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INFLUÊNCIA DA HETEROGENEIDADE ESPACIAL, COBERTURA VEGETAL E ALTITUDE NA DIVERSIDADE DE PEIXES EM DUAS BACIAS NEOTROPICAIS

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Dourados – MS
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"Com paciência e perseverança muito se alcança."

Theophile Gautier

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Sumário

RESUMO

Riachos são sistemas ecológicos muito importantes que promovem uma heterogeneidade estrutural responsável por manter a fauna aquática. A altitude, a integridade vegetal e a heterogeneidade espacial são alguns dos principais fatores que influenciam a diversidade ictiofaunística, sendo imprescindíveis à manutenção das características naturais das assembleias aquáticas. Assim, o objetivo deste trabalho foi testar a hipótese de que a diversidade nas assembleias de peixes de riachos em duas bacias hidrográficas neotropicais é influenciada pela heterogeneidade espacial, integridade da vegetação e altitude. Amostragens foram realizadas em 80 locais, 32 na bacia do Apa e 48 na do Amambai. Para quantificar a importância da altitude, cobertura vegetal e heterogeneidade espacial sobre a riqueza rarefeita e equitabilidade, foram realizadas análises de covariância (ANCOVA), tendo as bacias hidrográficas como fator. Foram coletados 9.298 indivíduos, distribuídos em 117 espécies. *Astyanax* sp1 (19,21%) foi a espécie mais abundante na bacia do rio Apa, seguida por *Odontostilbe pequira* (14,47%). Por outro lado, *Astyanax* aff. *paranae* (29,60%) foi a espécie mais abundante na bacia do rio Amambai, seguida por *Phalloceros harpagos* (22,94%). Constatamos através da ANCOVA que a riqueza rarefeita não foi significativamente influenciada pela heterogeneidade espacial (F=0,04; p=0,699) e vegetação ripária (F=0,044; p=0,237), contudo, foi influenciada pela altitude (F=24,129; p<0,001) e diferiu entre bacias (F=5,695; p=0,019). De forma similar, a equitabilidade não apresentou influência significativas em relação à heterogeneidade espacial (F=0,123; p=0,727) e vegetação ripária (F=0,172; p=0,6793), entretanto, apresentou diferenças entre altitude (F=11,817; p<0,001) e bacias (F=8,532; p=0,004). A partir dos resultados, podemos inferir que a diversidade de espécies difere entre bacias e ao longo do gradiente longitudinal.

Palavras-chave: Ictiofauna, Bacia do Rio Apa, Bacia do Rio Amambai.

ABSTRACT

Streams are important ecological systems, promoting a structural heterogeneity responsible for maintaining the aquatic fauna. Riparian vegetation integrity and spatial heterogeneity are one of the main factors affecting the freshwater fish diversity, being necessary to maintaining the natural characteristics of aquatic assemblages. Thus, the aim of this work was to test the following hypothesis: is freshwater fish diversity influenced by spatial heterogeneity, vegetation integrity and altitude in streams of two neotropical river basins. Samplings were carried out in 80 sites, 32 in Apa and 48 in Amambai river basin. In order to quantify the importance of altitude, vegetation integrity and spatial heterogeneity on rarefied richness and evenness, covariance analysis (ANCOVA) were performed, using the river basins as a factor. A total of 9,298 fish were collected, belonging to 117 species. *Astyanax* sp1 (19.21%) was the most abundant species in Apa river basin, followed by *Odontostilbe pequira* (14.47%). On the other hand, *Astyanax* aff. *paranae* (29.60%) was the most abundant species in Amambai river basin, followed by *Phalloceros harpagos* (22.94%). After performing the covariance analysis (ANCOVA), the rarefied richness had no significant influence on the spatial heterogeneity (F=0.04; p=0;699) and riparian vegetation (F=0.044; p=0.237), but it had significant influence of altitude (F=24.129; p<0.001) and presented difference between river basins (F=5.695; p=0.019). Similarly, the evenness had no significant influence on spatial heterogeneity (F=0.123; p=0.727) and riparian vegetation (F=0.172; p=0.679), however, it presented influence of altitude (F=11.817; p<0.001) and between river basins (F=8.532; p=0.004). According to our findings, we can infer that freshwater fish species diversity differs between river basins throughout the longitudinal gradient.

Key words: Ichthyofauna, Apa River Basin, Amambai River Basin.

CAPÍTULO 1 - CONSIDERAÇÕES GERAIS

Rios, riachos e zonas ripárias

A água é um recurso precioso e imprescindível, e por ser basicamente precursora da vida em todo o planeta, sustenta a biodiversidade, sendo recurso estratégico na produção de alimentos e integrante essencial em todos os ciclos naturais (TUNDISI & MATSUMURA-TUNDISI, 2005). Apesar disso, de toda a água existente no planeta, apenas 0,009% é doce e está disponível em ambientes lóticos e lênticos (TUNDISI & TUNDISI, 2011).

Dentre os ecossistemas lóticos, destacam-se os rios, pois são considerados sistemas longitudinais (cabeceira-foz) conectados e dependentes dos demais componentes do ecossistema aos quais estão inseridos (COX & MOORE, 2011). Sendo caracterizados por apresentarem fluxo unidirecional, padrões de zonação e altos índices de relações ecológicas intra e interespecíficas, fluxo de energia e ciclagem de materiais e elementos químicos, bem como interações entre componentes bióticos e abióticos. Este padrão é regulado por sistemas orgânicos de energia em que processos ecológicos ocorridos em regiões de nascente influenciam diretamente regiões de foz, onde terminam os rios (MARGALEF, 1983; CROSS et al., 2013).

Outros sistemas ecológicos de grande importância, são os riachos, pois, apesar de seu tamanho, são responsáveis pela criação de uma heterogeneidade estrutural que é responsável pela manutenção da grande parte da diversidade da fauna aquática (LIMA & GASCON, 1999).

Riachos são ecossistemas caracterizados por uma alta variabilidade ambiental apresentando cursos d'água relativamente pequenos, constituídos por regiões denominadas como mosaicos, devido as suas diferentes categorias de hábitat, com regiões de "riffles", "pools" e "runs", termos que se referem a regiões de corredeiras, poços e águas correntes mais profundas do que corredeiras (CENEVIVA-BASTOS & CASATTI, 2007).

Muitas vezes estes ambientes são submetidos às mais variadas pressões, em consequência da contínua demanda humana por água, tanto para a indústria e a agricultura, quanto para própria subsistência. Devido à sua dimensão física reduzida, alterações antrópicas, como poluição, degradação de hábitats, introdução de espécies exóticas e

ocupação de zonas ripárias, têm ações negativas e imediatas em suas bacias de drenagem (DUDGEON et al., 2006).

Nota-se grande perda na integridade de riachos devido à ocupação de zonas ripárias (PAULA & GESSNER, 2010). Fato que vem sendo base para diversos trabalhos, que buscam evidenciar a importância da zona ripária para a integridade de riachos, uma vez que ela é condição necessária na estabilidade do ambiente, e essencial para o fornecimento de abrigo e nutrientes para diferentes organismos (SANTOS et al., 2015; LOBÓN-CERVIÁ et al., 2016; CASATTI et al., 2012; MERRITT et al., 2012).

A mata ripária é determinante na estabilidade de riachos, por evitar o assoreamento e erosão das margens, além de servir como barreira física, também funciona como um bloqueio para a entrada de sedimentos, fertilizantes e pesticidas que podem ser levados aos corpos d'água pela chuva. Esse bloqueio evita possíveis eventos de eutrofização nestes ambientes (ALLAN, 2004; CASATTI et al., 2012; SANTOS et al., 2015).

Destaca-se ainda como função desta região de mata, ser fonte de abrigo para a fauna de riachos, contribuindo para a entrada de material alóctone, com galhos, troncos e folhas (CASTRO & CASATTI, 1997; LOWE-MCCONNELL, 1999; UIEDA & MOTTA, 2007). Estes materiais são a principal fonte de energia, principalmente em riachos de cabeceira, pois fornecem abrigo para macroinvertebrados aquáticos (KIKUCHI & UIEDA, 2005) que são o principal recurso alimentar para a ictiofauna nestes ambientes.

Desta forma, em riachos de pequeno porte, a vegetação ripária está totalmente relacionada ao funcionamento e a manutenção da biodiversidade local, pois itens alóctones são fundamentais para a ictiofauna, seja de maneira direta, por meio de material vegetal e insetos que caem no leito dos riachos, ou indireta, por meio do consumo de macroinvertebrados que utilizam itens alóctones como recurso alimentar.

Caracterização da área de estudo

Sub-bacia do Rio Apa

A sub-bacia do Rio Apa é uma das sub-bacias componente do Alto Rio Paraguai. Ela está localizada no extremo sul da bacia do alto rio Paraguai, na fronteira entre o estado de Mato Grosso do Sul com o Paraguai. Compreendendo uma área física de 12.181,32 km² e possuindo uma extensão de aproximadamente 500 km de sua nascente, na Cabeceira do Rio Apa, localizada no distrito do município de Ponta Porã, até desaguar na margem esquerda

do Rio Paraguai, a sub-bacia do rio Apa abrange sete munícipios brasileiros (Ponta Porã, Antônio João, Bela Vista, Caracol, Porto Murtinho, Bonito e Jardim) e dois departamentos paraguaios (Amambay e Concepcíon) (SILVA et al., 2015).

A bacia é formada em um terreno acidentado e conta com dezenas de quedas d'água que atuam como barreiras para a migração de peixes na região (SÚAREZ et al., 2007). A bacia encontra-se em boas condições ambientais devido aos níveis populacionais serem relativamente baixos ao seu entorno (CHERNOFF et al., 2001).

Sub-bacia do Rio Amambai

A sub-bacia do Rio Amambai é componente do Alto Rio Paraná e está localizada na região Sudeste de Mato Grosso do Sul, possuindo uma extensão de aproximadamente de 290 km desde sua nascente até sua foz, cruzando nove municípios (Amambai, Aral Moreira, Caarapó, Iguatemi, Itaquirai, Juti, Laguna Carapã, Naviraí e Ponta Porã) (MOLINA et al., 2014).

Biodiversidade em rios e riachos

Os peixes constituem um grupo muito diversificado, constituído por mais de 34.000 espécies, o que representa mais da metade de todas as 60.000 espécies de vertebrados viventes (NELSON, 2016). Somente na América do Sul existem cerca 5.160 espécies válidas, o que representa 28% de todos os peixes já registrados (REIS et al., 2016).

A ictiofauna de água doce da América do Sul é a mais diversa do planeta, contando com aproximadamente 5.160 espécies de peixes descritas, concentradas em 739 gêneros, 69 famílias e 20 ordens. Este número representa cerca de um terço de toda fauna de peixes do mundo (REIS et al., 2016). Dentro dessa grande diversidade, cerca de 3.599 espécies concentram-se apenas nas três maiores bacias hidrográficas da América do Sul: a bacia Amazônica, a do Orinoco e a bacia do Paraná-Paraguai (também chamada de bacia de La Plata), que juntas abrangem 11.300.000 km^2 (REIS et al., 2016, ALBERT & REIS, 2011).

A bacia do Paraná-Paraguai é a segunda maior bacia hidrográfica e terceira mais diversificada da América do Sul, abrangendo Argentina, Bolívia, Brasil, Paraguai e Uruguai, banhando também o bioma do Pantanal que está situado na divisa entre Bolívia, Brasil e Paraguai, sendo a maior planície alagada do planeta. A bacia é representada por 44 famílias, 242 gêneros e 924 espécies de peixe, sendo que 444 espécies (48%) são endêmicas. Devido

ao seu tamanho, a bacia do Paraná- Paraguai é subdivida em outras bacias: alto rio Paraná, alto rio Paraguai e Uruguai em sua porção final, representando 48,7%, 35,3% e 16,0%, respectivamente, da bacia (ALBERT & REIS, 2011).

O Estado de Mato Grosso do Sul é banhado por duas das principais bacias do sistema hidrográfico Paraná-Paraguai, as bacias do Alto Rio Paraná, em sua porção leste, e a bacia do Alto Rio Paraguai, a oeste (FROELICH et al., 2017).

A fauna íctica do estado corresponde, até o momento, a 356 espécies de peixes, 201 espécies ocorrendo na região drenada pelo Alto Rio Paraná, enquanto o Alto Rio Paraguai apresenta 257 espécies (FROELICH et al., 2017). Apesar do elevado número de espécies conhecidas no estado, essa riqueza pode ser ainda maior, uma vez que a composição e diversidade de espécies habitantes de riachos, sobretudo nas regiões de cabeceira do estado, são ainda pouco estudadas.

Grande parte das espécies de peixes de água doce possuem preferência por riachos (NELSON, 2016). Segundo estimativas de OYAKAWA & MENEZES (2011), realizadas no estado de São Paulo, espécies de pequeno porte, que são encontradas principalmente em riachos, são responsáveis por 80% da diversidade íctica.

Neste sentido, o conhecimento da diversidade e organização das comunidades de espécies de riachos deveria ser considerado como prioridade, tendo em vista suas demandas ecológicas especificas, seu alto grau de endemismo e o elevado potencial de descoberta de novas espécies (CASTRO & MENEZES, 1998; NELSON, 2016).

Determinar um padrão de distribuição de organismos aquáticos e relacioná-lo com o gradiente ambiental e espacial sempre foi um dos principais objetivos da ecologia de sistemas aquáticos continentais (THORP et al., 2006). Desta forma, conceitos e teorias foram desenvolvidas e servem como alicerce para a atual compreensão destes padrões. Vannote et al. (1980) propuseram o conceito do "rio contínuo" (*River Continuum Concept* - RCC) que propõem que uma rede de drenagem é definida como um gradiente longitudinal contínuo de diferentes condições físicas e químicas no qual as estratégias adotadas por organismos que ali habitam estão intimamente ligadas ao sistema fluvial, que por sua vez é caracterizado pelos atributos físicos da rede de drenagem como: altitude, profundidade, largura, temperatura e entre outros. O conceito de Vannote et al. (1980) diz que os sistemas aquáticos lóticos são contínuos de transformações longitudinais, porém não incluiu efeito de pulsos de

inundações (THORP et al., 2006). Um outro conceito complementar ao *River Continuum Concept* é o *Process Domain Concept* – PDC ou "domínio de processos" elaborado por Montgomery (1999). Neste conceito os corpos d'água são apresentados como resultados de condições ambientais já citadas no RCC e também processos geomorfológicos representados pela geologia, topografia e clima local. Estes dois conceitos trazem como base a importância da vegetação ripária, especialmente em riachos de primeira ordem, considerados heterotróficos, já que a heterotrofia neste sistema é resultado do sombreamento promovido pela mata ripária, que ameniza a incidência de raios solares e consequente produção primária (VANNOTE et al., 1980; MONTGOMERY, 1999).

Outro importante fator que explica as características das assembleias em riachos é a heterogeneidade espacial ambiental (KERR & PACKER, 1997) que pontua que em ecossistemas com maiores variações de características físicas ambientais, ou seja, maior disponibilidade de potenciais habitats, também há maior riqueza de espécies, uma vez que haveria menor sobreposição de nichos. Vários outros estudos têm apontado fatores que podem atuar em sinergia para a estruturação das comunidades aquáticas e algumas delas ainda carecem de dados empíricos para testá-las.

Estudos avaliando a influência dos parâmetros físico-químicos (MELO et al., 2009; DIAS & TEJERINA-GARRO, 2010) e da heterogeneidade espacial (WOHL, 2016; PALLER et al., 2016) sobre a composição da assembleia de peixes tem provado sua eficácia, sugerindo que os padrões de diversidade são resultados da interação entre assembleias com fatores locais e regionais (LEIBOLD et al., 2004). Embora estudos sobre peixes de riacho ainda serem escassos, deve-se considerar sua importância como preditores de processos ecossistêmicos. Neste sentido, a avaliação dos padrões de diversidade por variáveis ambientais em ambientes submetidos as interferências antrópicas podem contribuir para mudanças de maiores escalas em processos ecológicos.

Desta forma, o objetivo geral do estudo foi avaliar se altitude, a heterogeneidade espacial e a vegetação ripária influenciam no padrão de diversidade de assembleias de peixes das sub-bacias do Rio Apa, Alto Rio Paraguai, e do Amambai, Alto Rio Paraná.

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CAPÍTULO 2 – INFLUENCE OF SPATIAL HETEROGENEITY, RIPARIAN VEGETATION AND ALTITUDE ON FISH SPECIES DIVERSITY IN STREAMS OF TWO NEOTROPICAL BASINS

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Abstract:

Streams are important ecological systems, promoting a structural heterogeneity responsible for maintaining the aquatic fauna. Riparian vegetation integrity and spatial heterogeneity are one of the main factors affecting the freshwater fish diversity, being necessary to maintaining the natural characteristics of aquatic assemblages. Thus, the aim of this work was to test the following hypothesis: is freshwater fish diversity influenced by spatial heterogeneity, vegetation integrity and altitude in streams of two neotropical river basins? Samplings were carried out in 80 sites, 32 in Apa and 48 in Amambai river basin. In order to quantify the importance of altitude, vegetation integrity and spatial heterogeneity on rarefied richness and evenness, covariance analysis (ANCOVA) were performed, using the river basins as a factor. A total of 9,298 fish were collected, belonging to 117 species. *Astyanax* sp1 (19.21%) was the most abundant species in Apa river basin, followed by *Odontostilbe pequira* (14.47%). On the other hand, *Astyanax* aff. *paranae* (29.60%) was the most abundant species in Amambai river basin, followed by *Phalloceros harpagos* (22.94%). After performing the covariance analysis (ANCOVA), the spatial heterogeneity (F=0.04; p=0;699) and riparian vegetation (F=0.044; p=0.237) had no significant influence on rarefied richness, but altitude (F=24.129; p<0.001) had significant influence on the rarefied richness and presented difference between river basins (F=5.695; p=0.019). Similarly, spatial heterogeneity (F=0.123; p=0.727) and riparian vegetation (F=0.172; p=0.679) had no significant influence on evenness, however, altitude (F=11.817; p<0.001) presented influence and it was observed differences between river basins

(F=8.532; p=0.004). According to our findings, we can infer that freshwater fish species diversity differs between river basins throughout the longitudinal gradient.

Keywords: Freshwater fish, richness, Upper Paraguay River Basin, Apa river, Upper Paraná River Basin.

INTRODUCTION

There are approximately 34,700 species of freshwater fish (Eschemeyer & Fong 2018), with more than 5,160 species described in South America (Reis *et al.*, 2016). Brazil has the largest hydrographic system in the world, as well as one of the richest and most diverse freshwater ichthyofaunas (Menezes, 1996; Magalhães *et al.*, 2011), with approximately 3,500 valid species.

The Paraná-Paraguay river basin is the second most important river basin in Brazil by area and fish diversity, with more than 924 fish species recognized (Reis *et al.*, 2016). However, its species composition is still not fully known as a consequence of the irregular distribution of sampling (Carvalho & Langeani, 2013; Froehlich *et al.*, 2017). The majority of recent described species are small (less than 15cm) and occupy mainly streams (Castro, 1999; Langeani *et al.*, 2005).

Streams are important ecological systems, promoting a structural heterogeneity responsible for maintaining the aquatic fauna (Lima & Gascon, 1999). These environments are structurally complex and varied and can be divided into mosaics, with riffle, pool and potions regions, substantially increasing the availability of microhabitats (Langeani *et al.*, 2005; Brown, 2007), which are delimited by different combinations of flow, depth and substrate composition (Lemes & Garutti, 2002; Camana *et al.*, 2016). Heterogeneous environments provide more resources to communities, increasing the availability of niches and, therefore, having greater levels of species diversity (Silva *et al.,* 2010)

Another important factor in the maintenance of water regime, environmental stability and the provision of resources for the ichthyofauna in streams is the riparian vegetation, which may compromise the integrity of streams microhabitats when absent (Ferreira & Casatti, 2006). In addition, the quotient between channel area and drainage area in streams is low, making the terrestrial environment to have great influence on the ichthyofauna in these ecosystems (Karr & Schlosser, 1978).

Streams serve as shelter for species with restricted distribution and that, sometimes, have not even been known by science (Araújo & Tejerina-Garro, 2007). As riparian vegetal integrity and heterogeneity are one of the main factors affecting the freshwater fish diversity, it is necessary to maintain the natural characteristics around streams (Aquino *et al.*, 2009).

Therefore, the aim of this work was to answer the following question: Are richness and evenness in fish assemblages of Apa, Upper Paraguay, and Amambai, Upper Paraná, river basins influenced by spatial heterogeneity, riparian vegetation and altitude? Our hypothesis is that spatial heterogeneity and vegetation cover are positively related to fish diversity and altitude (longitudinal position) and fish diversity is negatively influenced by them, independent of differences in species composition between sampled basins.

MATERIAL AND METHODS

Study site

In Mato Grosso do Sul State, there is an interface between two of the main river basins of Brazil - Upper Paraná River and Upper Paraguay River basins. Although these river basins belong to the same hydrographic system (Paraguay-Paraná system), they have marked differences in species composition, with a strong relationship between upper Paraguay and Amazon basins (Albert & Reis, 2011). In this study, the sub-basins of Apa (Paraguay) and Amambai (Paraná) river were sampled.

The Amambai River basin has an extension of approximately 354 km (Molina *et al.*, 2014). Located in the southeast part of Mato Grosso do Sul State, it is part of the Upper Paraná River Basin. The Amambai River Basin is associated with several kinds of land use and human occupation, including agricultural areas (e.g., pastures and sugar cane crops), urban areas (e.g., industrial buildings and urban infrastructure) and farms, which have reduced the native vegetation cover (Viana *et al.*, 2017).

Located in the southwest part of Mato Grosso do Sul state, the Apa River Basin is part of the Upper Paraguay River Basin, and it has an extension of approximately 500 km (Silva *et al*., 2015). Although agriculture, cattle ranching and logging are the main activities in the area, human population levels are relatively low in this basin (Chernoff *et al.*, 2001).

Sampling

Fish were caught from 2014 to 2016 in 80 sites, 32 in the Apa and 47 in the Amambai sub-basins distributed along the entire basins (Figure 1).
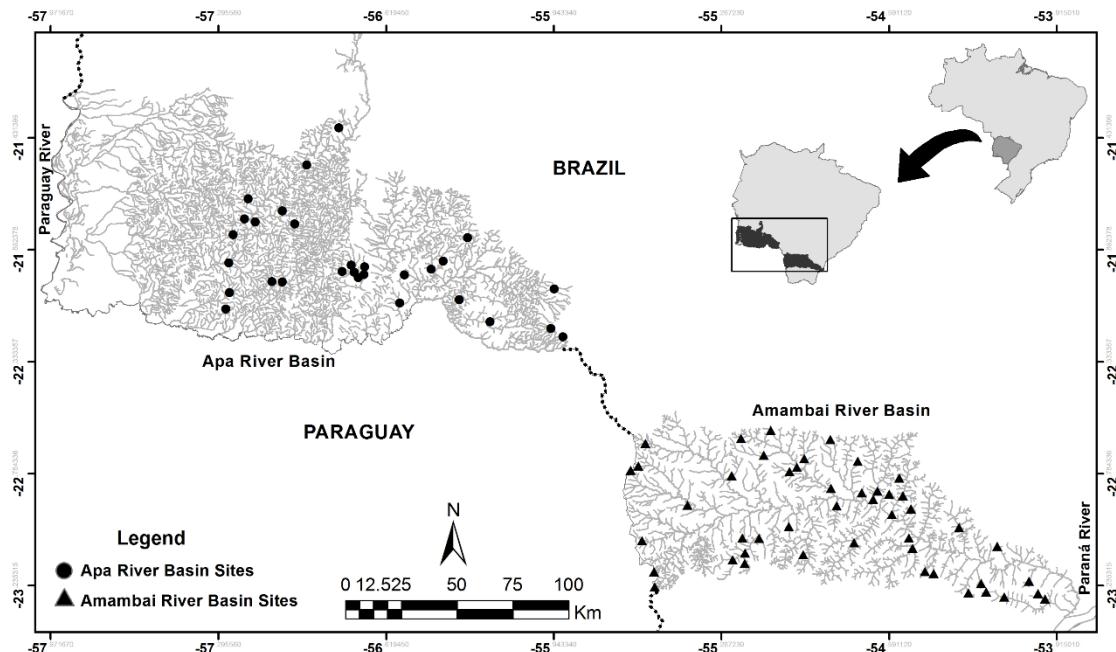
Figure 1. Sampled sites of Apa sub-basin, Upper Paraguay river basin, and Amambai sub-basin, Upper Paraná river basin, Brazil.

The samples were taken mainly during the daylight, with a rectangular sieve measuring 1.2 x 0.8 m (2 mm mesh size). Streams with larger volume were also sampled, using a seine net 1.5 x 5 m (2 mm mesh size) and gillnets with different mesh sizes (15, 20, 30, 40 and 50 mm) between adjacent knots. At each location, samplings were performed in stretches of approximately 100 m length.

In the field, collected fish were fixed in 10% formaldehyde and after 72 hours they were transferred to 70% alcohol. The identification was based on Britski *et al.* (2007) and Graça & Pavanelli (2007) as well as consultations with experts from Universidade Estadual de Maringá (UEM-NUPELIA) and the ichthyological museum of the Universidade Federal de Mato Grosso do Sul (UFMS) whenever necessary.

At each location, the following environmental variable were measured: geographical coordinates, altitude, water speed (m/s), depth (m) and width (m). In addition, sediment granulometry and presence and absence of floating or submerged macrophytes were obtained. Stream depth, width, water speed, sediment granulometry, macrophytes cover (floating and submerged) were obtained at five approximately equidistant points in each sampled stretch (100m). Sediment granulometry measurements were made using an arbitrary classification scale where 0: corresponds to silt/clay; 1: sand/organic matter; 2: gravel/pebble; 3: boulder and 4: blocks and rocks. Similarly, the quantification of the abundance of macrophytes in each site followed the classification in which 0: corresponded to absence of vegetation; 1: < 20% of vegetation; 2: from 20 until <40% of vegetation; 3: from 40 until <60% of vegetation and 4: more than 60% of vegetation.

The riparian vegetation along the sampling sites was determined by a supervised classification of land using LANDSAT 8 imagery, from 2014, 2015 and 2016. It was created buffers with 1 km radius at each site, in which the main forms of land use in the basin were quantified: agriculture and livestock farming, built-up area, forest fragments and wetlands. For the interpretation of aerial images, a visual classification was used by selecting polygons in the areas of interest, calculating their areas and percentages for each buffer. All land use analyses were performed on QGIS 2.8.2 (QGIS Development team, 2015).

Data Analysis

Due to the difference in number of individuals collected in each stream, we used rarefied richness and evenness as diversity estimators of fish assemblages (Gotelli & Graves, 1996). Evenness was defined as H'/Hmax, where H' is the Shannon diversity index and Hmax (maximum diversity) is the logarithm of species richness, both estimates were converted into Log2 values.

A Principal Coordinates Analysis (PCoA) was performed to visualize differences in species composition among samples sites into and between sub-basins using a Bray-Curtis coefficient and relative abundance of sampled species. A distance-based permutational multivariate analysis of variance (PERMANOVA) was also performed to quantify differences in species composition (Anderson, 2001). The significance of statistical

difference was estimate using 999 permutations. This procedure was made using "*adonis*" function in vegan package (Oksanen *et al.,* 2017).

Spatial environmental data (width, depth, water speed, sediment, floating macrophytes and submerged macrophytes) was used to construct an index that expressed the spatial heterogeneity in each stream site, using a centroid mean distance in a principal coordinates analysis to each of the five measurements. Then, after standardization of variables, we performed a PCoA using Euclidean distance, comparing each of individual measure in relation to site centroid. This procedure was made using the "*betadisper*" command in vegan package.

In order to quantify the importance of environmental variables on rarefied richness and evenness we performed analysis of covariance (ANCOVA), using rarefied richness and evenness as response variables, sub-basins as factor and spatial heterogeneity, altitude and riparian vegetation cover as covariables. The vegetation cover (%) was previously transformed in squared root arcsin.

RESULTS

A total of 9,298 individuals belonging to 6 orders, 25 families and 117 species were caught in streams at Amambai and Apa River sub-basins. Streams at Amambai river basin presented 4,067 individuals (53 species), while Apa streams presented 5,231 individuals, belonging to 77 species. The species accumulation curve (Figure 2) showed that, regardless of the number of sites sampled, the greatest richness is observed in the Apa river basin.
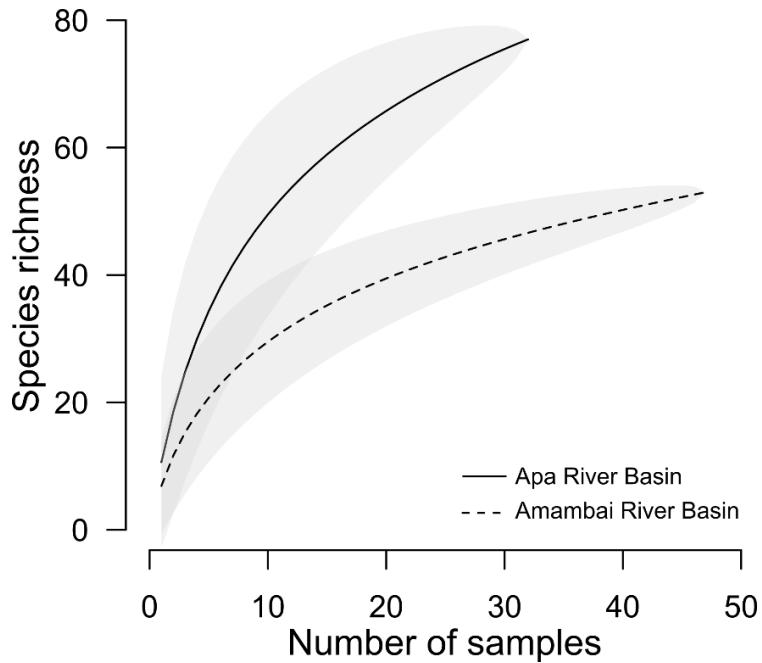
Figure 2. Species accumulation curve based on fish sampled from 2104 to 2016 in streams of Apa sub-basin, Upper Paraguay River basin, and Amambai sub-basin, Upper Paraná River basin, Brazil.

Characiformes and Siluriformes were the richest orders in both basins (Apa River Basin: 44 and 25 species, respectively; Amambai River Basin: 26 and 16, respectively). Regarding to abundance, Characiformes predominated in both basins: Apa River Basin: 90.51%; Amambai River Basin: 56.94%. However, species distribution was not uniform between the basins: 54.70% of species were unique to the Apa River, while 34.18% of species were unique to the Amambai River, and only 11.11% of species occurred in both basins. The low number of common species was reflected in statistical differences in species composition when we performed a distance-based permutational multivariate analysis of variance (F=10.046, p<0.001) (Figure 3).
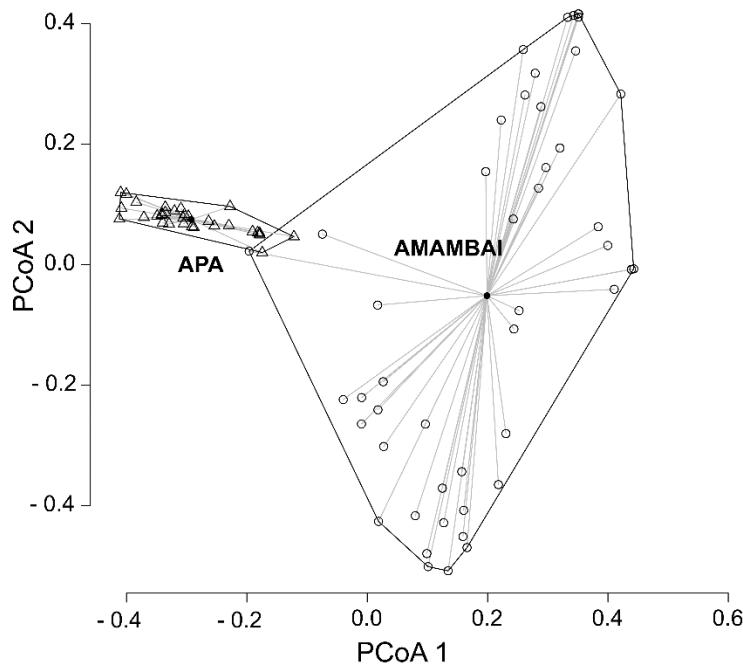
Figure 3. Principal Coordinates Analysis (PCoA) run on Bray-Curtis dissimilarity matrix of fish sampled in streams of Apa and Amambai sub-basins.

Astyanax sp1 (19.21%) was the most abundant species in Apa river basin, followed by *Odontostilbe pequira* (14.47%), both occurred exclusively in Apa river basin. In the Amambai river basin, the most abundant fish species was *Astyanax* aff. *paranae* (29.60%), followed by *Phalloceros harpagos* (22.94%), both also occurred exclusively in Amambai river basin (Table 1 – in Apêndice).

After performing the ANCOVA, we found significant differences in rarefied richness between basins (F=5.695; p=0.019) and significant influence of altitude (F= 24.129; p<0.001); however, we did not observe significant influence of spatial heterogeneity (F= 0.04; p= 0.699) or riparian vegetation cover (F=0.044; p=0.237). Similarly, we found significant difference on evenness between sub-basins (F= 11.817; p<0.001) and significant influence of altitude (F= 8.532, p=0.004), but we did not find significant influence of spatial heterogeneity (F=0.123; p=0.727) or riparian vegetation cover (F=0.172; p=0.6793) (Figure 4).
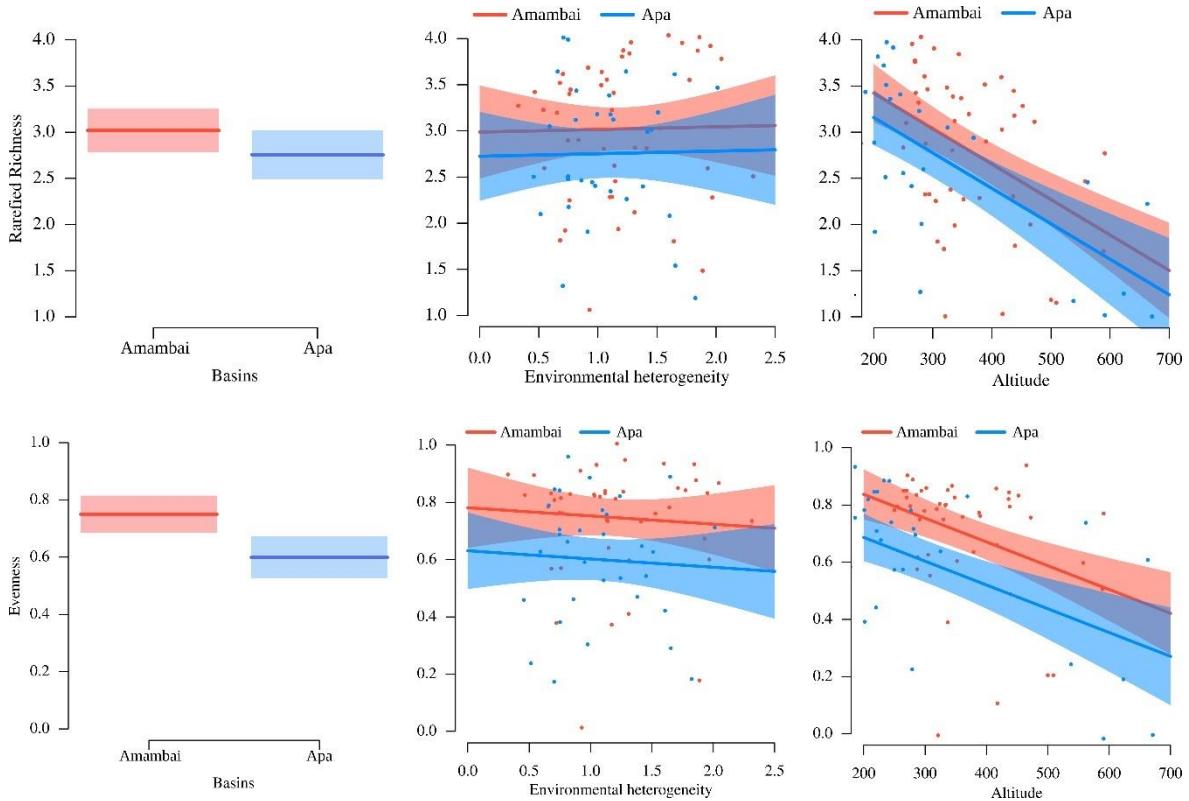
Figure 4. Mean values and confidence intervals of rarefied richness and evenness to sampled basins and relation of altitude, spatial heterogeneity and forest remnants in Apa and Amambai sub-basins in Paraguay-Paraná basin from 2014 to 2016.

DISCUSSION

Brazil holds about 10% of fish species in the world (Froehlich *et al.*, 2017), but there is still much to know about the composition of fish assemblages along its river basins (Silva-Filho *et al.*, 2011 Ferreira *et al.*, 2017), especially far from large urban regions. The most complete inventories of the ichthyofauna of the Upper Paraná and Paraguay River basins have listed 368 and 269 species, respectively (Britski *et al.,* 2007; Langeani *et al.,* 2007; Creta *et al.,* 2016; Ferreira *et al.,* 2017; Froehlich *et al.,* 2017). Even though these numbers might have increased due to the lack of knowledge about headwater streams compared to the main rivers of these basins, where most of the studies have been carried out (Polaz *et al.,* 2014). In this sense, the present study found 14.4% of the fish richness that occurred in the Upper Paraguay River basin and 28.62% of the richness of species from the Upper Paraná River basin.

The richness found in this work represents approximately 12.7% of fish richness of the whole Paraguay/Paraná basin (La Plata Basin – Reis et al., 2016); however, these two sub-basins represent only approximately 0.87% of the area of La Plata Basin. Therefore, the species richness of Amambai and Apa sub-basins may be considered as representative. Other studies about diversity of fish assemblages in these basins are mainly focused on small-scale areas (eg. Súarez *et al.,* 2007, Botini *et al.,* 2015; Severo-Neto *et al.,* 2015; Teresa *et al.,* 2010; Tondato *et al.,* 2013; Krinski *et al.,* 2015; Oliveira *et al.,* 2015; Castro & Vizzoto 2013), an exception is a large-scale species composition analysis of Ferreira et al., (2017) about stream fish composition in headwater portion of Miranda sub-basin in Upper Paraguay basin.

The observed predominance of the Characiformes and Siluriformes in the present study is a biogeographic pattern for the neotropical region (Lowe-Mcconnell, 1999; Langeani *et al.*, 2007; Stefani & Smith, 2014; Reis *et al.*, 2016; Ferreira *et al.*, 2017). In a similar way, the high number of species belonging to Characidae and Loricariidae families is an expected result (Froehlich *et al.*, 2017; Oliveira *et al.*, 2015, Vizzotto & Castro 2015, Mehanna & Penha, 2011).

The high abundances of *Astyanax* (*Astyanax* sp1 and *Astyanax* aff. *paranae*) in both sub-basins are consistent with previous studies carried out in the Upper Paraná and Paraguay River basins (Araújo *et al.*, 2011; Costa-Pereira *et al.*, 2012; Froehlich *et al*., 2017). The genus *Astyanax* is a species-rich characid with 164 valid species (Eschemeyer & Fong 2018) widely distributed in the neotropical region, with a great capacity of dispersion, inhabiting from caves, streams, lakes, and lagoons to rivers, even changing trophic category according to the stretch of stream (Bennemann *et al.*, 2011). Moreover, the genus *Astyanax* is characterized by a high relative fecundity rate, fast development, and absence of parental care, and with its opportunistic feeding behavior, allow the species of this genus to colonize a variety of new environments, predominating over other species (Agostinho *et al.*, 1999; Ceneviva-Bastos & Casatti, 2007; Gomiero & Braga, 2008).

Although the studied river basins are located in the same hydrographic system (La Plata basin) and have certain geographic proximity, there is a great difference regarding to physical characteristics such as the geomorphology of the Upper Paraguay River that differs from the Upper Paraná basin (Froehlich *et al.*, 2017). The Apa river basin, in the Paraguay basin, is steeper and has a rugged relief with waterfalls of up to 30 meters that act as

migratory barriers for fish within the river basin; therefore, most of the species present in the highest parts of the basin are the same ones that have persisted over time, since the origin of the Pantanal. However, there may also be species that have a high dispersal capacity, being able to pass through several waterfalls (Súarez *et al.*, 2007). In a complementary manner, Upper Paraguay River basin has more than one event of connections with Upper Madeira River Basin (Albert et al. 2011) generating significant differences between Upper Paraguay and Upper Paraná basins. According to Froehlich *et al.* (2017), the Upper Paraná River and Upper Paraguay River basins share 100 species in the state of Mato Grosso do Sul, out of a total of 356 species. In other words, about 28% of the total species that compose the state's fauna. From these 100 species, less than a half (49 species) are native to these basins. In this study only 11% of the total species were recorded in both river basins. Then, common species are probably species with wide distributions or species that have been captured in headwaters, as also observed for some species, as *A. lineatus* (Ferreira *et al.*, 2017A), corroborating the hipothesis of Assine (2004) that, during the Cretaceous period, part of the Upper Paraná River basin extended to where the Pantanal nowadays is, suggesting that during the origin of the Pantanal, headwater streams captured portions of tributaries of the Upper Paraná River (Menezes *et al.* 2008, Albert & Reis, 2011).

The composition of fish assemblages in streams is the result of interactions between many environmental variables such as local, biotic, regional and biogeographic factors (Hoeinghaus *et al.*, 2007, Súarez *et al.*, 2011). Among these factors, spatial heterogeneity and vegetation cover are essential in the fish assemblages structure, considering that the availability of resources and their conditions in the environment directly influence the distribution and coexistence of species in natural environments (Casatti *et al.*, 2006; Teresa & Casatti, 2012; Molina *et al.*, 2017). Although we found no influence of spatial heterogeneity and riparian vegetation cover on fish diversity of the studied sub-basins, many studies have shown their importance in these environments (Teresa & Romero, 2010; Teresa & Casatti, 2012; Massicotte *et al.*, 2015; Yang *et al.*, 2015), as increased spatial heterogeneity and riparian vegetation are believed to be the main determinants of high species richness. The contradictory results observed in our study (the non-significant influence of spatial heterogeneity) can be explained by the hypothesis that local heterogeneity measured in our study is not adequate to evaluate fish species diversity, that is more efficiently predicted by regional and biogeographical scales (Yang *et al.*, 2015). While niche theory (Hutchinson, 1957) predicts a positive relationship between richness and

spatial heterogeneity, by increasing opportunities for niche partition, a more recent model combining niche and island biogeography theories has suggested that area limitation, dispersal limitation and isolation may create negative relationships between species diversity and habitat heterogeneity (Kadmon & Allouche 2007). Nonsignificant influence of heterogeneity-diversity, as showed in our study, has also been linked with micro-fragmentation (Laanisto *et al.*, 2013). Increasing heterogeneity also raises the potential number of species that may exist in a given area, however, at the same time, the amount of effective area available for each species decreases and, therefore, reducing population sizes and raising the probability of stochastic events of extinction (Hortal *et al.*, 2009; Kadmon & Allouche, 2007; Allouche *et al.,* 2012).

In addition to the significant difference between the studied basins, the environmental descriptor that presented the greatest significant influence on the values of rarefied richness and evenness was the altitude. There are several studies on fish diversity and distribution suggesting that differences in altitude influence freshwater fish assemblages (Camana *et al.,* 2016; Tondato *et al.,* 2010; Valério *et al.,*2007; Súarez *et al.,* 2007). Altitude acts as a limitation for some species due to elevation, which prevents the migration of several stream species, promoting a contrasting difference between species occurring in the highest portions of the basins, with species of lower portions (Costa *et al.*, 2017). Therefore, altitude is an environmental descriptor capable of summarizing other hydrological characteristics that may influence the assemblages of stream fish, once other environmental variables such as temperature, depth, water flow and even predation levels are associated with altitude (Tondato *et al.*, 2010, Súarez *et al.*, 2011). This result is confirmed by the River Continuum Concept (RCC) proposed by Vanotte *et al.,* (1980) in which physical components, as altitude, gradually change from headwaters to downstream habitats in streams and rivers, influencing biological communities to adjust themselves though species substitution.

In this way, our results suggest that despite the biogeographical differences present in the studied basins, the patterns of species variation seem to be mainly determined by the altitude. These results can be used to point out the need for conservation measures for these assemblages, since communities such as those of Upper Paraguay River that have barriers, which are often isolated and have reduced migration rates, are extremely vulnerable to changes around it.

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SUPPLEMENTARY MATERIAL

Table 1. List of species sampled from 2014 to 2016 in Apa sub-basin, Upper Paraguay river basin, and Amambai sub-basin, Upper Paraná river basin, Mato Grosso do Sul state, Brazil.

Taxon	Sub-basins	
	Apa	Amambai
CHARACIFORMES		
Family Acestrorhynchidae		
Acestrorhynchus pantaneiro Menezes 1992	X	-
Family Anostomidae		
Leporellus vittatus (Valenciennes 1850)	X	-
Leporinus amblyrhynchus Garavello & Britski, 1987	X	X
Leporinus friderici (Bloch, 1794)	X	X
Leporinus lacustris Amaral Campos, 1945	X	-
Leporinus striatus Kner, 1858	X	-
Family Bryconidae		
Brycon hilarii (Valenciennes, 1850)	X	-
Salminus brasiliensis (Cuvier, 1816)	-	X
Salminus hilarii Valenciennes, 1850	-	X
Family Characidae		
Aphyocharax dentatus Eigenmann & Kennedy, 1903	X	-
Aphyocharax nattereri (Steindachner, 1882)	X	-
Aphyocharax rathbuni Eigenmann, 1907	X	-
Astyanax fasciatus (Cuvier, 1819)	-	X
Astyanax lacustris (Lütken 1875) (syn. *Astyanax asuncionensis*)	X	X
Astyanax lineatus (Perugia, 1891)	X	-
Astyanax marionae Eigenmann, 1911	X	-
Astyanax aff. *paranae* Eigenmann, 1914	-	X
Astyanax sp. 1	X	-
Brachychalcinus retrospina Boulenger, 1892	X	-
Bryconamericus exodon Eigenmann, 1907	X	-
Bryconamericus stramineus Eigenmann, 1908	-	X
Bryconamericus turiuba Langeani Lucena *et al.*, 2005	-	X
Hemigrammus lunatus Durbin, 1918	X	-
Hemigrammus marginatus Ellis, 1911	-	X
Hyphessobrycon anisitsi (Eigenmann, 1907)	-	X
Hyphessobrycon eques (Steindachner, 1882)	X	-
Hyphessobrycon luetkenii (Boulenger, 1887)	X	-
Jupiaba acanthogaster (Eigenmann, 1911)	X	-
Knodus moenkhausii (Eigenmann & Kennedy, 1903)	-	X
Moenkhausia bonita Benine *et al.*, 2004	X	X

Moenkhausia dichroura (Kner, 1858)	X	-
Moenkhausia forestii Benine *et al*., 2009	X	X
Moenkhausia oligolepis (Günther, 1864)	X	-
Odontostilbe paraguayensis Eigenmann & Kennedy, 1903	X	-
Odontostilbe pequira (Steindachner, 1882)	X	-
Oligosarcus pintoi Amaral Campos, 1945	X	X
Phenacogaster tegatus (Eigenmann, 1911)	X	-
Piabarchus analis (Eigenmann, 1914)	X	-
Piabarchus torrenticola Mahnert & Géry, 1988	X	-
Piabina argentea Reinhardt, 1867	-	X
Poptella paraguayensis (Eigenmann, 1907)	X	-
Psellogrammus kennedyi (Eigenmann, 1903)	X	-
Serrapinnus notomelas (Eigenmann, 1915)	-	X
Serrapinnus calliurus (Boulenger, 1900)	X	-
Xenurobrycon macropus Myers & Miranda Ribeiro, 1945	X	-
Family Crenuchidae		
Characidium gomesi Travassos, 1956	-	X
Characidium laterale (Boulenger, 1895)	X	-
Characidium sp.	X	-
Characidium sp. 1	X	-
Characidium zebra Eigenmann, 1909	X	X
Family Curimatidae		
Cyphocharax gillii (Eigenmann & Kennedy, 1903)	X	-
Steindachnerina brevipinna (Eigenmann & Eigenmann, 1889)	X	-
Family Erythrinidae		
Hoplias sp. 1	-	X
Hoplias sp. 2	X	X
Hoplias sp. 3	-	X
Family Iguanodectidae		
Bryconops melanurus (Bloch, 1794)	X	-
Family Lebiasinidae		
Pyrrhulina australis Eigenmann & Kennedy, 1903	X	X
Family Parodontidae		
Apareiodon afnis (Steindachner, 1879)	X	-
Apareiodon piracicabae (Eigenmann, 1907)	-	X
Parodon nasus Kner, 1859	-	X
Family Prochilodontidae		
Prochilodus lineatus (Valenciennes, 1837)	X	X
CYPRINODONTIFORMES		
Family Poeciliidae		
Phalloceros harpagos Lucinda, 2008	-	X
Phallotorynus pankalos Lucinda *et al*., 2005	-	X

Family Rivulidae

Melanorivulus apiamici (Costa, 1989)	-	X

GYMNOTIFORMES

Family Gymnotidae

Gymnotus inaequilabiatus (Valenciennes, 1839)	-	X
Gymnotus paraguensis Albert & Crampton, 2003	X	-
Gymnotus sp.	-	X

Family Hypopomidae

Brachyhypopomus sp.	X	-

Family Sternopygidae

Eigenmannia virescens (Valenciennes, 1836)	X	-
Eigenmannia trilineata López & Castello, 1966	-	X

PERCIFORMES

Family Cichlidae

Apistogramma trifasciata (Eigenmann & Kennedy, 1903)	X	-
Cichlasoma dimerus (Heckel, 1840)	X	-
Cichlasoma paranaense Kullander, 1983	-	X
Crenicichla britskii Kullander, 1982	-	X
Crenicichla lepidota Heckel, 1840	X	-
Geophagus brasiliensis (Quoy & Gaimard, 1824)	-	X
Satanoperca pappaterra (Heckel, 1840)	X	-
Tilapia rendalli (Boulenger, 1897)	-	X

SILURIFORMES

Family Aspredinidae

Bunocephalus doriae Boulenger, 1902	X	-

Family Callichthyidae

Callichthys callichthys (Linnaeus, 1758)	X	-
Corydoras aeneus (Gill, 1858)	X	X
Corydoras aurofrenatus Eigenmann & Kennedy, 1903	X	-

Family Heptapteridae

Cetopsorhamdia iheringi Schubart & Gomes, 1959	-	X
Imparfnis borodini Mees & Cala, 1989	-	X
Imparfnis schubarti (Gomes, 1956)	-	X
Imparfinis sp.	X	-
Imparfnis stictonotus (Fowler, 1940)	X	-
Phenacorhamdia tenebrosa (Schubart, 1964)	-	X
Pimelodella gracilis (Valenciennes, 1835)	X	-
Rhamdia quelen (Quoy & Gaimard, 1824)	X	X

Family Loricariidae

Ancistrus sp. 1	X	-
Ancistrus sp. 2	X	-
Farlowella paraguayensis Retzer & Page, 1997	X	-

Hisonotus insperatus Britski & Garavello, 2003	-	X
Hypostomus albopunctatus (Regan, 1908)	-	X
Hypostomus ancistroides (Ihering, 1911)	-	X
*Hypostomus basilisko*Tencatt, Zawadzk & Froehlich 2014	X	-
Hypostomus boulengeri (Eigenmann & Kennedy 1903)	X	-
Hypostomus cochliodon Kner 1854	X	-
Hypostomus iheringii (Regan, 1908)	-	X
*Hypostomus khimaera*Tencatt, Zawadzki & Froehlich 2014	X	-
Hypostomus aff. *latirostris* (Regan 1904)	X	-
Hypostomus sp.	-	X
Hypostomus strigaticeps (Regan, 1908)	-	X
Loricaria luciae Thomas, *et al.* 2013	X	-
Otothyropsis marapoama Ribeiro, Carvalho & Melo, 2005	-	X
Otothyropsis polyodon Calegari *et al.*, 2013	-	X
Otothyropsis sp.	X	-
Otocinclus cf. *bororo* Schaefer, 1997	X	-
Rineloricaria aurata (Knaack, 2003)	X	-
Rineloricaria lanceolata (Günther, 1868)	X	X
Rineloricaria latirostris (Boulenger, 1900)	-	X
Rineloricaria parva (Boulenger, 1895)	X	-
Family Pimelodidae		
Pimelodus maculatus La Cepède, 1803	X	-
Family Pseudopimelodidae		
Pseudopimelodus mangurus (Valenciennes, 1835)	X	-
Family Trichomycteridae		
Ituglanis eichorniarum (Miranda Ribeiro, 1912)	X	-
SYNBRANCHIFORMES		
Family Synbranchidae		
Synbranchus marmoratus Bloch, 1795	X	X

NORMAS DA REVISTA

AUTHOR GUIDELINES

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Book

Bradley-Johnson, S. (1994). Psychoeducational assessment of students who are visually impaired or blind: Infancy through high school (2nd ed.). Austin, TX: Pro-ed.

Chapter in an Edited Book

Borstrøm, I., & Elbro, C. (1997). Prevention of dyslexia in kindergarten: Effects of phoneme awareness training with children of dyslexic parents. In C. Hulme & M. Snowling (Eds.), *Dyslexia: Biology, cognition and intervention* (pp. 235–253). London: Whurr.

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