**Taxonomic and phylogenetic beta diversity in headwater streams of the Paraná and Paraguay basins.**

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**Abstract**

Describing the variation among ecological communities and searching for their causes is a key task in ecological studies. Patterns of species replacement and richness differences along environmental gradients or biogeographic realms shed light on different ecological and evolutionary mechanisms acting on community structure. In aquatic ecosystems communities from different watersheds are supposed to host distinct species, quantify and understand how much of these differences in species composition are affected by environmental and biogeographical factors is already an open question for these environments, particularly in the tropical region. In this work, we investigated the patterns of taxonomic and phylogenetic composition of headwaters streams of Paraná and Paraguay basins to understand how ecological and biogeographical factors affect the assembly of fish communities in these two basins. We quantified the taxonomic and phylogenetic beta-diversity by decomposing them into nestedness and turnover components. We showed that environmental and biogeographical history affected different aspects of headwaters stream fish communities in different ways. Whereas pH affected both taxonomic and phylogenetic turnover, water velocity was responsible for phylogenetic turnover of fish clades. Ph was also the main driver of phylogenetic nestedness. Our results indicated a greater effect of local environmental factors acting in determine the structure of the fish community in stream headwaters of this region through a combination of species sorting mechanism (water velocity and pH) and phylogenetic habitat filtering (pH). Depicting beta diversity among its different components allowed to shed light the balance of different mechanisms acting on community assembly of headwaters streams.

Keywords: phylobetadiversity; tropical streams; community assembly; turnover; nestedness

**Introduction**

Compositional patterns in ecological communities is a result of numerous factors acting both contemporary and historically to determine the assembly of currently species in space and time (1). The patterns of variation exhibited in community composition can reveal the imprints that different factors leave on these communities (2) (e.g. Kissling et al. 2012). In this sense, beta diversity metrics that considers both taxonomic and lineages distribution through space have been showed effective to reveal the balance among contemporary and historical/biogeographical factors acting on community organization (4–8).

Particularly, the investigation of the variation in communities of distinct biogeographic areas have the potential to reveal factors associated with the history of each ecoregion acting on the determination of community structure (9) (Vilhena and Antonelli, 2015). We can ask if the variation among communities are mainly determined by these historical constraints (for example, related to the biogeographical barriers and vicariance), environmental factors, or a balance among these two (10) (Graham and Fine, 2008). For example, (11) Duarte et al (2014) showed how taxonomic and phylogenetic beta diversity patterns reveal that differences in tree composition of Atlantic Forest complex are mainly due to recent events occurring in the evolutionary history of the group. In aquatic ecosystems, (12) Petry et al. (2016) stressed the importance of historical connectivity of coastal lagoons acting on the assembly of fish communities. Therefore, investigate taxonomic and phylogenetic variation from different regions offers a more complete portrait of mechanisms acting on community structure than when use only the traditional taxonomic dimension of biodiversity.

Particularly, in neotropical freshwater ecosystems, the understanding of the balance among historical and local environmental factors is rarely addressed. Paraná and Paraguay basins comprise two of the main tributaries in South America, each one with particularities in their environmental characteristics and biotas, being considered as two distinct bioregions (13) (Albert and Reis, 2011). In this perspective, compare headwaters stream fish communities from Paraná and Paraguay basins, separated by long distances, offers an adequate model to address the biogeographical perspective on the study of fish community assembly.

The current knowledge about headwaters streams of Paraná and Paraguay Basins, suggested that the biogeographical isolation resulted in different stream fish composition among these basins (Valerio et al., 2007), and that Upper Paraná river basin constitute an area of high endemism due to Sete Quedas Fall, that works as a biogeographical barrier. Furthermore, the characteristics of headwaters streams in Paraguay basin (accidented with waterfalls) resulted in a more variable fish fauna than in Paraná Basin due to the higher isolation of the streams (14,15). Therefore, the differences in taxonomic composition can be a result of differences in historical processes acting on the distribution of lineages, however, this is an open question at the moment. Furthermore, little is known about the mechanisms associated to the variation in community composition of headwaters streams (e.g. species sorting, habitat filtering (16)).

In this work, our aim was to assess the patterns of taxonomic and phylogenetic beta diversity in headwaters stream fish communities of Paraguay and Paraná Basins, testing for the influence of local and biogeographical factors acting in the species composition and lineages among these two distinct aquatic bioregions. We hypothesize that Paraná and Paraguay basins presented differences in clade composition, and that these differences are mainly associated with lineages replacement (phylogenetic turnover), due to the long time of separation of these two fish fauna. Furthermore, we also hypothesize that Paraguay basin will present more variation in taxonomic composition among its streams than Paraná Basin, since the first present steeper slopes that results in more isolated streams than the streams of Paraná basin (15).

Specifically we test if 1) the variation in taxonomic beta diversity is also accompanied with a variation in clade composition, indicating distinct evolutionary histories in the species occurrence between these two basins and 2) which factors (local or regional) were the main drivers for the differences in phylogenetic and taxonomic composition between the headwaters streams.

**Methods**

*Sampling sites*

The sampled streams were located within an area of 200 km length between the Paraguay and Paraná basins, near S20° 54’ to 22° 18’ latitude and W54° 56’ to 56° 05’ longitude (Figure 1).

Figure 1

We collected samples bimonthly in 10 streams of each basin (Figure 1), from January to November 2004, totalizing 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 Paraguay catchments is uneven, with many waterfalls higher than 30 m, which act as barriers to fish migration within the basin (15,17). Stream depths varied from 0.11 m to 1.75 m, water velocity from 0.056 m\*s–1 to 1.90 m\*s–1 and altitude from 264 m to 641 m. The streams, inserted in areas with small human population, are relatively well preserved, with most of the natural riparian vegetation remaining. Names and codes for the streams are shown in Figure 1.

We took samples during daylight with a rectangular sieve of 1.2 to 0.8 m, with a 2 mm mesh size and standardized sampling effort (20 throws) at each site, and approximately 50 m 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.

In all analyzed streams, we collected a set of key 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. We obtained 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. To measure altitude (m) we used the altimeter of a GPS. We characterized all streams using mean values of stream depth and velocity.

*Phylogenetic hypothesis for stream fish communities of Paraná and Paraguay basins*

We build a phylogenetic hypothesis for Paraná and Paraguay headwaters streams adapting a molecular time-calibrated tree for bony fishes proposed by (18) Rabosky et al. (2019). The adaptation of the original phylogeny consisted in replace the taxa presented in the original phylogeny by sister taxa of species presented in the species pool of Paraná and Paraguay streams (or the closest specie available in the original phylogeny). The original phylogeny was downloaded with use of R package fishtree (19) (Chang et al. 2019). The R code used to perform all the manipulations of original phylogeny are presented in Appendix S1 of Supplementary material. In summary, we followed a sequential procedure in which we started by replacing all species from the original phylogeny with the species of the same genus presented in our species pool. When the genre was not presented in the original phylogeny we inserted the species in the most related species of the same family and for species that do not presented neither species of the same genre nor family representatives we inserted the species at the order level (e.g. *Synbranchus marmoratus*). After realized all insertions the phylogenetic tree was pruned to maintain only the species that occur in our communities.

The final phylogenetic hypothesis used in this work is shown in Figure 2. We assembled an R function to automate the process of phylogenetic tree edition described above and can be assessed at https://github.com/GabrielNakamura/function\_phyloMatch\_fishtree.

Figure 2

*Assessing turnover and nestedness components of taxonomic and phylogenetic beta diversity*

To quantify the amount of beta diversity in each of the two basins, and to determine how much of this variation is due to differences in richness (nestedness component) and/or species replacement (turnover component), we used the method proposed by (20) Baselga et al. (2012). Beta diversity was calculated as being the pairwise dissimilarity among sites obtained through Sorensen index (Sorensen, 1948). The turnover is represented by Simpson index (21) (Simpson, 1943) and correspond to the dissimilarity among a pair of sites (or communities) removing the effect caused by richness differences. Finally, nestedness component was obtained through the subtraction of turnover component (Simpson) from total beta diversity (Sorensen). Beta diversity values ranged from 0 to 1, where 1 correspond to completely different communities (20,22) (Baselga 2010, Baselga, 2012). We used an occurrence community matrix (presence/absence) and the phylogenetic hypothesis presented in Figure 2 to obtain all components of taxonomic and phylogenetic beta diversity for headwaters stream fish communities.

We analyzed the relationship between environmental and biogeographical (basins) variables and patterns of spatial distribution of different components of functional and taxonomic beta diversity through the use of a permutational multivariate analysis of variance with distance matrices (*adonis2* function in vegan package, Anderson, 2002 (23)). We used the adonis2 function presented in vegan, opting to evaluate the marginal effects of each variable, that corresponded to the effect of each variable when controlling the effects of all other variables in the model.

We tested the hypothesis that Paraguay fish fauna were more variable than that in Paraná through the use of a multivariate analysis of homogeneity of groups dispersion (24) (Anderson, 2006). We performed this test using *betadisper* function from vegan package (25) (Oksanen, XXXX). The analysis consists in calculate the mean distance of sample units to the group centroid that these units belong. We performed this analysis using betadisper function with distance matrices that contained the pairwise dissimilarities of each stream regarding the total, the turnover and the nestedness component of taxonomic and phylogenetic beta diversity. We test if the mean distance of each group differs from one another by performing a permutation test, where a null F distribution is generated and compared to the observed F of the original distances (using *permutest* function in vegan package). All analyses and calculations were performed in R (R Development Core Team, 2014). All p values were stated with nominal error of α=0.05. All data and codes used to perform the analysis can be accessed through GitHub repository https://github.com/GabrielNakamura/MS\_Parana\_Paraguai.

**Results**

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

Greater differences in pH resulted in greater differences of species composition that are related to differences in both taxonomic turnover as well as in phylogenetic turnover and nestedness among streams (Table 1 and 2). Streams with higher pH presented only a subset of lineages of streams with low pH. Besides that, greater differences in water velocity among streams were the main driver of turnover of fish lineages among streams (Table 2). The higher differences in phylogenetic turnover (0.73) was found among Santa Virgem (Paraná) and Fundo (Paraguay) streams, whereas in phylogenetic nestedness was found among São Bento and Apa streams (0.53).

Accordingly to our analysis of homogeneity of variances Paraná and Paraguay basins showed a similar variation in both turnover (F= 1.14, p= 0.30 for taxonomic and F= 0.001, p= 0.97 for phylogenetic dimension) and nestedness components (F= 0.19, p= 0.65 for taxonomic and F= 0.13, p= 0.73) inside each basin, with Paraguay streams presenting a taxonomic and phylogenetic composition slightly more variable than the streams in Paraná basin (Figure 3), but not significant different from Paraná communities. Therefore, we reject the hypothesis that Paraguay basin would present a significant more variable fish fauna than the Paraná headwaters streams, despite the differences in slopes and the presence of waterfalls (Figure 1).

**Discussion**

Our findings showed that the variation among headwaters streams are determined by a combination of environmental factors associated with different processes driving stream fish communities. These factors act in different ways on the distribution of species and lineages among streams of Paraná and Paraguay basin. Our work complements what was already known about headwaters stream fish communities in this region (15,26) (Súarez et al, 2007; Valério et al. 2011), by depicting the environmental factors that also act in the determination of phylogenetic variation of stream fish communities. We also showed for the first time that despite Paraguay headwaters streams are located in a more homogeneous region than Paraguay headwaters streams, the second do not presented a significant difference in the variation of phylogenetic and taxonomic composition of fish communities, as it was believed (15) (Valério et al).

By depicting beta-diversity into its turnover and nestedness components we showed that some environmental factors act mainly by promoting species replacement (pH and water velocity) and other by selecting only a subset of lineages that are tolerant to the environmental characteristics of streams (pH). Together, these results evidence a process of phylogenetic habitat filtering (6), mediated by pH, joint with species sorting mediated mainly by functional traits of species associated with water velocity (e.g. body format) and pH (16).

We reinforce the patterns recognized in other works, regarding the difference among fish fauna presented between Paraná and Paraguay basins (15,26), but showing that this variation do not presented a deep evolutionary base that cause the differences among fish fauna of headwater streams of Paraná and Paraguay, evidencing that species from similar lineages inhabit both basins, despite the different biogeographical history of them (Brea and Zucol, XXXX).

From a conservation perspective, our results sustain the importance to maintain the environmental integrity of these streams. Environmental modification, like land use, affect directly on water characteristics (e.g Ph level), that can promote a depauperation of fish lineages and exert great impact on the capacity of these communities to respond to environmental modification (27) (Fialho et al 2008). Also, the proliferation of small hydroelectric plants (SHPs) comprises another threat to these communities (28) (Benejam et al 2016), since water velocity comprises an important characteristic to maintain lineage turnover along streams. The alteration of water velocity promoted by SHPs could compromise the variation of functional strategies conducting to a lineage and functional homogenization of headwater stream fish fauna.

We conclude by stressing the importance to consider different dimensions of biodiversity in community studies, joint with the distinction among the components of variation in beta diversity (nestedness and turnover), since they are affected in different ways by environmental and biogeographical factors and allow to identify the main drivers of community assembly. Furthermore, we reveal that a combination of phylogenetic habitat filtering and species sorting are the most probable mechanisms structuring both the taxonomic and phylogenetic composition of headwater stream fish communities in Paraná and Paraguay basins.

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**Figure captions**:

**Fig. 1** Headwaters streams sampled in an interface region of the Paraná and Paraguay basins. Blue circles represent streams of the Paraná basin, and triangles represent streams of the Paraguay basin. The stars represent waterfalls. The color indicate the variation in altitude.

Fig 2: Phylogenetic hypothesis for fish collected in headwater stream of Paraná and Paraguay basins.

**Fig. 3** Turnover and nestedness components of taxonomic and phylogenetic beta diversity in streams of the Paraná and Paraguay basins. Clockwise from top left: total, turnover and nestedness components of taxonomic beta diversity, and in the bottom line the total, turnover and nestedness components of phylogenetic beta diversity. Filled circles correspond to Paraná streams and triangles to Paraguay streams.