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Taxonomic and phylogenetic beta diversity in headwater stream fish communities of the Paraná and Paraguai River basins

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Patterns of species replacement and richness differences along environmental gradients or ecoregions shed light on different ecological and evolutionary mechanisms acting on community structure. Communities of aquatic ecosystems of different watersheds are supposed to host distinct species and lineages. Quantifying and understanding the degree to which these differences are affected by environmental and biogeographical factors remains an open question for these environments, particularly in the Neotropical region. We investigated patterns of taxonomic and phylogenetic composition of headwater streams of the Paraná and Paraguai River basins to understand how local and biogeographical factors affect the assembly of fish communities. We also quantified taxonomic and phylogenetic beta diversity by decomposing them into nestedness and turnover components. We found that local environmental factors are the main factors influencing the composition of stream fish communities. Whereas pH affected both taxonomic and phylogenetic turnover, water velocity was responsible for phylogenetic turnover and pH was the main driver of phylogenetic nestedness. Our results indicate an effect of local environmental factors in determining the structure of headwater stream fish communities through a combination of a species sorting mechanism (water velocity and pH) and phylogenetic habitat filtering (pH).

Keywords: Community assembly, Nestedness, Phylobetadiversity, Tropical streams, Turnover.

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Padrões de substituição de espécies ou diferenças de riqueza ao longo de gradientes ambientais ou ecoregiões lançam luz sobre diferentes processos e mecanismos ecológicos atuando na estruturação das comunidades. Supõe-se que comunidades aquáticas pertencentes a diferentes bacias pertençam a linhagens evolutivas distintas. Quantificar e entender o grau em que tais diferenças são resultado de fatores ambientais locais e/ou processos biogeográficos ainda é uma questão pouco explorada. Neste estudo nós investigamos os padrões de composição taxonômica e filogenética em riachos de cabeceira das bacias dos Rios Paraná e Paraguai, para entender como fatores locais e biogeográficos afetam a estruturação das comunidades de peixes. Nós quantificamos a diversidade beta taxonômica e filogenética decompondo estas em aninhamento e substituição. Encontramos que os fatores ambientais locais são os principais determinantes da composição das comunidades de peixes destes riachos. Enquanto o pH afetou tanto a substituição de linhagens e de espécies, a velocidade da água foi responsável por uma substituição de linhagens, enquanto o pH foi o principal responsável pelo aninhamento de linhagens. Nossos resultados indicam a importância dos fatores locais através da combinação entre mecanismos de preferência de nicho (velocidade da água e pH) e filtragem ambiental de linhagens (pH).

Palavras-chave: Aninhamento, Estrutura de comunidades, Filobetadiversidade, Riachos tropicais, Substituição.

INTRODUCTION

The presence of species in space and time is the result of numerous factors acting both contemporary and historically (Graham, Fine, 2008), and the imprints that different factors leave on community composition can be captured by describing the patterns of variation of species among communities within or among different regions (Graham, Fine, 2008; Kissling *et al*., 2012; Gerhold *et al*., 2015). Community structure has traditionally been described by focusing on quantifying differences or similarities in the distribution of species among assemblages (through the use of the so called beta diversity measures) (Legendre, De Cáceres, 2013). However, diversity can present variation that goes beyond taxonomic resolution, including multiple dimensions like functional (variation in species attributes) and phylogenetic (variation in evolutionary history) diversity (Stevens, Tello, 2014; Nakamura *et al*., 2019).

Differences in species composition can indicate the importance of different contemporary factors acting on niche preferences of species that determine their distribution or events associated with deep evolutionary history that influenced the current occurrence of species in communities (*e.g*., vicariance events, historical dispersal, speciation and diversification) (Pyron, 2014; Gerhold *et al*., 2018). Although very useful for understanding the drivers of community structure, analyzing community composition strictly from a taxonomic perspective does not allow the direct evaluation of species characteristics that can be important in mediating species occurrence in communities, which may be due to a balance among niche based processes (Duarte, 2011; Roa-Fuentes *et al*., 2020), dispersal capacities (Mikkelson, 2005; Siqueira *et al*., 2020) or interspecific interactions (Godoy *et al*., 2014).

Since evolutionary relatedness of species can be indicative of overall similarity of characteristics (Wiens, Graham, 2005), assessing the phylogenetic composition of communities and its variation in space can serve as a more direct link among ecological and historical processes influencing species occurrence (Cavender-Bares *et al*., 2009). In the case when communities differ in both taxonomic and phylogenetic composition, the taxonomic based approach alone is sufficient for linking relationships among community composition and possible mechanisms structuring these communities, considering species differences (Safi *et al*., 2011). However, in the case when differences in community composition are not followed by their evolutionary/functional distinctness (for example, communities that host species with high phylogenetic similarity), the taxonomic approach alone will fail to identify the causes of variation underlying community assembly (Tucker, Cadotte, 2013; Ochoa-Ochoa *et al*., 2020).

The phylogenetic structure of communities can reflect events of the evolutionary history of species (Leibold *et al*., 2010; Pillar, Duarte 2010; Duarte 2011; Peres-Neto *et al*., 2012; Debastiani *et al*., 2015), or contemporary factors acting on species characteristics, thus allowing the relative importance of niche (Leibold *et al*., 2004) and biotic (*e.g*., competitive asymmetry among co-occurring species (Godoy *et al*., 2014) and historical factors (Kissling *et al*., 2012) to be inferred. Examples of the phylogenetic approach are more common for terrestrial communities, mainly plants (*e.g*., Duarte, 2011; Duarte *et al*., 2014; Carlucci *et al*., 2017) than aquatic environments, despite its potential to unveil important ecological processes in the latter. For example, Saito *et al.* (2016a) showed that high phylogenetic diversity in tropical streams can be associated with historical stability of these environments, which host ancient macroinvertebrate species from different lineages. In another study in a tropical riverine system using Odonata as a study model, Saito *et al.* (2016b) suggested that species sorting is the main driver in determining the community structure of damselfly assemblages.

Despite the complex and rich evolutionary history of fish freshwater diversity (Albert *et al*., 2020), which make this group an interesting model for the study of community assembly from phylogenetic and taxonomic perspectives, there have been few studies of fish communities in tropical aquatic ecosystems using both dimensions. To the best of our knowledge, existing examples are restricted to case studies (Nakamura *et al*., 2017, 2020) that did not directly intend to unveil the factors influencing the community assembly process, or to a more applied perspective for understanding anthropogenic effects on stream fish assemblages restricted to the same region (Roa-Fuentes *et al*., 2019, 2020). So, describing community patterns and their ecological and evolutionary causes for tropical fish communities of different bioregions can fill an important gap in the understanding of fish community ecology of the tropical region.

The Paraná and Paraguai River basins comprise two of the main tributaries in South America, each with its own particular environmental characteristics and biotas, and thus are considered to be two distinct bioregions (Albert, Reis, 2011). From this perspective, a comparison of the taxonomic and phylogenetic diversity of the communities between Paraná and Paraguai River basins, offers an opportunity to address the biogeographical perspective of the study of fish community assembly.

Current knowledge about headwater streams of the Paraná and Paraguai basins suggests that biogeographical isolation resulted in them possessing different stream fish compositions (Valério *et al*., 2007), and that Upper Paraná river basin constitutes an area of high endemism due to Sete Quedas Falls, which functions as a biogeographical barrier. Furthermore, the presence of numerous waterfalls in Paraguai River basin, are responsible for a greater isolation of the streams and a more variable fish fauna than in the Paraná basin (Súarez *et al*., 2007; Valério *et al*., 2007). Therefore, differences in taxonomic composition can be the result of differences in historical processes acting on the distribution of lineages between the two basins. However, this remains an open question at the moment and little is known about the mechanisms associated with variation in community composition in headwater streams [*e.g*., species sorting, habitat filtering (Leibold *et al*., 2004) or/and historical processes].

In this work, our aim was to assess patterns of taxonomic and phylogenetic beta diversity in headwater stream fish communities of the Paraguai and Paraná river basins and to investigate how local and biogeographical factors act on the assembly of these communities in these distinct aquatic bioregions. We hypothesize that the two basins differ in clade composition due to differences in the diversification and evolutionary history of their fish faunas and that these differences are mainly associated with replacement of lineages (phylogenetic turnover between Paraná and Paraguai basins), due to the long period of time that these two fish faunas have been separated. Furthermore, we also hypothesize that the Paraguai River basin will have more variation in taxonomic and phylogenetic composition among its streams than will the Paraná river basin, since the former possesses steeper slopes producing more isolated streams than those of the latter (Valério *et al*., 2007). Finally, we also hypothesize that environmental differences among streams will also influence phylogenetic and taxonomic structure of fish assemblages by niche mediated processes, since stream fish are sensitive to variation in environmental factors, even though the local environmental gradients of headwater streams are much narrow than in other riverine systems.

Thus, we specifically tested if (1) variation in taxonomic beta diversity is accompanied by variation in clade composition, which would indicate distinct evolutionary histories in species occurrence between the two basins; and determined (2) which factors (local or regional) were the main drivers of the differences in phylogenetic and taxonomic composition among the headwater streams.

MATERIAL AND METHODS

Sampling sites. The sampled streams are located within a 200-km-long area in the Paraguai and Paraná basins, near 20°54' to 22°18'S latitude and 54°56' to 56°05'W longitude (Fig. 1).

We collected samples bimonthly in 10 streams of each basin (Fig. 1), from January to November 2004, for a total of 60 samples per basin. In each basin, we selected streams from four sub-basins, according to their accessibility from the road, their position in the hydrographic basin and their environmental integrity (visual assessment). The geographic relief of the Apa and Miranda sub-basins in the Paraguai basin is uneven, with many waterfalls higher than 30m, which act as barriers to fish migration (Súarez *et al*., 2007; Valério *et al*., 2007). Stream depths in this basin varied from 0.31 to 0.84m,

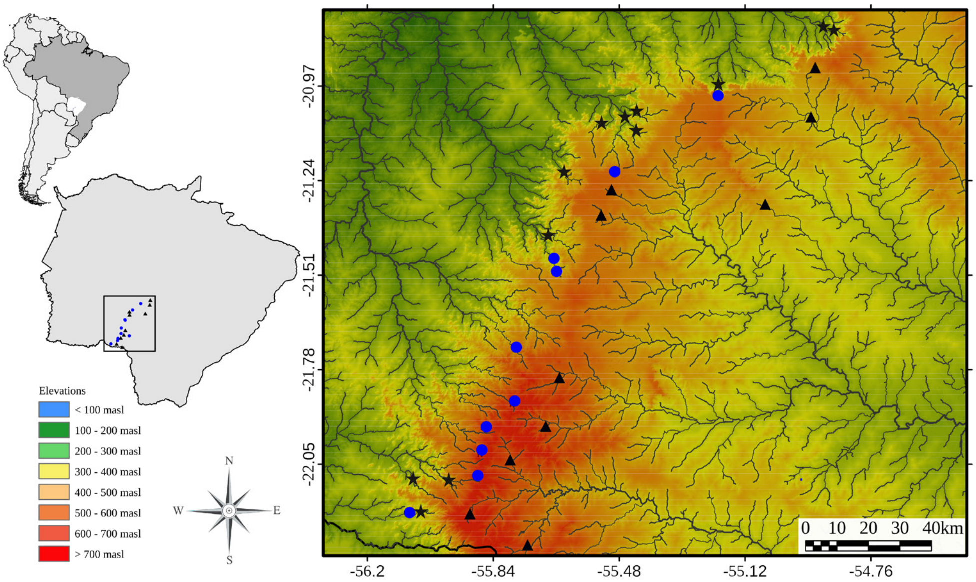

FIGURE 1 | Headwater streams sampled in the Paraná and Paraguai River basins. Blue circles represent streams of the Paraguai basin, and black triangles represent streams of the Paraná basin. The stars represent waterfalls. The color indicates the variation in altitude.

water velocity from 0.33 to 0.81m*s^{-1} and altitude from 344 to 571m. Stream depths in the Paraná basin varied from 0.20 to 0.46m, water velocity from 0.16 to 0.85m*s^{-1} and altitude from 264 to 641m. The streams are located in areas with little human habitation and are relatively well preserved with most of their natural riparian vegetation remaining. The locations of all the sampled streams are shown in Fig. 1.

We took samples during daylight with a rectangular sieve (1.2 x 0.8m, 2mm mesh) and standardized sampling effort at each site with 20 throws and approximately 50m long hauls. We chose this method to avoid complications related to the use of electrofishing in streams with low water conductivity, difficult access, and variable turbidity that prevents the visual detection of fish.

We measured a set of key local environmental variables known to influence stream fish communities: pH, conductivity (mS/cm), water temperature, and dissolved oxygen using a YSI model 556 field multi probe, and turbidity with a portable turbidity meter. Stream depth was recorded from 10 locations in each stream, using a graduated wooden stick. We measured water velocity (m/s) three to five times in different parts of the stream using the float method. We measured altitude (m) with the altimeter of a GPS unit. We characterized all streams using mean values for stream depth and velocity. All mean values for environmental variables are provided in Tab. 1.

We built a phylogenetic hypothesis for the fish of the Paraná and Paraguai headwater streams by adapting a molecular time-calibrated tree for bony fishes (Rabosky *et al.*, 2018). Rabosky´s phylogeny was adapted by replacing missing taxa by sister taxa of species presented in the species pool of the sampled streams of the Paraná and Paraguai basins (or the closest species available in the original phylogeny). The phylogeny was downloaded using the R package fishtree (Chang *et al.*, 2019). To build our phylogeny containing species of the local pool we followed a sequential procedure in which we

TABLE 1 | Mean values of environmental variables measured in 20 headwater streams of the Paraná and Paraguai River basins.

Stream	Turbidity	pH	Conductivity (mS/cm^{-1})	Dissolved O2 (%)	Altitude (m)	Temperature (°C)	Stream width (m)	Stream depth (m)	Water velocity (m/s^{-1})	Basin
Agua Fria	10.51	7.26	0.012	77.03	554	22.31	1	0.39	0.39	Paraná
Boa Vista	10.20	6.61	0.019	77.73	511	19.51	7	0.65	0.52	Paraná
Buriti	7.05	7.29	0.020	76.13	466	20.84	3.80	0.25	0.525	Paraguai
Apa	3.87	5.89	0.005	79.25	591	22.42	0.60	0.33	0.540	Paraguai
Cab. Dourados	2.86	7.59	0.029	72.45	641	20.67	1.50	0.22	0.340	Paraná
Moças	4.43	7.28	0.128	59.86	366	19.94	2	0.32	0.430	Paraguai
Feio	25.96	7.67	0.125	86.41	470	21.34	5	0.34	0.660	Paraguai
Fundo	4.20	7.36	0.017	72.76	619	20.30	1.10	0.41	0.179	Paraguai
Lagoa Cheia	7.05	7.004	0.03	77.65	528	19.42	3.08	0.32	0.720	Paraná
Lagoinha	6.20	6.87	0.010	65.80	541	20.37	5.80	0.31	0.670	Paraná
Mosqueteiro	6.60	6.87	0.020	76.41	623	20.12	1.80	0.33	0.370	Paraguai
Miranda	6.61	7.53	0.111	78.33	542	19.21	1.20	0.20	0.160	Paraguai
Nioaque	4.01	7.05	0.100	83.03	386	20.13	6.00	0.35	0.790	Paraguai
Nova Alvorada	6.15	7.26	0.090	66.91	432	22.81	2.30	0.55	0.370	Paraná
Passa Tempo	29.55	7.10	0.040	57.86	358	19.60	1.70	0.52	0.400	Paraná
S. Bento	10.85	7.27	0.030	75.76	344	21.11	6	0.84	0.800	Paraná
S. Virgem	5.05	7	0.020	76.18	571	20.24	6	0.70	0.730	Paraná
Taquarussu	6.96	7.04	0.030	65.66	490	20.29	4.50	0.22	0.850	Paraguai
Turvo	10.45	7.51	0.026	75.91	523	21.54	4.30	0.49	0.810	Paraná
Ita	4.36	7.07	0.160	69.86	264	20.88	4.00	0.46	0.440	Paraguai

started by replacing species from the original phylogeny with the species of the same genus presented in our species pool. When the genus was not presented in the original phylogeny, we inserted the species in the most closely related species of the same family, and for species that did not present neither species of the same genus nor family, representatives we inserted the species at the order level (*e.g.*, *Synbranchus marmoratus* in our dataset). After all insertions, the phylogenetic tree was pruned to maintain only the species that occur in the sampled streams. The final phylogenetic hypothesis is shown in Fig. 2. We assembled an R function to automate the process of phylogenetic tree edition described above, which is freely available and can be assessed at https://github.com/GabrielNakamura/function_phyloMatch_fishtree.

To quantify beta diversity in each of the two basins, and to determine how much of its variation is due to differences in richness (nestedness component) and/or species replacement (turnover component), we used the method proposed by Baselga (2010). Beta diversity was calculated as pairwise dissimilarity among communities obtained through the Sorensen index (Sorensen, 1948). Turnover is represented by the Simpson index (Simpson, 1943) and corresponds to the dissimilarity between a pair of sites (or communities) removing the effect caused by differences in richness. Finally, the nestedness component was obtained through the subtraction of the turnover component (Simpson) from total beta diversity (Sorensen). Beta diversity values ranged from 0 to 1, where 1 corresponds to completely different communities (Baselga, 2010; Baselga, Orme, 2012). We used a community occurrence matrix (presence/absence) and the

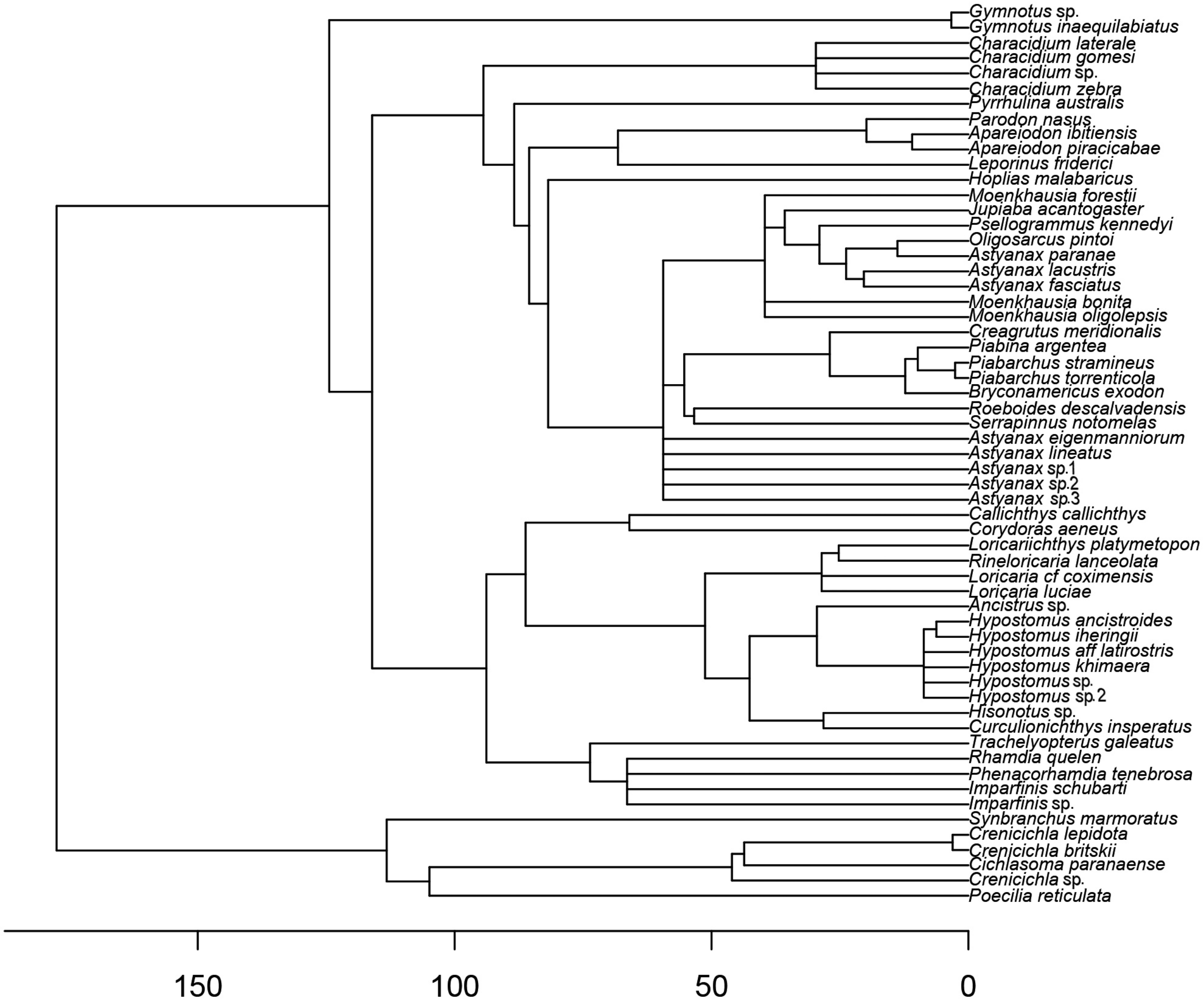

FIGURE 2 | Phylogenetic hypothesis for fish collected in headwater streams of the Paraná and Paraguai River basins.

phylogenetic hypothesis presented in Fig. 2 to obtain all components of taxonomic and phylogenetic beta diversity for the headwater stream fish communities. We analyzed relationships between environmental and biogeographical (basins) variables and patterns of spatial distribution of different components of phylogenetic and taxonomic beta diversity through the use of a permutational multivariate analysis of variance with distance matrices through adonis2 function in vegan package (Anderson *et al*., 2006), that evaluate the marginal effects of each variable, which corresponds to the effect of each variable when controlling the effects of all other variables in the model.

We tested the hypothesis that the fish fauna of the Paraguai basin is more variable than that of the Paraná basin through multivariate analysis of homogeneity of groups dispersion (Anderson, 2006). This analysis consists of calculating the mean distance of sample units from the centroid of the group to which these units belong. We performed this analysis using the *betadisper* function with distance matrices that contained the pairwise dissimilarities of each stream regarding the total, the turnover and the nestedness components of taxonomic and phylogenetic beta diversity. We tested for differences among group mean distances by performing a permutation test, where a null F distribution is generated and compared to the observed F of the original distances (using the *permutest* function in the vegan package).

Due to the lack of complete knowledge of the phylogenetic relationships among neotropical fish some species were added as polytomies in our phylogenetic hypothesis. The lack of resolution in some genera (*e.g.*, *Astyanax* Baird & Girard, 1854 and *Hypostomus* Lacepède, 1803) can be a source of bias in estimates of phylogenetic beta diversity indexes and, consequently, in their relationship with environmental/biogeographical factors.

The use of molecular markers could be a solution to problems related to lack of resolution in phylogenetic trees, however, it is very costly and, in some cases, operationally unfeasible due to the lack of an accessible data base of molecular markers. Therefore, in order to account for the effects of the phylogenetic uncertainty in the patterns of phylogenetic structure we derived 1000 random solutions for all polytomies presented in the synthesis phylogenetic tree, following the procedure described in Martins *et al*. (2013). In summary, this procedure searches for all nodes with more than two daughter taxa (a polytomy). For each polytomy node a new node and a new edge is created, the branch length of this new edge is set from a random uniform distribution and the lengths of descendant edges are adjusted to preserve the ultrametricity of the tree (a requirement for phylogenetic metrics used in this work). The resolution of polytomy nodes was repeated 1000 times for each node to account for all possible solutions for the polytomies. For each randomly fully-solved tree we calculated all indices of phylogenetic beta diversity and used each one as the response variable in an adonis2 model with environmental variables. This procedure resulted in a distribution of F and p values for all environmental variables, and we compared this distribution with that observed for our synthesis phylogeny. The distributions for F and p values resulting from this procedure are presented in **S1**.

All analyses and calculations were performed in R (R Development Core Team, 2020). All p values are stated with a nominal error of $\alpha = 0.05$. All data and codes needed to reproduce the analysis performed in this study can be accessed through GitHub repository https://github.com/GabrielNakamura/MS_Parana_Paraguai.

RESULTS

We found that variation in both taxonomic and phylogenetic composition among headwater stream fish communities was mainly due to species turnover between the two basins (Fig. 3). Phylogenetic and taxonomic turnover accounted for 83% of the mean variation in, respectively, mean total phylogenetic and taxonomic beta diversity.

Greater differences in pH resulted in greater differences in species composition, which were related to both taxonomic turnover as well as to phylogenetic turnover and nestedness among streams (Tabs. 2–3). Streams with high pH levels had only a subset of lineages of streams with low pH levels. Besides that, differences in water velocity

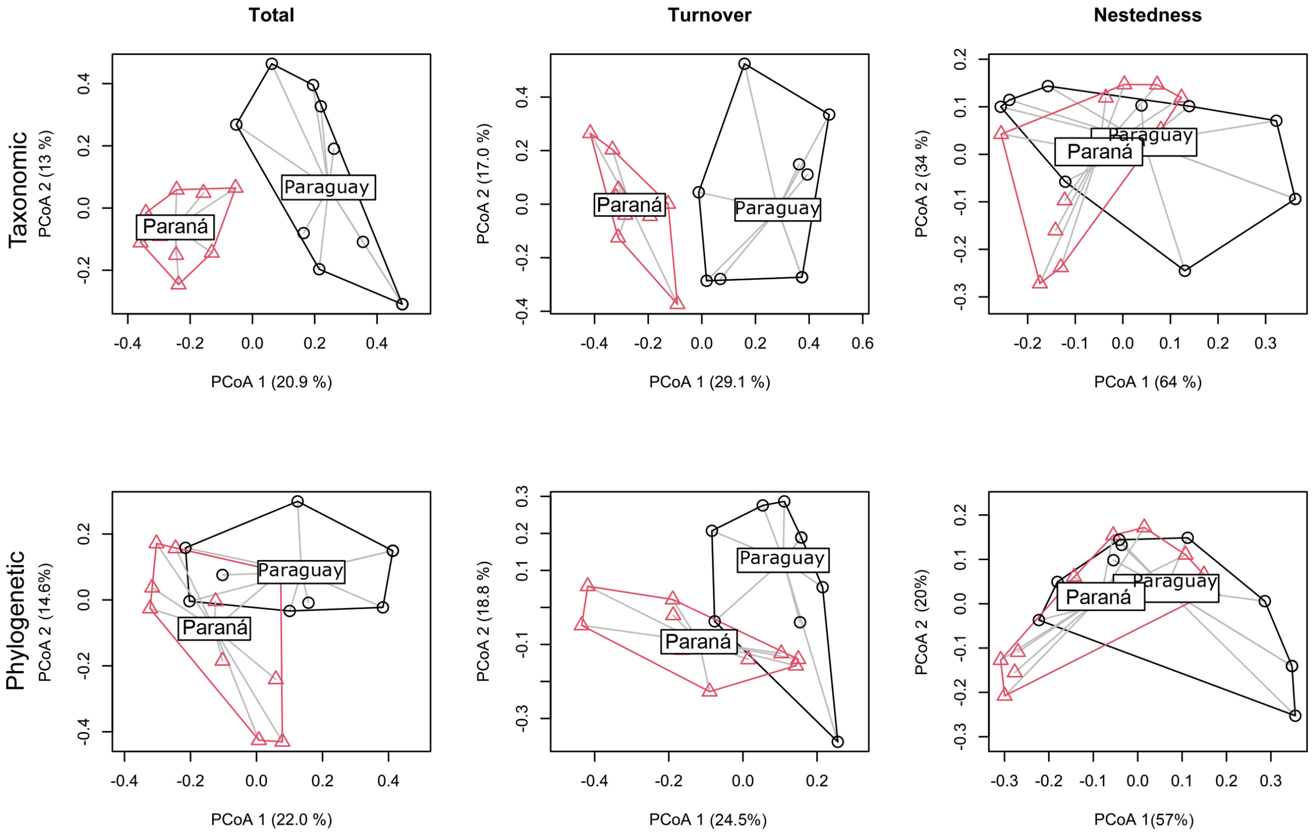

FIGURE 3 | Turnover and nestedness components of taxonomic and phylogenetic beta diversity in streams of the Paraná and Paraguai River basins. Clockwise from top left: total, turnover and nestedness components of taxonomic beta diversity; at the bottom, total, turnover and nestedness components of phylogenetic beta diversity. Filled circles correspond to Paraná streams and triangles to Paraguai streams.

among streams was the main driver of turnover of fish lineages among streams (Tab. 2). The highest phylogenetic turnover (0.73) was found between Santa Virgem (Paraná) and Fundo (Paraguai) streams, whereas the highest phylogenetic nestedness was found between São Bento and Apa streams (0.53).

According to our analysis of homogeneity of variances, the Paraná and Paraguai basins had similar variation in both turnover (F= 1.14, p= 0.30 for taxonomic and F= 0.001, p= 0.97 for phylogenetic dimensions) and nestedness (F= 0.19, p= 0.65 for taxonomic and F= 0.13, p= 0.73 for phylogenetic dimensions) components within each basin, with streams of the Paraguai basin having slightly more variable taxonomic and phylogenetic composition than the streams of the Paraná basin (Fig. 3), but not significantly. Therefore, we rejected the hypothesis that the Paraguai basin has a significantly more variable fish fauna than does the Paraná basin, despite the differences in slopes and the presence of waterfalls (Fig. 1).

All these patterns related to phylogenetic structure among stream fish communities and the variance in phylogenetic structure in each basin remains the same even when we consider the phylogenetic uncertainty of the phylogeny due to polytomies at some nodes. Since the patterns found for the synthesis phylogeny was the same as that when we took phylogenetic uncertainty into account, we decided to present here only the

TABLE 2 | Results from adonis2 analysis relating different components of taxonomic beta diversity and environmental variables. Values in bold indicate response variables that presented a significant effect (p-value < = 0.05) in beta diversity.

		Total			Turnover			Nestedness		
	Df	R^2	F	p-value	R^2	F	p-value	R^2	F	p-value
Turbidity	1	0.05068	1.45	0.177	0.06457	2.13	0.096	0.01856	0.30	0.634
pH	1	**0.09597**	**2.75**	**0.005**	**0.15957**	**5.27**	**0.002**	-0.14894	-2.47	0.971
Conductivity	1	0.04479	1.28	0.25	0.06478	2.14	0.116	-0.11458	-1.90	0.956
Dissolved O2	1	0.01497	0.42	0.902	0.00875	0.28	0.841	0.02684	0.44	0.598
Altitude	1	0.04238	1.21	0.279	0.02439	0.80	0.517	0.11041	1.83	0.324
Temperature	1	0.0425	1.21	0.294	0.04255	1.40	0.263	-0.03705	-0.61	0.831
Depth	1	0.03863	1.10	0.362	0.02842	0.93	0.463	-0.00199	-0.033	0.697
Water velocity	1	0.05208	1.49	0.176	0.06563	2.16	0.102	0.03257	0.54	0.571
Basin	1	0.06341	1.81	0.116	0.05951	1.96	0.136	-0.01378	-0.22	0.748
Residual	10	0.34882	–	–	0.30265	–	–	0.60189	–	–

TABLE 3 | Results from adonis2 analysis relating different components of phylogenetic beta diversity and environmental variables. Values in bold indicate response variables that presented a significant effect (p-value < = 0.05) in beta diversity.

		Total			Turnover			Nestedness		
	Df	R^2	F	p-value	R^2	F	p-value	R^2	F	p-value
Turbidity	1	0.04122	1.28	0.265	0.07972	2.84	0.061	-0.00169	-0.63	0.869
pH	1	**0.11142**	**3.46**	**0.004**	**0.10489**	**3.74**	**0.03**	**0.16215**	**6.06**	**0.04**
Conductivity	1	0.06094	1.89	0.092	0.08256	2.94	0.053	0.02187	0.81	0.436
Dissolved O_2	1	0.03452	1.07	0.395	0.05591	1.99	0.148	-0.01541	-0.57	0.973
Altitude	1	0.0672	2.08	0.074	0.04794	1.71	0.202	0.11249	4.21	0.08
Temperature	1	0.03723	1.15	0.334	0.05076	1.81	0.202	0.01435	0.53	0.528
Depth	1	0.04771	1.48	0.182	0.07644	2.72	0.073	-0.01281	-0.47	0.963
Water velocity	1	0.06314	1.96	0.087	**0.10219**	**3.64**	**0.03**	0.00637	0.23	0.692
Basin	1	0.051	1.58	0.15	0.08362	2.98	0.05	-0.00242	-0.09	0.867
Residual	10	0.32175	–	–	0.28027	–	–	0.26715	–	–

results derived from the synthesis phylogeny and show the analysis accounting for phylogenetic uncertainty in **S1** and **S2**. Additionally, a list containing the names of all species collected in this study with their respective classification and register numbers in scientific collection are presented in **S3.**

DISCUSSION

By decomposing beta diversity into its turnover and nestedness components we were able to show that some environmental factors, namely pH and water velocity, act mainly by promoting species replacement, and another (pH) by selecting a subset of lineages that are tolerant to the environmental characteristics of streams. Together, these results evidence a process of phylogenetic habitat filtering (Duarte, 2011), mediated by pH, together with species sorting probably mediated by functional traits of species associated with water velocity (*e.g*., body form) and pH (Leibold *et al*., 2004), evidenced by differences among lineages. As far as we know, our findings represent the first evidence of ecological mechanisms underlying the assembly process of headwater stream fish communities of the Paraná and Paraguai River basins.

Our work advances beyond what was already known about headwater stream fish communities in this region (Súarez *et al*., 2007; Valério *et al*., 2007), by depicting the environmental factors that also act in determining phylogenetic variation among stream fish communities. These findings provide a more complete picture of headwater stream fish communities since evaluating phylogenetic and taxonomic composition together provides a more direct link among compositional patterns and mechanisms mediating community assembly (Graham, Fine, 2008). We also showed for the first time that despite the Paraguai headwater streams being located in a more heterogeneous region than the Paraná headwater streams, those of the former did not have greater variation in phylogenetic and taxonomic composition than the those of the latter, as was previously believed (Valério *et al*., 2007), reinforcing the greater importance of local hydrological characteristics rather than characteristics of the relief were there streams are located.

Our findings also reinforce the patterns recognized in other works regarding differences in the fish fauna of the Paraná and Paraguai basins (Súarez *et al*., 2007; Valério *et al*., 2007), yet showed that this variation did not have a sufficiently deep evolutionary influence to generate differences in fish lineages between the headwater streams of the two basins, with similar lineages inhabiting both basins despite their different biogeographical histories (Brea, Zucol, 2011).

It is well known that, in general, abiotic factors are more influential in upstream areas of watersheds (Reyes-Gavilán *et al*., 1996) than in downstream areas. In this sense, and considering that we sampled headwaters, our results evidenced these general patterns. Acidification of water, besides being one of the most influential factors for fish fitness in streams (Fialho *et al*., 2008), is the predominant characteristic of running waters because of the leaching of iron from watershed surfaces by rain (Giller, Malmqvist, 1998). Water pH influences fish preferences for reproduction habitat (Deitos *et al*., 2002) and determines fish growth (Ferreira *et al*., 2001). Acidic waters result in significant ion losses due to osmoregulatory disturbances of fish (Shuter *et al*., 1989) and disturbs a variety other physiological processes, including oxygen affinity, digestion and osmotic balance (Almeida-Val, 1995). In this way, the replacement of species and the selection of subsets of fish lineages are expected to be mediated mainly by pH, even given a narrow pH gradient when compared with other studies, as pointed out by Winemiller *et al*. (2008), and as we evidenced here.

Specifically, the Apa stream had the lowest pH (5.89) and was mainly responsible for the pattern of phylogenetic nestedness. The Apa stream community also influences phylogenetic turnover, since it hosts only two species with very restricted occurrences in streams of the Paraná and Paraguai basin (*Astyanax* sp1 and *Hypostomus khimaera* Tencatt, Zawadzki & Froehlich, 2014), therefore, representing only a subset of lineages (Characidae and Loricariidae). This indicates a restriction of functional space mediated by pH restraining the occurrence of some lineages typical of headwater streams of the Paraná and Paraguai basins (*e.g*., Cichlidae, Crenuchidae, Heptapteridae, Parodontidade).

Water velocity is also recognized an important factor affecting the compositional patterns of stream fish communities at the local scale. Teresa, Casatti (2012) showed how mesohabitat types (based on water velocity and depth) sort fish species of distinct functional types in lowland streams. We found that streams with low water velocity, such as Fundo stream in our data set (0.17m/s), possess species from lineages with traits characteristic of lentic water, such as *Synbranchus marmoratus* Bloch, 1795, that are not found in streams with high water velocity, which, in turn, possess species of families with traits characteristic of riffle habitats (*e.g*., species of the genus *Hypostomus*, *Piabina argentea* Reinhardt, 1867 and *Hisonotus* sp.). These results complement what was already known regarding the importance of water velocity in determining community composition of these streams (Valério *et al*., 2007) by evidencing the importance of hydrological characteristics to maintain species and evolutionary variation in these communities.

Great attention has been given to the influence of extensive environmental modification and its influence on associated fauna in large rivers from both a theoretical perspective (*e.g*., the river continuum concept proposed by Vannote *et al*., 1980), and an applied perspective focused on anthropogenic effects on fish fauna (Casatti *et al*., 2012; Roa-Fuentes *et al*., 2019, 2020). Here we showed that even the small range of variation in environmental conditions typical of well-preserved headwater streams is capable of influencing the structure of fish communities and maintaining variation in both taxonomic and evolutionary aspects of biodiversity.

The patterns found for the relationship between phylogenetic structure and environmental variables were robust even when accounting for phylogenetic uncertainty due to the lack of resolution within some genera (*e.g*., *Astyanax* and *Characidium* Reinhardt, 1867). Previous studies have emphasized that the use of a synthesis phylogeny (*sensu* Li *et al*., 2019) is not a major concern for common analysis of community phylogenetic structure (Li *et al*., 2019). We endorse this view by showing that uncertainties, mainly associated with recent nodes in our phylogeny, did not imply a substantial problem for interpreting ecological patterns in headwater stream fish communities. Despite our findings, the synthesis phylogeny we used clearly needs improved resolution of phylogenetic relationships among some genera, like *Astyanax* and *Hypostomus*.

From a conservation perspective, our results reinforce the importance of maintaining the environmental integrity of these streams. Environmental modifications, such as land use, directly affect the characteristics of water (*e.g*., pH level), which can act as an important filter of fish species by selecting only specific lineages that possess adaptations (*e.g*., to low pH habitats) and, consequently, modify fish composition (Fialho *et al*., 2008; Araújo, Tejerina-Garro, 2009). Since anthropogenic activities, such as farming and cattle ranching, are associated with alterations to the pH of streams, mainly via the release of calcium into aquatic environment from the terrestrial habitat, it is important to stress how these potential modifications can affect not only the identity of species in streams, but also entire fish lineages, which in turn can have effects on a broad time scale and ecosystem functions. The proliferation of small hydroelectric plants comprises another threat to fish communities (Benejam *et al*., 2016; Couto, Olden, 2018), since water velocity is an important characteristic for maintaining variation of species lineages throughout headwater streams. The alteration of water velocity promoted by such dams could also compromise variation in ecosystem services throughout headwater streams by promoting biotic homogenization, which can have long term effects due to the influence of water velocity on the distribution of species lineages.

We conclude by stressing the importance of considering different dimensions of biodiversity in community studies, together with distinguishing the components of variation in beta diversity (nestedness and turnover), since they are affected in different ways by environmental factors and allow the main drivers of community assembly to be identified in a more directly way than when using the taxonomic dimension alone. We revealed that a combination of phylogenetic habitat filtering and species sorting are the most probable mechanisms structuring both the taxonomic and phylogenetic compositions of headwater stream fish communities in the Paraná and Paraguai River basins. We also revealed that some well-recognized patterns related to regional characteristics (associated with relief and basin) were not supported, stressing the importance of local hydrological characteristics of headwater streams for sustaining both phylogenetic and taxonomic compositions.

Finally, as a suggestion for future studies, and based on our results regarding the importance of pH on the distribution of fish lineages even with a limited range of variation, we encourage investigation into the importance of edaphic composition on a broader scale because it may shed light on possible historical imprints related to the importance of geological history on the distribution of species lineages in stream fish communities of the Paraná and Paraguai River basins.

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AUTHOR’S CONTRIBUTION

Gabriel Nakamura: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing-original draft, Writing-review and editing.

Wagner Vicentin: Data curation, Writing-review and editing.

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The authors declare no competing interests.

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