17.431944, -53.242778	<i>P. pankalos</i> , -23.710556, -54.435833
<i>C. septentrionalis</i> , -17.519722, -53.259167	<i>P. pankalos</i> , -23.644722, -55.201389
<i>C. septentrionalis</i> , -17.418889, -53.233333	<i>P. pankalos</i> , -23.0443, -55.1143
<i>C. septentrionalis</i> , -17.3146991729736, -53.2153015136719	<i>P. pankalos</i> , -23.6447222, -55.2013889
<i>C. septentrionalis</i> , -17.3146991729736, -53.2153015136719	<i>P. pankalos</i> , -23.6324996948242, -55.0158004760742
<i>C. septentrionalis</i> , -17.51972, -53.25917	<i>P. psittakos</i> , -27.173498, -56.770692
<i>C. septentrionalis</i> , -17.723611111111, -53.261388888889	<i>P. psittakos</i> , -27.173498, -56.770692
<i>C. septentrionalis</i> , -17.73055555556, -53.283611111111	<i>P. psittakos</i> , -27.3999996185, -56.7999992371
<i>Cnesterodon</i> sp. C, -25.214994, -50.981299	<i>P. psittakos</i> , -27.3999996185, -56.7999992371
<i>Cnesterodon</i> sp. C, -25.0644444, -51.2944444	<i>P. victoriae</i> , -27.38938, -56.841932
<i>P. dispilos</i> , -25.5424995422, -55.8569450378	<i>P. victoriae</i> , -21.31444, -51.83194

P. victoriae, -21.12083, -51.775
P. victoriae, -21.00056, -51.70139
P. victoriae, -31.5833339691, -60.6833343506
P. victoriae, -20.787158, -51.70465
P. victoriae, -25.149609, -54.505736
P. victoriae, -25.585638, -54.616247
P. victoriae, -29.498056, -56.595278

APPENDIX C - Matrix file constructed to run with the parsimony analysis.

APPENDIX D - Six equally most parsimonious trees obtained with the parsimony analysis.

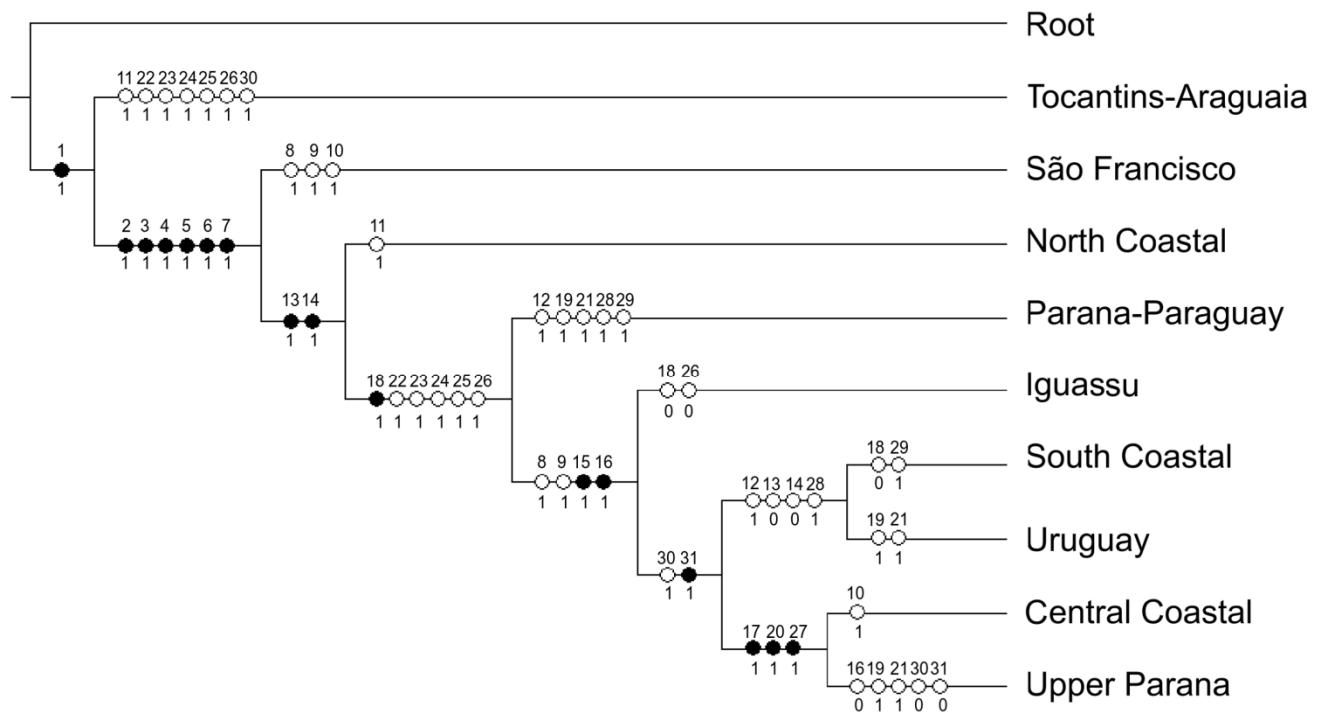


Figure S1 First most parsimonious general cladogram evidencing the results of Brooks Parsimony Analysis and the historical relationships among the delimited areas using Cnesterodontini as a study case. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

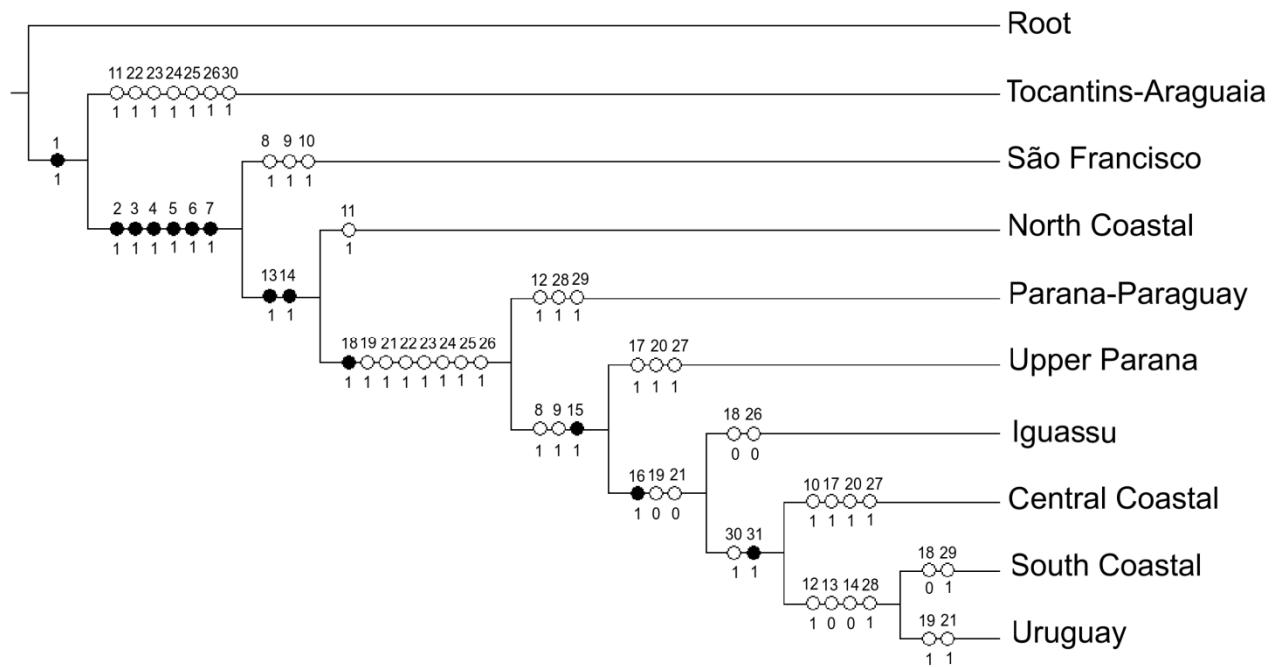


Figure S2 Second most parsimonious general cladogram evidencing the results of Brooks Parsimony Analysis and the historical relationships among the delimited areas using *Cnesterodontini* as a study case. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

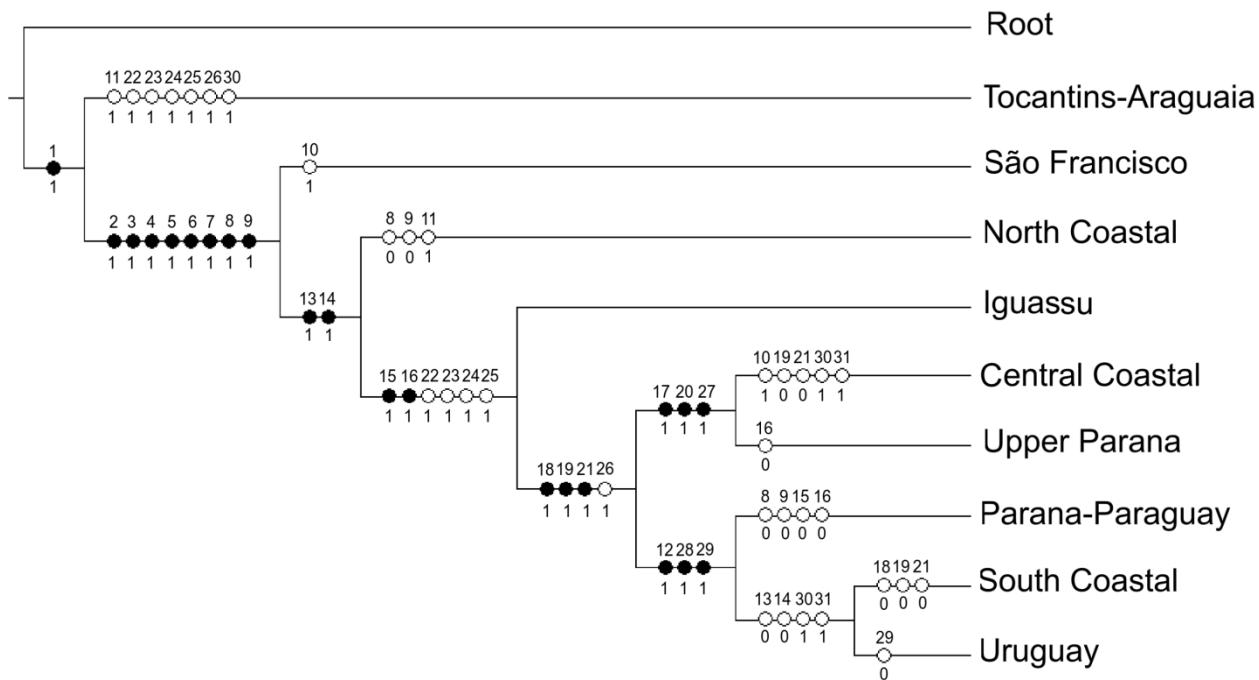


Figure S3 Third most parsimonious general cladogram evidencing the results of Brooks Parsimony Analysis and the historical relationships among the delimited areas using *Cnesterodontini* as a study case. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

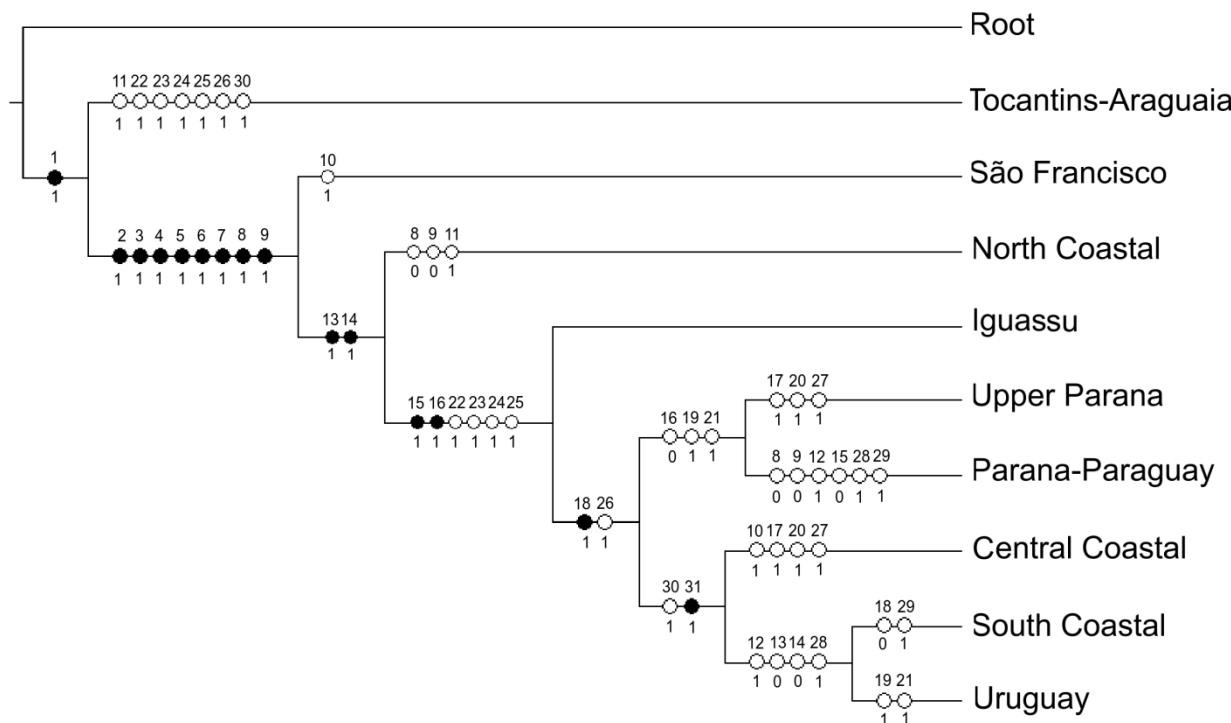


Figure S4 Fourth most parsimonious general cladogram evidencing the results of Brooks Parsimony Analysis and the historical relationships among the delimited areas using *Cnesterodontini* as a study case. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

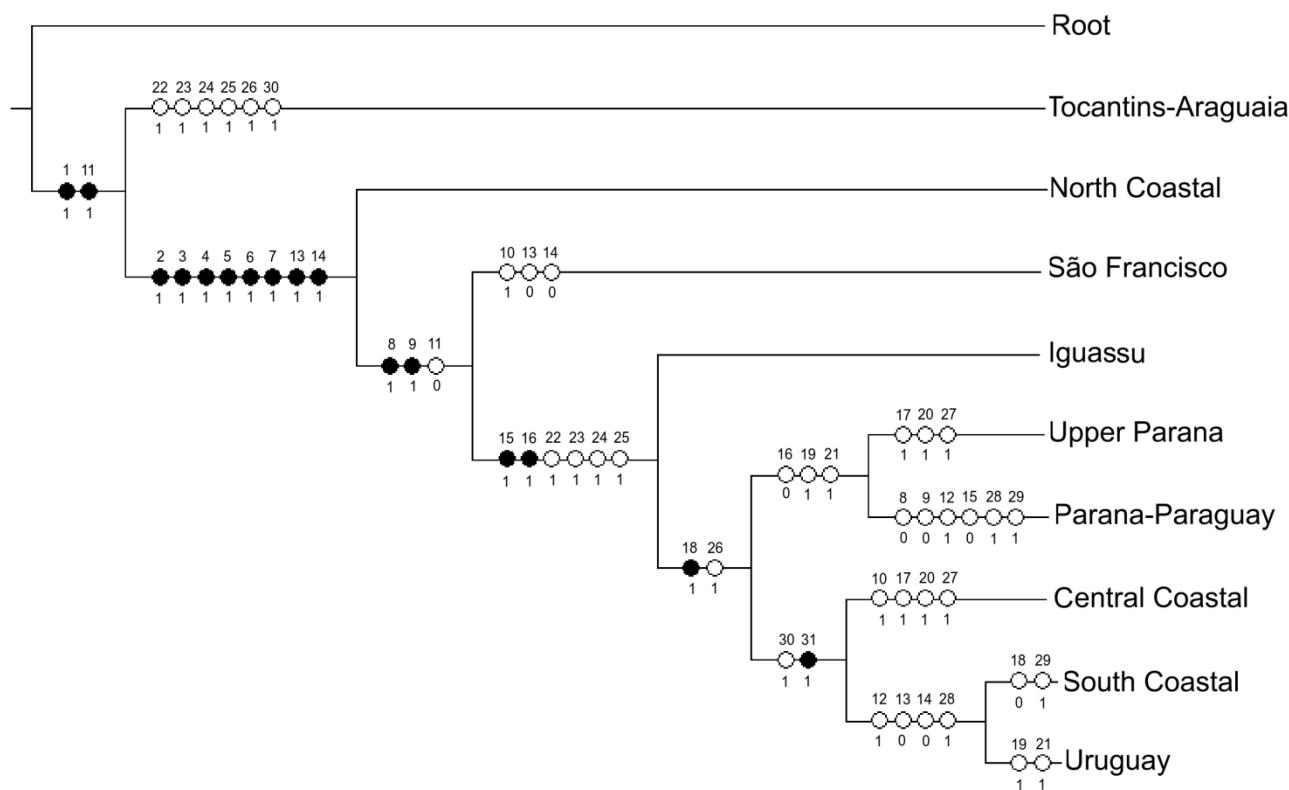


Figure S5 Fifth most parsimonious general cladogram evidencing the results of Brooks Parsimony Analysis and the historical relationships among the delimited areas using Cnesterodontini as a study case. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

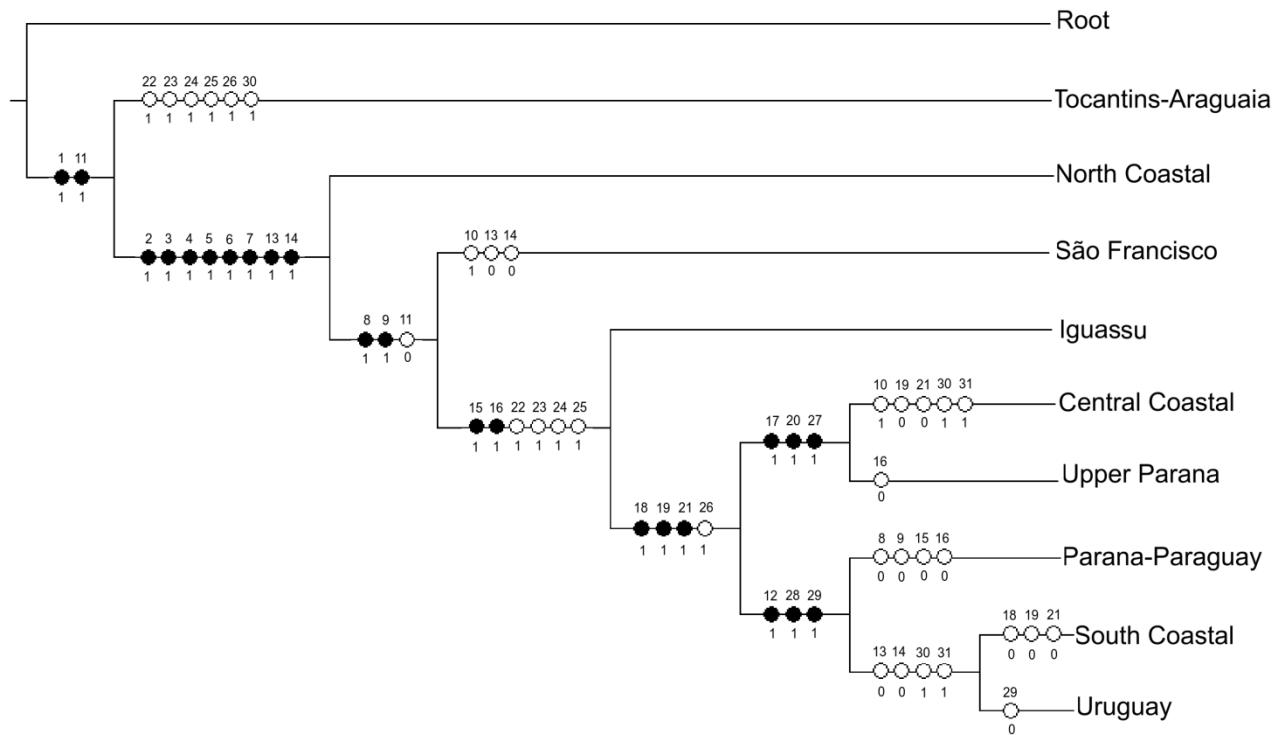


Figure S6 Sixth most parsimonious general cladogram evidencing the results of Brooks Parsimony Analysis and the historical relationships among the delimited areas using *Cnesterodontini* as a study case. Numbers above each dot represent the components numbered from 1 to 31 according to Figure 2.2. Black and white dots are synapomorphies and homoplasies, respectively. Homoplasies are parallelisms (1) or reversals (0).

APPENDIX E - Geographic distribution of the 108 native fish species (including endemics = *) from the Ivaí River basin (Reis, Frota, Deprá, et al., 2020) on its distinct ichthyofaunistic regions delimited by the NMDS axes.

Classification	Regions		
	Upper	Middle	Lower
CHARACIFORMES			
Acestrorhynchidae			
<i>Acestrorhynchus lacustris</i> (Lütken, 1875)	X	X	X
Anostomidae			
<i>Leporinus amablyrhynchus</i> Garavello & Britski, 1987	X	X	X
<i>Leporinus friderici</i> (Bloch, 1794)		X	X
<i>Leporinus octofasciatus</i> Steindachner, 1915	X	X	X
<i>Megaleporinus obtusidens</i> (Valenciennes, 1837)		X	X
<i>Schizodon nasutus</i> Kner, 1858	X	X	X
Bryconidae			
<i>Brycon nattereri</i> Günther, 1864	X		
<i>Brycon orbignyanus</i> (Valenciennes, 1850)		X	X
<i>Salminus brasiliensis</i> (Cuvier, 1816)			X
Characidae			
Aphyocharacinae			
<i>Aphyocharaxanisitsi</i> Eigenmann & Kennedy, 1903			X
<i>Aphyocharax</i> sp.	X		
Characinae			
<i>Galeocharax gulo</i> (Cope, 1870)	X	X	X
Cheirodontinae			
<i>Odontostilbe avanhandava</i> Chuctaya, Bührnheim & Malabarba, 2018			X

<i>Serrapinnusnotomelas</i> (Eigenmann, 1915)		X	X
Stethaprioninae			
<i>Astyanaxlacustris</i> (Lütken, 1875)	X	X	X
<i>Astyanax</i> sp.	X	X	X
<i>Hyphessobryconboulengeri</i> (Eigenmann, 1907)	X		
<i>Moenkhausiabonita</i> Benine, Castro & Sabino, 2004			X
<i>Oligosarcus paranensis</i> Menezes & Géry, 1983	X	X	X
<i>Oligosarcuspintoi</i> Amaral Campos, 1945	X	X	X
<i>Psalidodonbockmanni</i> (Vari & Castro, 2007)	X	X	X
<i>Psalidodon</i> aff. <i>fasciatus</i> (Cuvier, 1819)	X	X	X
Stevardiinae			
<i>Bryconamericuscoeruleus</i> Jerep & Shibatta, 2017	X	X	
<i>Bryconamericus</i> aff. <i>iheringii</i> (Boulenger, 1887)	X	X	
<i>Bryconamicusturiuba</i> Langeani, Lucena, Pedrini & Tarelho-Pereira, 2005	X	X	X
* <i>Diapoma</i> sp.	X		
<i>Piabarchus</i> aff. <i>stramineus</i> (Eigenmann, 1908)	X	X	X
<i>Piabinaargentea</i> Reinhardt, 1867	X	X	X
<i>Planaltinakaingang</i> Deprá, da Graça, Pavanelli, Avelino & Oliveira, 2018	X	X	
Crenuchidae			
<i>Characidiumgomesi</i> Travassos, 1956		X	
* <i>Characidiumheirmostigmata</i> da Graça & Pavanelli, 2008	X	X	
<i>Characidium</i> aff. <i>zebra</i> Eigenmann, 1909		X	X
Curimatidae			

<i>Cyphocharax cf. corumbae</i> (Pavanelli & Britski, 1999)	X	X	
<i>Cyphocharaxnagelii</i> (Steindachner, 1881)		X	X
<i>Steindachnerinainsculpta</i> (Fernández-Yépez, 1948)			X
Erythrinidae			
<i>Hopliasargentinensis</i> Rosso, González-Castro, Bogan, Cardoso, Mabragaña, Delpiani & Díaz de Astarloa, 2018		X	X
<i>Hopliasintermedius</i> (Günther, 1864)	X	X	
<i>Hoplias</i> sp.	X	X	X
Parodontidae			
<i>Apareiodonaffinis</i> (Steindachner, 1879)	X	X	X
<i>Apareiodon piracicabae</i> (Eigenmann, 1907)	X	X	
<i>Apareiodonvladii</i> Pavanelli, 2006	X	X	
<i>Parodonnasus</i> Kner, 1859		X	X
Prochilodontidae			
<i>Prochiloduslineatus</i> (Valenciennes, 1837)		X	X
Serrasalmidae			
<i>Piaractusmesopotamicus</i> (Holmberg, 1887)			X
<i>Serrasalmusmaculatus</i> Kner, 1858			X
GYMNOTIFORMES			
Apterontidae			
<i>Apterontus aff. albifrons</i> (Linnaeus, 1766)		X	X
<i>Apterontus cf. caudimaculosus</i> de Santana, 2003		X	
<i>Apterontusellisi</i> (Alonso de Arámburu, 1957)		X	
SILURIFORMES			

Auchenipteridae			
<i>Glanidiumcesarpintoi</i> Ihering, 1928	X	X	
<i>Tatianeivai</i> (Ihering, 1930)		X	X
Callichthyidae			
<i>Callichthyscallichthys</i> (Linnaeus, 1758)			X
<i>Corydorasaeneus</i> (Gill, 1858)	X	X	X
<i>Corydorasehrhardti</i> Steindachner, 1910	X		
* <i>Corydoraslacrimostigmata</i> Tencatt, Britto & Pavanelli, 2014	X	X	
Cetopsidae			
<i>Cetopsisgobiooides</i> Kner, 1858		X	
Heptapteridae			
<i>Cetopsorhamdiaiheringi</i> Schubart & Gomes, 1959	X	X	X
<i>Heptapterusmustelinus</i> (Valenciennes, 1835)	X	X	
<i>Imparfinisborodini</i> Mees & Cala, 1989	X	X	X
<i>Imparfinismirini</i> Haseman, 1911	X	X	X
<i>Imparfinis schubarti</i> (Gomes, 1956)	X	X	X
<i>Phenacorhamdiatenebrosa</i> (Schubart, 1964)	X	X	X
<i>Pimelodellaavanhandavae</i> Eigenmann, 1917	X	X	
<i>Pimelodellagracilis</i> (Valenciennes, 1835)	X	X	X
<i>Rhamdiaquelen</i> (Quoy & Gaimard, 1824)	X	X	X
Loricariidae			
Hypostominae			
<i>Ancistrus</i> sp.	X	X	
<i>Hypostomusalbopunctatus</i> (Regan, 1908)	X	X	

<i>Hypostomusancistroides</i> (Ihering, 1911)	X	X	X
<i>Hypostomuscommersoni</i> Valenciennes, 1836		X	
<i>Hypostomushermannii</i> (Ihering, 1905)	X	X	
<i>Hypostomusheringii</i> (Regan, 1908)		X	X
<i>Hypostomusmargaritifer</i> (Regan, 1908)	X	X	
<i>Hypostomusnigromaculatus</i> (Schubart, 1964)	X		
<i>Hypostomuspaulinus</i> (Ihering, 1905)	X	X	
<i>Hypostomusregani</i> (Ihering, 1905)		X	
* <i>Hypostomus robertsoni</i> Dias & Zawadzki, 2021	X	X	
<i>Hypostomusstrigaticeps</i> (Regan, 1908)		X	
<i>Hypostomustopavae</i> (Godoy, 1969)		X	
* <i>Hypostomus</i> sp. 2	X	X	
* <i>Hypostomus</i> sp. 3	X	X	
<i>Megalancistrusparananus</i> (Peters, 1881)		X	X
Loricariinae			
<i>Loricaria</i> sp.		X	X
<i>Proloricariaprolixa</i> (Isbrücker & Nijssen, 1978)		X	X
<i>Rineloricarialatirostris</i> (Boulenger, 1900)	X	X	
<i>Rineloricaria pentamaculata</i> Langeani & de Araujo, 1994	X	X	X
Neoplecostominae			
* <i>Neoplecostomus</i> sp.	X	X	
Otothyridinae			
<i>Curculionichthysinsperatus</i> (Britski & Garavello, 2003)		X	
* <i>Curculionichthysoliveirai</i> (Roxo, Zawadzki & Troy,	X	X	X

2014)			
<i>Hisonotus francirochai</i> (Ihering, 1928)		X	
* <i>Hisonotuspachysarkos</i> Zawadzki, Roxo & da Graça, 2016	X	X	
<i>Otothyropsisbiamnicus</i> Calegari, Lehmann & Reis, 2013		X	X
Pimelodidae			
<i>Iheringichthyslabrosus</i> (Lütken, 1874)	X	X	X
<i>Pimelodusmicrostoma</i> Steindachner, 1877	X		
<i>Pimelodusparanaensis</i> Britski & Langeani, 1988		X	
<i>Pseudoplatystomacorruscans</i> (Spix & Agassiz, 1829)			X
<i>Sorubimlima</i> (Bloch & Schneider, 1801)			X
<i>Steindachneridionscriptum</i> (Miranda-Ribeiro, 1918)		X	X
Pseudopimelodidae			
<i>Rhyacoglanisparanensis</i> Shibatta & Vari, 2017		X	
Trichomycteridae			
<i>Cambevadavisi</i> (Haseman, 1911)	X	X	X
<i>Cambevadiabola</i> (Bockmann, Casatti & de Pinna, 2004)	X		
* <i>Cambevahoracioi</i> Reis, Frota, Fabrin & da Graça, 2019	X	X	
SYNBRANCHIFORMES			
Synbranchidae			
<i>Synbranchusmarmoratus</i> Bloch, 1795	X	X	X
CICHLIFORMES			
Cichlidae			
<i>Cichlasomaparanaense</i> Kullander, 1983		X	X

<i>Crenicichlabritskii</i> Kullander, 1982		X	X
<i>Crenicichlaharoldoi</i> Luengo & Britski, 1974		X	
<i>Crenicichlajaguarensis</i> Haseman, 1911		X	
<i>Geophagusiporangensis</i> Haseman, 1911	X	X	
CYPRINODONTIFORMES			
Poeciliidae			
* <i>Cnesterodon</i> sp.	X		
<i>Phallocerosharpagos</i> Lucinda, 2008	X	X	X