



Drivers of phylogenetic structure in Amazon freshwater fish assemblages

Laís Salgueiro¹ | Fernanda A. S. Cassemiro² | James S. Albert³ |
Renata G. Frederico⁴ | Max Hidalgo⁵ | Bernard Hugueny⁶ | Céline Jézéquel⁶ |
Hernan Ortega⁵ | Pablo A. Tedesco⁶ | Gislene Torrente-Vilara⁷ | Jansen Zuanon⁸ |
Thierry Oberdorff⁶ | Murilo S. Dias⁹

¹Programa de Pós-Graduação em Ecologia (PPG-ECL), Universidade de Brasília (UnB), Brasília, Distrito Federal, Brazil

²Programa de Pós-Graduação em Ecologia e Evolução, Universidade Federal do Goiás, Goiânia, GO, Brazil

³Department of Biology, University of Louisiana at Lafayette, Lafayette, Louisiana, USA

⁴Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, MG, Brazil

⁵Departamento de Ictiología, Museo de Historia Natural, Universidad Nacional Mayor San Marcos (MUSM), Lima, Peru

⁶Laboratoire Évolution et Diversité Biologique (EDB), Université de Toulouse, CNRS 5174, IRD 253, Toulouse, France

⁷Departamento de Ciências do Mar, Campus Baixada Santista (UNIFESP), Universidade Federal de São Paulo, Santos, SP, Brazil

⁸Instituto Nacional de Pesquisas da Amazônia (INPA), Coordenação de Biodiversidade, Manaus, Brazil

⁹Departamento de Ecologia, Universidade de Brasília, Brasília, Distrito Federal, Brazil

Correspondence

Laís Salgueiro, Programa de Pós-Graduação em Ecologia (PPG-ECL), Universidade de Brasília (UnB), Brasília, Distrito Federal, Brazil.
Email: laissalgueiroribeiro@gmail.com

Murilo S. Dias, Departamento de Ecologia, Universidade de Brasília, Brasília, Distrito Federal, Brazil.
Email: msdias@unb.br

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Abstract

Aim: Phylogenetic relatedness among species can provide useful information on the diversification history and past dispersal events that may have shaped contemporary assemblages. Here, using of the most comprehensive fish occurrence database currently available and a global molecular phylogeny of ray-finned fishes, we evaluate the respective roles of historical and contemporary processes in generating and maintaining fish assemblage phylodiversity patterns among 97 sub-drainages covering the Amazon River basin.

Location: Amazon River basin.

Taxon: Freshwater fishes.

Methods: Using a large comprehensive database of freshwater fish species distributions, and a global molecular phylogeny of ray-finned (actinopterygian) fishes, we estimated historical and contemporary environmental effects on sub-drainage fish phylodiversity patterns using three phylogenetic metrics standardized for richness effect: Phylogenetic Diversity (ses.PD), Mean pairwise Phylogenetic Distance between species capturing patterns at older evolutionary timescales (ses.MPD), and Mean Nearest Taxon Distance capturing patterns at younger evolutionary timescales (ses.MNTD).

Results: We found significant effects of elevation gradients, contemporary climate, and water types on assemblage phylodiversity patterns. Furthermore, we found

Murilo S. Dias and Thierry Oberdorff have contributed equally.



significant relationships among the three phylogenetic metrics used, and between these metrics and the distance of sub-drainages to the Amazon River mouth, representing the Amazon basin West-East longitudinal gradient.

Main conclusions: Phylogenetic diversity showed a highly non-random spatial distribution across the Amazon basin. Beyond significant regional effects of several contemporary and historical drivers, there was a significant West-East decline in sub-drainage assemblages phylogenetic clustering, along with an increase in phylogenetic diversity. These latter patterns suggest deeper evolutionary divergences among taxa located to the East, and more recent radiations in the Western sub-drainages. Based on these findings and given that assemblages are, on average more species-rich in sub-basins of the Western part of the basin than in their Eastern relatives, we conclude that Western Amazon can be seen as an evolutionary “cradle” of biodiversity for freshwater fishes.

KEYWORDS

Amazon drainage basin, habitat fragmentation, historical contingencies, marine incursion, phylogenetic relatedness, water type, West-East gradient

1 | INTRODUCTION

To understand the heterogeneous distribution of life on Earth, it is important to evaluate both historical and contemporary drivers on the origination, dispersal, extinction, adaptation, and coexistence of species at regional spatial scales (Brown, 2014; Hovikoski et al., 2007; Mittelbach et al., 2007; Ricklefs, 2007). Historical drivers often involve past geological and/or climatic events such as the formation of biogeographical barriers to species colonization (e.g. rivers and mountains formation; Rangel et al., 2018) or historical climatic variability (e.g. Dobrovolski et al., 2012; Mascarenhas et al., 2019; Svenning et al., 2015). Evaluating such historical effects often requires the use of phylogenetic data that can provide information on the diversification history and past dispersal events that may have shaped contemporary species assemblages (Albert et al., 2021; Faith, 1992; Lomolino et al., 2009; Miller et al., 2005; Pigot & Etienne, 2015). For example, assemblages formed by many phylogenetically related species (i.e. clustered assemblages) indicate the action of in situ diversification (Tucker et al., 2017; Webb, 2000). On the other hand, assemblages composed of species from distinct evolutionary lineages (i.e. overdispersed assemblages) indicate the role of dispersal assemblage formation (Craig et al., 2020; Dexter et al., 2017; Tucker et al., 2017; Webb, 2000). Understanding these processes can shed light on evolutionary community assembly and on the effects of historical drivers on current distribution patterns (Crouch et al., 2019; Dexter et al., 2017; Graham, 2003; Leprieur et al., 2016; Pyron & Burbrink, 2014; Qian et al., 2020; Sandel et al., 2020).

The Amazon River basin is a major biodiversity hotspot (Antonelli, Ariza, et al., 2018; Malhi et al., 2008), which holds the highest freshwater biodiversity on Earth (Tisseuil et al., 2013). The Amazon dwelling freshwater fishes represent ~15% (>2400 validated species) of all freshwater fish species currently described worldwide (Jézéquel,

Tedesco, Bigorne, et al., 2020; Tedesco et al., 2017). Despite recent advances in describing fish diversity patterns in the Amazon basin (e.g. Albert, Carvalho, et al., 2011; Albert et al., 2011, 2020; Dagosta & Pinna, 2019; Dagosta et al., 2021) or contribution of particular fish lineages (e.g. Melo et al., 2021), only one study so far has attempted to quantitatively analyse multiple basin-wide drivers and separate those effects in a unique framework (Oberdorff et al., 2019). Using 97 sub-drainage basins covering the entire Amazon System, this study revealed prominent influences of current climatic conditions and habitat size on sub-drainage species richness, whereas habitat size, current and past climatic stability, and isolation by natural waterfalls better explained their endemic richness. All these drivers are already well known to promote or slow down extinction, speciation or immigration processes, ultimately shaping riverine fish assemblage structure and diversity (Albert et al., 2020; Hugué et al., 2010).

More surprisingly, Oberdorff et al. (2019) also highlighted a negative upriver-downriver (West-East) gradient in species richness. This pattern is contrary to the expectation of increasing diversity at more downriver locations along fluvial systems. This reversed gradient in species richness was associated to the peculiar history of the Amazon drainage network, which, after having been isolated as Western and Eastern basins since the Paleogene (from ~65 Ma) (Hoorn et al., 2010), only began flowing eastward most probably during mid to late Miocene (from ~9 to 5 Ma) (Hoorn et al., 2017; Latrubesse et al., 2010). During the early Miocene (from ~23 Ma), Western Amazon was occupied by a mega wetland, known as the Pebas System (Wesselingh, 2006), periodically connected to the Caribbean Sea (Bicudo et al., 2019; Jaramillo et al., 2017) and subjected to multiple marine incursions (Hoorn et al., 2010; McDermott, 2021). This wetland system was separated from the fluvial Eastern Amazon possibly by the Purus Arch (Figueiredo et al., 2009). Following this scheme, the unexpected reverse gradient in species

richness found by Oberdorff et al. (2019) suggests that the main historical centre of fish diversity was located westward with a potential second centre of origin located eastward, but much smaller in size and diversity, and that current fish dispersal and adaptation processes from the westward centre are currently progressing eastward, but not yet achieved (Oberdorff et al., 2019).

Considering the phylogenetic dimension may provide further information on the evolutionary processes that shaped Amazon contemporary fish species assemblages. A pattern of phylogenetic diversity congruent with the noticed reverse pattern of species richness will strengthen the hypothesis recently proposed by Fontenelle et al. (2021) that Western Amazon may act as a species pump (*sensu* Haffer, 1969) for Eastern sub-drainages, i.e. diversity-rich sub-drainages in Western Amazon gradually spreading lineages, due to higher speciation and persistence of older lineages, to species-poor sub-drainages in Eastern Amazon. Still, if Western Amazon has acted as a species pump for the whole Amazon basin with currently incomplete species range expansion, this would predict a phylogenetic pattern geographically structured with high phylogenetic diversity and high phylogenetic clustering (i.e. closely related species due to extensive local in situ speciation in this region) in Western assemblages and a progressive decrease along the Amazon Basin West-East gradient. Alternatively, if fish lineages have experienced frequent long-distance dispersal throughout their history, we should expect random patterns of phylogenetic diversity in sub-drainage assemblages, with respect to relatedness and to the West-East gradient.

Here, relying on a large comprehensive database of freshwater fish species distribution in the Amazon River basin (Jézéquel, Tedesco, Bigorne, et al., 2020) and taking advantage of a recently published, global molecular phylogeny of actinopterygian fishes (Rabosky, 2020; Rabosky et al., 2018), we analysed phylogenetic diversity patterns of fish assemblages in our 97 sub-drainages. Examining the same diversity drivers as previously defined in Oberdorff et al. (2019), we determine which of these drivers were most closely associated with sub-drainage patterns of phylogenetic diversity using three complementary metrics: the phylogenetic diversity *sensu stricto* (the total phylogenetic branch length present among species in a given assemblage; PD, Faith, 1992), the mean pairwise distance (the mean of all pairwise phylogenetic distances among species in a given assemblage; MPD, Webb et al., 2002), and the mean nearest taxon distance (the mean of the phylogenetic distance between each species and its closest relative in a given assemblage; MNTD, Webb et al., 2002). While PD measures the overall phylogenetic diversity of an assemblage, the two other metrics represent an average relatedness among fish species composing this assemblage, giving, respectively, information on the deep and recent evolutionary history of taxa within assemblages (Cadotte et al., 2010; Swenson, 2009; Webb, 2000).

We expect these three phylodiversity metrics (i.e. PD, MPD and MNTD), after controlling for species richness effects, to be significantly related to the same drivers already found to act significantly on sub-drainage richness and endemism patterns (Oberdorff et al., 2019). More specifically, we expect (i) high phylogenetic diversity

due to persistence of lineages (low extinction and high in situ diversification) in sub-drainages that have been climatically stable over time; (ii) assemblages located in highly fragmented sub-drainages (i.e. with a large number of natural waterfalls) to present low phylogenetic diversity due to few immigration events and high phylogenetic clustering due to high in situ diversification; (iii) assemblages located in sub-drainages that have suffered from past marine incursions to present low phylogenetic diversity due to increased lineages extinction and high phylogenetic clustering due to in situ diversification of few remaining lineages. Moreover, and as previously said, we expect under the hypothesis proposed by Fontenelle et al. (2021) of Western Amazon acting as a fish species pump for Eastern assemblages (iv) a significant West-East gradient of decreasing phylogenetic diversity and phylogenetic clustering, as Western assemblages should display higher speciation rates and persistence of older lineages compared to their Eastern relatives.

2 | MATERIALS AND METHODS

2.1 | Biological data and phylogeny

Fish occurrence records have been compiled and constantly updated under the AmazonFish project (www.amazon-fish.com) by mobilizing and integrating all information available in published articles, books, gray literature, online databases, worldwide museums and Universities, expeditions conducted during the project, and by checking for systematic reliability and consistency for each species recorded. The published database (Jézéquel, Tedesco, Bigorne, et al., 2020), covering a time span of almost two hundred years (1834–2019), currently contains 21,500 sites, 232,936 georeferenced records for 2406 valid native freshwater fish species from 514 genera and 56 families. To our knowledge, the database is the most accurate and complete compilation of fish distributional and regional species richness data for the Amazon River basin. Even if sampling gaps do exist, mainly located in regions either difficult to access due to the topography of the basin and/or located in protected areas (i.e. indigenous lands and strictly protected areas) (Jézéquel, Tedesco, Bigorne, et al., 2020; Jézéquel, Tedesco, Darwall, et al., 2020), sampling sites constituting the database are evenly distributed all over the basin, minimizing the risk to bias the results of our analyses (see Appendix Fig. S1.1 in Supporting Information). As we were interested in riverine fishes, we excluded all species from the genus *Orestias* (i.e. 15 species) as species diversification within this genus mostly occurred in lentic habitats from the Andes highlands (Scott et al., 2020).

We defined fish assemblages from species presence/absence within each of the 97 sub-drainages covering the entire Amazon River basin. The full description of sub-drainages delineation is detailed in Oberdorff et al. (2019). Briefly, we classified our sub-drainage basins based on the HydroBASIN framework (Lehner & Grill, 2013) and combined different HydroBASIN levels to retain sub-drainages >20,000 km² to optimize sampling effort (see Appendix Fig. S1.1 in Supporting Information). Even if sampling effort within

sub-drainages was higher, on average, in the Amazon mainstream than in other parts of the basin, a previous analysis using three completeness descriptors showed that 70% of the 97 sub-drainages may be considered relatively well surveyed, with no bias in their geographic distribution (Jézéquel, Tedesco, Darwall, et al., 2020).

We obtained phylogenetic information on Amazon fishes from the most recent and largest ray-finned fish supertree (Rabosky, 2020; Rabosky et al., 2018; Chang et al., 2019). Overall, the backbone of this global supertree consists of 11,638 species with available genetic data (27-gene multi-locus alignment), and 130 fossil-constrained nodes to produce a time-calibrated phylogeny. Taxa with no genetic information (19,888 species) were further inserted into this phylogeny based on the monophyly of its most restrictive taxonomic rank (e.g. order, family, genus) (Rabosky et al., 2018). To determine divergence time for species without genetic information and thus resolve the problem of branch lengths and polytomies, Rabosky et al. (2018) sampled from a distribution of waiting times conditioned on rank-specific estimates of speciation rates, resulting in 100 fully sampled ray-finned fish phylogenies and more accurate placement of species without genetic information (see Rabosky et al. (2018) for a full description of the methodology). For the Amazon basin, we found 635 species (27% of the entire Amazon fish fauna) for which genetic data were available (hereafter, genetic tree) and 1451 species for which inclusion was based on taxonomy, resulting in 2086 fish species (87% of the entire fish fauna) in the final pruned tree (hereafter, genetic-taxonomic tree).

2.2 | Phylogenetic fish assemblage metrics

We first calculated phylogenetic diversity *sensu stricto* (PD), mean phylogenetic diversity (MPD), and the mean nearest taxon distance (MNTD) metrics for each sub-drainage based on species occurrence records, our two phylogenetic trees (i.e. genetic and genetic-taxonomic trees), and the cophenetic distance (i.e. the pairwise distance between tips, which is the sum of branch length between species). PD measures the total branch length of all species occurring in a given sub-drainage assemblage (Faith, 1992), whereas the other two metrics represent an average of phylogenetic relatedness among fish species (i.e. phylogenetic dispersion of clades) composing each sub-drainage basin assemblage (Cadotte et al., 2010; Swenson, 2009; Webb, 2000). MPD measures mean pairwise branch length distances between all species within a given assemblage, whereas MNTD measures the mean values of only the shortest branch length distances between species (Tucker et al., 2017). MPD gives this information on the deep evolutionary history of taxa, while MNTD gives information on the recent evolutionary history of taxa as calculated from the terminal structure of the tree (Webb, 2000). The use of these three distinct phylogenetic metrics is justified here as the historical events linked to the Amazon basin evolution occurred at various time scales (Li et al., 2019; Mazel et al., 2016).

A potential bias related to these three phylogenetic metrics (PD, MPD and MNTD) is their sensitivity to the total species richness

present in each assemblage (Cadotte et al., 2010; Sandel, 2018; Tucker & Cadotte, 2013). We removed this richness effect from the raw metric values (PD, MPD and MNTD) (Kembel et al., 2010; Miller et al., 2017; Webb et al., 2002) by calculating the richness-standardized versions of these measures (hereafter ses.PD, ses.MPD and ses.MNTD; or Phylogenetic Diversity Index, Net Related Index and Nearest Taxon Index, respectively, *sensu* Tsirogianis & Sandel, 2016) following the method proposed by Tsirogianis and Sandel (2016). The method consists in analytically calculating expected mean and standard deviation estimates of PD, MPD and MNTD for a given phylogenetic tree and a particular species set and using these estimates to calculate richness-standardized versions of metrics by subtracting the observed metric values from their expected mean and dividing by their expected standard deviation (Tsirogianis et al., 2012, 2014). This method is much faster and efficient than methods based on randomization approaches (Tsirogianis & Sandel, 2016). As the genetic-taxonomic data consists in 100 fully sampled ray-finned fish phylogenies, we calculated all these indices for each tree and averaged their values over all samples to provide a single value for each standardized metric.

For interpretation of the standardized metrics, positive ses.PD, ses.MPD and ses.MNTD values indicate, respectively, higher phylogenetic diversity, higher phylogenetic overdispersion and lower phylogenetic clustering compared to what is expected for a given assemblage containing the same number of species. Conversely, negative values indicate respectively lower phylogenetic diversity, lower phylogenetic overdispersion and higher phylogenetic clustering compared to what is expected for a given assemblage containing the same number of species. We used these standardized metrics in further analyses (see Table S1.2 for genetic-taxonomic tree values in Appendix 1 and Table S2.5 for genetic tree values in Appendix 2). All the ses calculations were performed under the R environment (R Core Team, 2020) using 'pd.query', 'mpd.query' and 'mntd.query' functions from the 'PhyloMeasures' package (Tsirogianis & Sandel, 2016).

Pearson correlation coefficients comparing the three ses metrics from the genetic tree with those from the genetic-taxonomic tree were all positive and significant ($r_{\text{ses.PD}} = 0.69$, $p < 0.001$; $r_{\text{ses.MPD}} = 0.83$, $p < 0.001$; $r_{\text{ses.MNTD}} = 0.45$, $p < 0.001$). However, we kept both trees (i.e. the genetic and genetic-taxonomic trees) for subsequent statistical model developments to control for potential biases linked to the paucity of species integrated in the genetic tree and for the absence of genetic information for a substantial number of species in the genetic-taxonomic tree. Given that both statistical models gave overall similar trends, we mostly discuss results obtained for the genetic-taxonomic tree but also provide model results from the genetic tree (Table S2.6 in Supporting Information).

2.3 | Historical and contemporary drivers

Large-scale biodiversity patterns can be explained by a range of ecological and historical drivers (Brown, 2014; Ricklefs, 2004), and most of these drivers also apply to freshwater fishes at large spatial scales

(Hugueny et al., 2010). These drivers can be summarized under climate/productivity, area/environmental heterogeneity, historical/evolutionary, and spatial hypotheses. Data sources and definitions of the drivers used in this study are presented in detail in Oberdorff et al. (2019), and we only provide here a brief overview of each of them. All predictors described below have been extracted for each of the 97 sub-drainage basins, providing a single mean value for each of them (Table S1.3).

We included variables related to the Amazon basin geological history from distinct time periods. We identified the sub-drainages potentially belonging (1) or not (0) to the Pebas System at ~23 Mya (*sensu* Hoorn et al., 2010), the surface area of each sub-drainage under seawater considering a sea-level rise of 25 m (<1 Mya) and of 100 m (~5 Mya) during recent Pleistocene marine incursions (Miller et al., 2005), and the Quaternary climate stability within the sub-drainages (from ~21 kya to present). We used Quaternary climate reconstructions of mean, max and min annual temperatures and precipitations at the Last Glacial Maximum (LGM; 21 kyr) from three Global Circulation Models (Community Climate System Model, Model for Interdisciplinary Research On Climate, and Max-Planck Institute; data available from www.worldclim.org/version1) (Hijmans et al., 2005) and calculated the difference between current and LGM mean values (from the three models) of the same variables to describe Current-LGM climate stability (Diff_CurrentLGM). We then performed a principal component analysis (PCA) on the current-past climatic differences and retained the first three axes explaining 88% of the total variation. We considered correlation coefficients higher than 0.25 (negative or positive) as the variables better explaining each PCA axis. PC1_Diff_CurrentLGM is positively associated to maximum precipitation (0.27), mean (0.52) and maximum (0.57) temperature, and negatively associated to minimum (-0.45) and annual precipitation (-0.32). PC2_Diff_CurrentLGM is positively related to a minimum temperature (0.29) and negatively associated with annual (0.63) and maximum (0.66) precipitation. The PC3_Diff_CurrentLGM is positively associated to a minimum precipitation (0.39) and minimum (0.87) temperature.

We estimated the fragmentation of sub-drainage basins, a key driver of freshwater fish diversity at a large scale (Dias et al., 2013), using the number of waterfalls within each sub-drainage (Waterfall) using data available from <http://wp.geog.mcgill.ca/hydrolab/hydrofalls/>. We further used the distance of each sub-drainage to the river mouth (km) (DistMouth) to represent the longitudinal gradient within the Amazon River network (see Oberdorff et al., 2019 for a detailed explanation).

To estimate the effect of current climate and productivity, we used the annual mean and seasonality (CV of intra-year monthly values) of temperature (Temp), precipitation (Prec), actual evapotranspiration (AET), potential evapotranspiration (PET), net primary productivity (NPP), solar radiation (SolRad), run-off (RO) and the lowest (or highest) value of the minimum (or maximum) temperature of the coldest (or warmest) month from WorldClim (version 1) (Hijmans et al., 2005). These variables measure the mean current climatic condition, the seasonal climatic variability, and the potential energy availability

within each sub-drainage basin. We also included elevation (mean, minimum, maximum, range; in m) as climate and elevation are usually linked. These global environmental variables (GlobEnv) were summarized through a principal components analysis (PCA) to reduce multicollinearity. We used the first four PCA axes, which explained 85% of total variability, as synthetic variables describing current climate and elevation gradient (Table S1.1 in Appendix 1). For results interpretation, we considered correlation coefficients higher than 0.25 (negative or positive) as the variables better explaining each PCA axis. The first axis of PCA (PC1_GlobEnv) is positively associated with net primary productivity seasonality (0.25) and negatively associated with minimum temperature (-0.26). The second axis (PC2_GlobEnv) is positively associated with precipitation seasonality (0.30), seasonal actual evapotranspiration (0.31), minimum (0.25) and maximum (0.37) potential net primary productivity and maximum temperature (0.30) and negatively associated with minimum precipitation (-0.29) and mean net primary productivity (-0.25). PC3_GlobEnv, the third axis of PCA, is positively associated with minimum solar radiation (0.29) and negatively correlated to annual precipitation (-0.27), maximum (-0.32) and annual (-0.28) actual evapotranspiration, maximum (-0.28) and seasonal (-0.30) potential evapotranspiration and temperature seasonality (-0.34). Finally, the fourth axis (PC4_GlobEnv) is positively related to mean (0.28), maximum (0.25), variability (0.26) and range (0.37) elevation, minimum potential evapotranspiration (0.37) and mean net primary productivity (0.32) and negatively related to potential evapotranspiration seasonality (-0.29) and temperature seasonality (-0.27) (see Table S1.1).

Habitat size and habitat diversity were estimated using the surface area of the sub-drainage basin (km²; Area), the network density (i.e. length of the riverine network divided by the surface area of the sub-drainage, a measure of habitat availability for fishes; NetwD), the land cover heterogeneity (i.e. a Shannon diversity index on the proportions of land cover classes within each sub-drainage basin; CoverDiv), and the soil heterogeneity (i.e. a Shannon diversity index on the proportions of each soil type within each sub-drainage basin; SoilDiv). These variables have been calculated and fully described in Oberdorff et al. (2019).

Amazon waters were divided into three distinct biogeochemical water types or "colours" known to affect fish assemblage structure (e.g. Bogotá-Gregory et al., 2020). We classified sub-drainages according to their main water type differentiated by sediment composition, geochemistry and optical characteristics (Venticinque et al., 2016). White waters have a predominantly Andean origin (e.g. the Madeira River and the Amazon mainstem), and are characterized by low transparency due to large amounts of sediment particles and a neutral pH (pH ~7). Nutrient poor blackwaters are mostly draining the Precambrian Guiana shield (e.g. the Negro River) and are characterized by their high acidity (pH <5). Clear waters are nutrient-poor, highly transparent and slightly acidic waters (pH ~6) that mostly drain the Brazilian and Guianas shields (e.g. the Tapajós and Xingu Rivers) (Sioli, 1984). The three water types were coded as categorical variables.

Finally, the number of sampling sites divided by the surface area of each sub-drainage (SamplingEffort) was also included in our

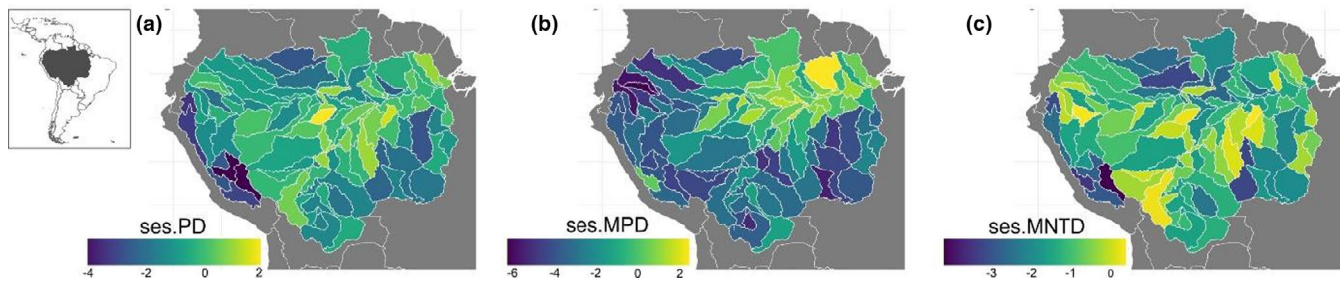


FIGURE 1 Standardized effect size (ses.) of phylogenetic metrics calculated using 2086 native Amazon freshwater fish species (genetic-taxonomic tree) from 97 sub-drainages of the Amazon drainage basin. Phylogenetic diversity *sensu stricto* (ses.PD; a), Mean Pairwise Distance (ses.MPD; b), and Mean Nearest Taxon Distance (ses.MNTD; c). Negative values of ses.MNTD and ses.MPD indicate phylogenetically clustered assemblages (i.e. assemblages predominantly composed of closely-related lineages), whereas positive ones indicate phylogenetically overdispersed assemblages (i.e. assemblages composed of distinct phylogenetic lineages). The inset map locates the Amazon basin in South America

models to control for a potential sampling effort effect as was previously noticed by Oberdorff et al. (2019).

2.4 | Statistical analyses

Prior to the analyses, we log-transformed ($\log[x+1]$) some predictors (i.e. surface area, number of waterfalls, sampling effort, elevation mean and elevation range) to reduce the effects of extreme values. As sea level predictors are proportions bounded between 0 and 1, we applied an arcsin square root transformation. Finally, we standardized predictors by subtracting the mean and dividing by two times the standard deviation in order to get comparable coefficients for our models (Gelman, 2008).

We fitted multiple linear regression models to determine the drivers of our three phylogenetic metrics (i.e. ses.PD, ses.MPD and ses.MNTD), and the significance of all predictors was determined by dropping individual variables from the full model and applying Likelihood Ratio Tests (LRT). The Variance Inflation Factor (VIF) was calculated for each predictor after model fitting. We found all values below 9 (Mean = 2.17, SD = 1.68), suggesting that multicollinearity was not an issue in our models. We checked the normality of residuals and model assumptions by drawing histograms of models' residuals and plotting model residuals against each predictor. Using Cook's distance, we checked and found no potential influential observations in our models. We used partial residuals plots from the four linear models to represent the partial relationship between predictors (e.g. distance of sub-drainages from the river mouth, 'DistMouth') and a given phylogenetic metric (ses.PD, ses.MPD, ses.MNTD) while controlling the effects of other predictors variables (Fox & Weisberg, 2019).

We also tested for spatial autocorrelation in model residuals by calculating the Moran's I statistic and its p -values, using the inverse of the watercourse distance among pairs of sub-drainages as weights. When spatial autocorrelation was detected, we constructed Moran's Eigenvector Maps (MEM) computed with watercourse distance. The spatial vectors related to the spatial structure for each predictor were obtained from a forward selection algorithm

that avoid type I error (Blanchet et al., 2008), and we included the selected MEMs as predictors in multiple regression models to control for spatial autocorrelation. After including MEMs in the models, spatial autocorrelation (Moran I values) disappeared from models residuals (all Moran' I were not significant), but we recalculated variance inflation factor (VIF) and found high VIF values (i.e. VIF > 10; Mean = 2.78, SD = 3.38) in the ses.PD models for some explanatory variables (i.e. distance, PC1_GlobEnv and MEM1, MEM3, VIF > 10). We obtained, however, similar responses when either including or excluding these variables from the models so that we maintained all variables in our models.

All analyses have been conducted in R environment (R Core Team, 2020) using 'GISTools', 'ggeffects', 'ggplot2', 'vegan', 'rgeos', 'effects', 'adespatial' and 'car' packages.

3 | RESULTS

3.1 | Spatial distribution of phylogenetic metrics

The ses.PD values varied between 1.88 and -4.32 (Mean = -0.75 , SD = 1.17; Figure 1a), with sub-drainages Coari (1.88) and Blanco Baures (1.87) having the highest phylogenetic diversity, and Ucayali2 (-4.33) and Urubamba (-4.32) those with the lowest values. Globally, high ses.PD values were found in sub-drainages located near the Amazon main course, and low values in peripheral sub-drainages. Values of ses.MPD vary between 2.43 and -6.25 , (Mean = -1.90 , SD = 2.15; Figure 1b), suggesting that phylogenetic clustering is more frequent than overdispersion in the sub-drainages analysed. The northeastern sub-drainages Trombetas1 (2.43) and Amazon9 (1.91) have the highest positive ses.MPD values, and the northwestern sub-drainages Curaray (-6.25) and Napo2 (-5.77) are the lowest. As for ses.PD, we observed that ses.MPD displays the highest values along the Amazon main course and the lowest values at the periphery of the Amazon basin. For ses.MNTD, more negative than positive values are also found (Mean = -1.03 , SD = 0.96, range = -3.98 – 0.58 ; Figure 1c), suggesting that most sub-drainages present a clustered phylogenetic pattern. The sub-drainages Maues

(0.58) and Jamanxim (0.58) have the highest positive ses.MNTD values, and Urubamba (-3.98) and Mantaro (-3.20) the lowest negative values. Phylogenetic metrics calculated from a genetic tree (635 fish species) presented a similar distribution as the three phylogenetic metrics calculated from the genetic-taxonomic tree (see Fig. S2.6 in Supporting Information).

Contrary to their respective raw values, Pearson correlations show that ses.PD, ses.MPD and ses.MNTD are unrelated to taxonomic metrics (richness and endemism; lower triangle, Table 1; see Table S2.4 in Supporting Information for genetic tree results). The raw values of all phylogenetic metrics showed high correlation values among each other and the other taxonomic metrics (upper triangle, Table 1; Table S2.4). Correlations were lower between richness-standardized metrics than between raw metrics (Table 1).

3.2 | Determinants of the three phylogenetic diversity metrics

None of our historical predictors showed an effect on ses.PD, which was only significantly negatively related to sub-drainage surface area and distance of the sub-drainage to the Amazon River mouth (Figure 2a; Table 2). We also observed a marginally significant positive effect of sub-drainage network density (i.e. the length of the riverine network divided by the surface area of the sub-drainage) on ses.PD. We found a significant spatial structure in model residuals, which was controlled for by including the three selected MEMs (Fig. S1.2).

The ses.MPD was positively related to the surface of sub-drainage covered by seawater at ~5 Mya (Figure 3b), negatively related to distance to the river mouth (Figure 2b) and related to current climate (positive effect of PC3_GlobEnv, and negative of PC4_GlobEnv) (Fig. S1.3–S1.4; Table S1.1). Values of ses.MPD were also significantly related to water types, white and clear waters having lower values compared to black waters (Fig. S1.5). Finally, significant

TABLE 1 Pearson Correlations between species richness, endemism, and each of the three phylogenetic metrics calculated from the genetic-taxonomic tree for 2086 freshwater fish species (Phylogenetic Diversity, PD; Mean Pairwise Distance, MPD; Mean Nearest Taxa Distance, MNTD). Values above the diagonal (upper triangle) refer to correlations between sub-drainages total species richness, endemic richness and the raw phylogenetic metric values (i.e. without controlling for richness). Values below (lower triangle) refer to correlations between sub-drainages total species richness, endemic richness and the standardized effect size (ses.) of the three phylogenetic metrics

	Richness	Endemism	PD	MPD	MNTD
Richness	–	–	0.98	0.43	–0.84
Endemism	0.44	–	0.38	0.02	–0.37
ses.PD	0.14	–0.21	–	0.52	–0.89
ses.MPD	0.09	–0.18	0.43	–	0.51
ses.MNTD	–0.04	–0.21	0.79	–0.01	–

negative effects of natural fragmentation (i.e. the total number of waterfalls; Figure 3a), sub-drainage surface covered by seawater at <1 Mya (Figure 3c), distance from the river mouth (Figure 2c), and surface area of sub-drainage were observed for ses.MNTD values.

For the above-mentioned predictors, similar trends emerged when running the analyses using the genetic instead of a genetic-taxonomic tree, even if the statistical significance of some of them changed slightly (see Table S2.6). This convergence between predictors trends for the statistical models using either the genetic-taxonomic tree or the genetic tree makes us confident that results we obtained using the genetic-taxonomic tree are not skewed by the absence of genetic information for a substantial part of the species.

4 | DISCUSSION

Both contemporary and historical drivers play important roles in explaining current patterns of diversity in Amazon taxa (Albert et al., 2021; Antonelli, Zizka, et al., 2018; Azevedo et al., 2020; Crouch et al., 2019; Coronado et al., 2015; Réjaud et al., 2020). Here, using a large data set on freshwater fish distribution in the Amazon River basin (Jézéquel, Tedesco, Bigorne, et al., 2020) and a recent phylogenetic supertree (Rabosky et al., 2018), we found marked and contrasting phylogenetic signatures of fish assemblages across the 97 sub-drainages covering the entire system, whatever the phylogenetic metric analysed (i.e. ses.PD, ses.MPD, ses.MNTD). These contrasting signatures were related to various historical and contemporary drivers most often similar to the ones related to richness and endemism patterns (Oberdorff et al., 2019). Besides studies performed at small spatial scales (Craig et al., 2020) or using taxonomic classification as a proxy for phylogeny (Dagosta et al., 2021), as far as we know, this is the first study applying a phylogenetic framework and depicting the determinants of fish assemblages at the scale of the whole Amazon River basin.

4.1 | Contemporary drivers of Amazon fish phylodiversity patterns

We found the effects of some contemporary environmental drivers on assemblage phylogenetic structure in our 97 sub-drainages. The third and fourth PCA axes describing current climate were respectively positively and negatively linked to ses.MPD values that capture the variation in phylodiversity at deep time scales. The former axis (PC3_GlobEnv) is linked to high energy availability and stable climatic conditions, whereas the latter (PC4_GlobEnv) is negatively related to high elevation and steep gradients (see Fig. S1.3 and S1.4 in Appendix 1). Both relationships indicate more overdispersed fish assemblages in sub-drainages located in the Eastern part of the basin and displaying low elevation and less steep gradients. Due to these specific environmental conditions, these sub-drainages may accumulate lineages due to lower extinction and higher colonization probabilities (Carvajal-Quintero et al., 2019; Oberdorff et al., 2019).

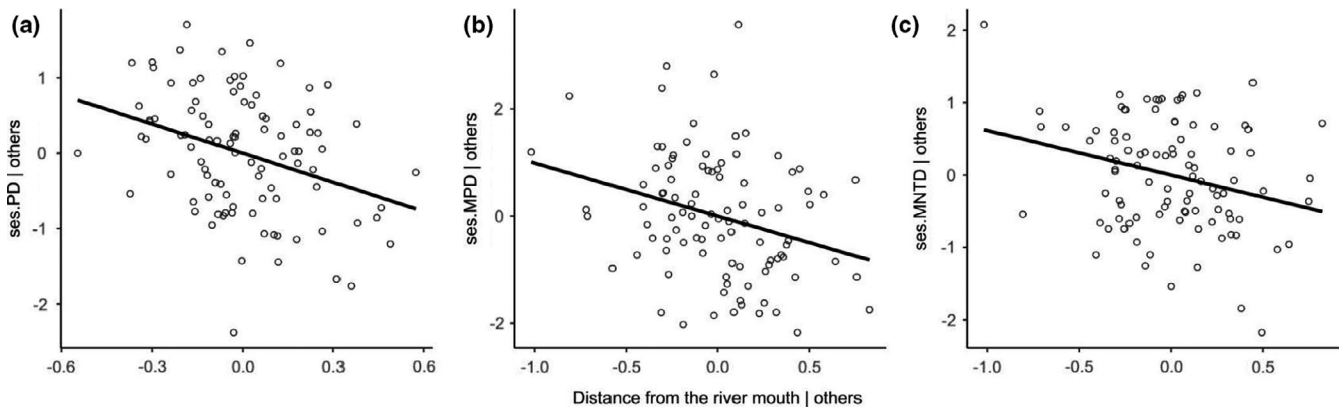


FIGURE 2 Partial regressions plots representing the significant relationships between the distance from the Amazon River mouth (DistMouth) with standardized effect size of fish communities Phylogenetic Diversity (ses.PD; a) in the 97 Amazonian sub-drainages and their Mean Pairwise Distance (ses.MPD; b) and Mean Nearest Taxon Distance (ses.MNTD; c), after controlling for all other predictors considered in our models. These partial effects (residuals) are calculated after excluding effects from all the other predictors in the model. The overall decrease in phylogenetic metrics from downriver (East) to upriver (West) is statistically significant (solid line, Table 2) (ses.PD, $p = 0.003$; ses.MPD, $p = 0.009$; ses.MNTD, $p = 0.016$)

High elevation areas, on the other hand, are more restricted to colonization by highly adapted species and subject to numerous random extinction events due to habitat harshness (Datry et al., 2016). This may result in species-poor assemblages with low lineage diversification over the long term.

Our results also depict an effect of water types on ses.MPD, black waters hosting assemblages more phylogenetically overdispersed than clear and white waters (Fig. S1.5). This pattern may be due to the characteristics of black water stained by tannins and humic acids leached from vegetation, causing low pH (pH~5 or lower) and low autochthonous productivity (Bogotá-Gregory et al., 2020). These characteristics create strong barriers to colonization for species unfitting these conditions that necessitate long term adaptation (Beheregaray et al., 2015; Crampton, 2019; Dagosta & Pinna, 2019; Gonzalez et al., 2006; Van Nynatten et al., 2015) and may have thus promoted lineages diversification through isolation processes.

4.2 | Historical drivers of Amazon fish phylodiversity patterns

Freshwater fishes are highly limited by connectivity among habitats (Carvajal-Quintero et al., 2019; Rahel, 2007; Tonkin et al., 2018; Wiens, 2002). The negative relationship was found between the number of waterfalls and the phylogenetic metric ses.MNTD (Figure 3a), capturing the variation in phylodiversity at the recent evolutionary time, suggests that intensely fragmented sub-drainages have assemblages formed by closely related species (i.e. assemblage showing a phylogenetic clustering pattern). This pattern can be attributed to allopatric speciation due to reduced population dispersal and consequently reduced gene flow among fragmented populations (Dias et al., 2013; Tedesco et al., 2012). Peripheral sub-drainages of the Amazon basin drain fragmented landscapes (e.g. ancient, cratonic rivers in the Brazilian and Guiana shields and recent Andean

mountains) (Bicudo et al., 2019; Hoorn et al., 2010) and contain high fish endemism levels (Oberdorff et al., 2019). Furthermore, although ses.PD and ses.MPD showed no significant link with habitat fragmentation by natural waterfalls, the low values of both metrics over the whole Amazon basin indicate lower phylogenetic diversity *sensu stricto* than expected in sub-drainages with comparable species richness. Together, these findings support the idea of recent speciation events coupled with both high extinction and/or low colonization rates in fragmented sub-drainages (Albert, Carvalho, et al., 2011).

The Amazon basin has been subject in the past to a series of marine incursions in both its Western (e.g. Bicudo et al., 2019; Hoorn et al., 2010) and Eastern (e.g. Christ et al., 2021) margins, and in more ancient (Eocene to Miocene periods in the West; Pozo and Pebas Systems) and more recent (Pleistocene marine incursions in the East) time scales. These marine incursions have favoured the adaptation of several marine lineages to freshwater environments (Bloom & Lovejoy, 2017; Fontenelle et al., 2021; Lovejoy et al., 2006) and probably led to high extinctions due to the concomitant elimination of freshwater habitats in the affected areas (Oberdorff et al., 2019). In agreement with this last hypothesis, we found a significant negative relationship between ses.MNTD and Eastern sub-drainages impacted by the last sea-level rise during the middle Pleistocene (<1 Mya, up to ~25 m in elevation) (Christ et al., 2021), meaning that assemblages in these sub-drainages are more phylogenetically clustered. This may be due to high extinction rates of lowland freshwater fish species in submerged areas that reduced their overall species richness, and diversification processes in the remaining, high elevation and isolated areas not affected by the seawater (Oberdorff et al., 2019). We also found that the phylogenetic metric ses.MPD, capturing the variation in phylodiversity at deep phylogenetic levels, was positively related to Eastern sub-drainages having experienced older and longer marine incursions (~5 Mya, from 50 to 100 m and a duration of ~0.8 Mya) (Haq et al., 1987). Given that phylogenetic diversity *sensu stricto* (ses.PD) is unrelated to marine incursions and

TABLE 2 Estimates, 95% confidence interval and p -values from the multiple regression models for phylogenetic diversity estimated using ses.PD, ses.MPD and ses.MNTD. Significant relationships ($p < 0.05$) estimated from Likelihood Ratio Tests are in bold

	ses.PD		ses.MPD		ses.MNTD	
	Estimates (CI)	p	Estimates (CI)	p	Estimates (CI)	p
(Intercept)	-1.05 (-1.95 to -0.15)	0.023	-1.00 (-2.22 to 0.22)	0.108	-1.27 (-2.09 to -0.45)	0.003
WaterColor [Clear]	-0.20 (-1.14 to 0.75)	0.683	-1.10 (-2.35 to 0.16)	0.085	-0.24 (-1.07 to 0.60)	0.579
WaterColor [White]	0.48 (-0.31 to 1.27)	0.229	-1.18 (-2.28 to -0.08)	0.036	0.28 (-0.45 to 1.02)	0.449
NetwD	0.19 (-0.02 to 0.40)	0.075	-0.12 (-0.42 to 0.18)	0.432	0.12 (-0.08 to 0.32)	0.230
Area	-0.33 (-0.63 to -0.03)	0.030	0.02 (-0.35 to 0.39)	0.909	-0.27 (-0.51 to -0.02)	0.033
SoilDiv	0.08 (-0.17 to 0.33)	0.506	0.24 (-0.10 to 0.58)	0.160	0.05 (-0.18 to 0.27)	0.686
DistMouth	-1.29 (-2.12 to -0.46)	0.003	-0.99 (-1.73 to -0.25)	0.009	-0.61 (-1.11 to -0.12)	0.016
CoverDiv	-0.12 (-0.47 to 0.23)	0.511	-0.01 (-0.50 to 0.48)	0.959	-0.01 (-0.34 to 0.32)	0.945
PC1_Diff_CurrentLGM	-0.08 (-0.45 to 0.29)	0.654	0.37 (-0.14 to 0.87)	0.151	0.14 (-0.20 to 0.48)	0.413
PC2_Diff_CurrentLGM	-0.01(-0.36 to 0.34)	0.959	-0.22 (-0.66 to 0.21)	0.315	0.01 (-0.28 to 0.31)	0.920
PC3_Diff_CurrentLGM	-0.04 (-0.35 to 0.27)	0.803	-0.19 (-0.61 to 0.23)	0.379	0.07 (-0.21 to 0.35)	0.615
PebasLake	0.08 (-0.72 to 0.88)	0.844	0.17 (-0.82 to 1.16)	0.726	0.20 (-0.46 to 0.86)	0.551
Seawater at <1 Mya	-0.26 (-0.63 to 0.11)	0.165	0.42 (-0.10 to 0.93)	0.111	-0.35 (-0.69 to 0.00)	0.050
Seawater at ~5 Mya	0.16 (-0.19 to 0.51)	0.358	0.71 (0.22 to 1.20)	0.005	-0.14 (-0.47 to 0.19)	0.397
Waterfall_log	-0.16 (-0.42 to 0.09)	0.201	0.20 (-0.14 to 0.53)	0.240	-0.29 (-0.51 to -0.06)	0.012
PC1_GlobEnv	0.46 (-0.19 to 1.11)	0.162	-0.07 (-0.82 to 0.67)	0.847	-0.15 (-0.65 to 0.35)	0.548
PC2_GlobEnv	-0.17 (-0.61 to 0.27)	0.449	0.45 (-0.08 to 0.98)	0.093	0.04 (-0.32 to 0.39)	0.827
PC3_GlobEnv	-0.17(-0.46 to 0.11)	0.225	0.55 (0.19 to 0.92)	0.003	-0.23 (-0.48 to 0.01)	0.063
PC4_GlobEnv	-0.14 (-0.38 to 0.11)	0.268	-0.43 (-0.75 to -0.10)	0.011	-0.00(-0.22 to 0.22)	0.989
SamplingEffort	-0.22 (-0.48 to 0.04)	0.096	-0.22(-0.58 to 0.15)	0.242	-0.22(-0.46 to 0.03)	0.084
MEM3	-0.47 (-0.84 to -0.11)	0.012	-	-	-	-
MEM1	0.18 (-0.40 to 0.77)	0.537	-	-	-	-
MEM6	-0.16 (-0.36 to 0.05)	0.127	-	-	-	-
R^2/R^2 adjusted	0.587/0.464		0.738/0.674		0.414/0.270	
Moran's I (p value)	-0.01 ($p = 0.67$)		-0.01 ($p = 0.60$)		-0.01 ($p = 0.14$)	

that many marine-derived species such as anchovies, flatfishes, pufferfishes, drums, needlefishes, and stingrays are present in these Eastern sub-drainages, we suggest that the increase in ses.MPD is at least partly related to the presence of marine-derived lineages in these sub-drainage assemblages (Dagosta & Pinna, 2019).

Our results show no significant effect of the categorical variable "Pebas Lake System" (*sensu* Hoorn et al., 2010) on assemblage phylogenetic diversity patterns. There was indeed no marked indication of structured phylogenetic diversity—overdispersed or clustered fish assemblages—that could have been produced by extinction, dispersal, and diversification of lineages within the Pebas System as defined in our study. However, the environmental attributes and geographical boundaries of the Pebas System remain uncertain and poorly understood (Bicudo et al., 2019; Fontenelle et al., 2021; Hoorn et al., 2010; McDermott, 2021). Given the rather rough categorical variable used here, we may have missed some key areas of the historical Pebas System, failing to capture any significant phylogenetic structure. This is possible as we did find a strong phylogenetic structure along the Amazon West-East gradient suggesting different histories between these two regions.

4.3 | Basin-wide drivers of fish phylogenetic diversity

We found a significant pattern of fish assemblage phylogenetic diversity along the Amazon West-East gradient, whatever the metric we used. Phylogenetic diversity *sensu stricto* (ses.PD) and ses.MPD metrics decrease from East to West (Figure 2) meaning that Western assemblages are less phylogenetically diverse than Eastern ones. This result is inconsistent with the prediction of higher lineages diversity in sub-drainages of the Western Amazon and thus refutes the hypothesis that this region acts as a broad species pump for the whole basin, as recently suggested by Fontenelle et al. (2021) based on a study of marine-derived lineages. This decrease in assemblage phylogenetic diversity (*sensu stricto*) from East to West, already highlighted by Dagosta et al. (2021) using a pure taxonomic-based tree, also contrast with the species richness gradient observed by Oberdorff et al. (2019) (i.e. sub-drainages fish species richness slightly but significantly increases from East to West). However, we also found here that Western assemblages are more phylogenetically clustered than Eastern ones (i.e. a decrease in ses.MNTD values from East to West, Figure 2). This finding suggests large and

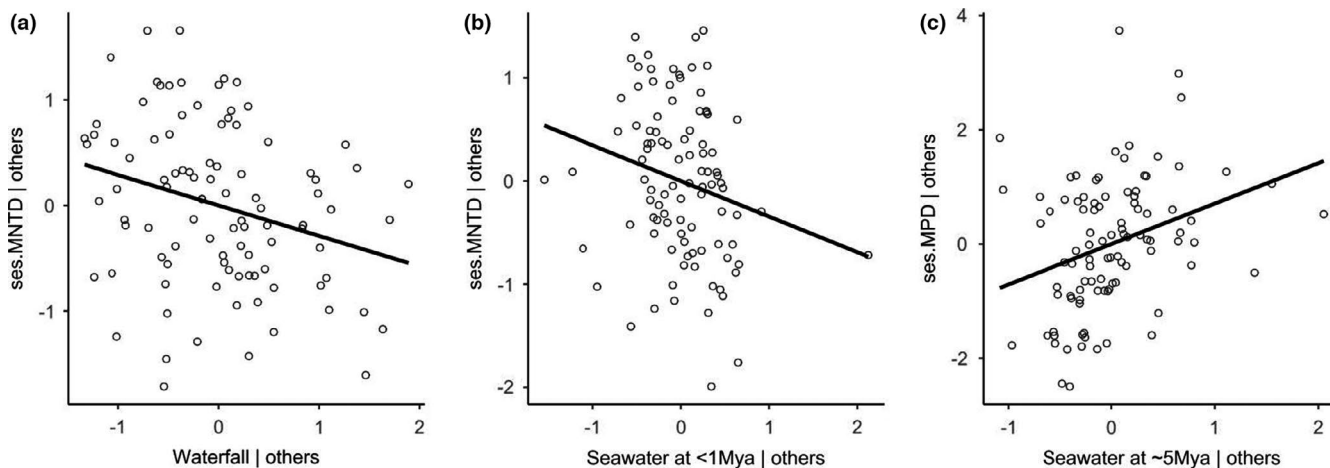


FIGURE 3 Partial regressions plots showing the effects of (a) the habitat fragmentation (Waterfall), (b) proportion of sub-drainages surface covered by seawater at <1 Mya (up to 25m in altitude) and (c) at ~5 Mya (up to 100m in altitude) on the standardized effect size of Mean Pairwise phylogenetic Distance (ses.MPD) and Mean Nearest Taxa phylogenetic Distance (ses.MNTD) of fish communities in the 97 Amazonian sub-drainages, after controlling for all other predictors considered in our models. These partial effects (residuals) are calculated after excluding effects from all the other predictors in the model. The overall decrease in ses.MNTD in more fragmented sub-drainages and in sub-drainages with more proportions of the area covered by seawater at <1 Mya are statistically significant ($p = 0.012$ and $p = 0.050$, respectively); the overall increase in ses.MPD in sub-drainages with more proportions of the area covered by seawater at ~5 Mya is statistically significant ($p = 0.005$) (solid line, Table 2)

recent radiations of fewer lineages in sub-drainages of the Western Amazon that may have generated higher overall species richness over time compared to more Eastern ones. Following these patterns, Western Amazon can be seen as an evolutionary cradle of biodiversity (i.e. location with unusually high rates of speciation) rather than a species pump region. In contrast, the lower phylogenetic diversity in Western assemblages compared to Eastern ones suggests either historical limited colonization events or most probably intense historical lineages extinction in this region.

Indeed, the repeated transitions between Eocene and Miocene periods from fluvial-like systems to wetlands (Pozo and Pebas Systems) (Antoine et al., 2016; Bicudo et al., 2019) produced strong habitat filtering for species that, together with complex salinity gradients due to periodical connections of the System to the Caribbean region, may have promoted lineage extinction events and remaining lineage diversification in the successive fluvio-lacustrine systems. The analysis of fossil records potentially available in this region may shed further light on these possible extinction processes (e.g. Chabain et al., 2017). In contrast, the proto-Amazon System flowing eastward of the Purus Arch has been geologically and hydrologically more stable than Western Amazon during the past 30 million years (Bicudo et al., 2019; Hoorn et al., 2010; Sacek, 2014), probably causing higher phylogenetic diversity *sensu stricto* due to accumulation and persistence of lineages in this area over a longer period of time (Coronado et al., 2015). Furthermore, fish assemblages in this Eastern region (downstream part of the Amazon current longitudinal gradient and near the historical West-East Amazon divide of Purus Arch) may benefit from lineages accumulation due to the conjunction of the three Amazon water types and to local colonization of species historically inhabiting both sides of the historical barrier (Albert et al., 2021; Dagosta et al., 2021).

To conclude, our study reveals a highly non-random spatial distribution of three phylogenetic metrics (ses.PD, ses.MPD, ses.MNTD) across the 97 sub-drainages covering the Amazon basin. We found Western Amazon sub-drainages hosting assemblages more phylogenetically clustered (higher rates of recent speciation) but less phylogenetically diverse than their Eastern counterparts. Even though some West-East fish dispersion events occurred (Albert et al., 2021), our finding goes against the hypothesis of Western Amazon acting as a historical species pump for its Eastern part but rather place Western Amazon as a current evolutionary cradle of biodiversity. Our results also suggest that diversification most often occurs within specific geographic areas (e.g. naturally fragmented areas, water type-dependence), and that the long-distance dispersal of species among major regions is less frequent (but see Fontenelle et al. (2021) for marine-derived lineages). It should be now relevant to analyse the variation in species composition between sub-drainage assemblages (i.e. taxonomic Beta diversity *sensu* Whittaker, 1960) since that will bring a more precise picture of the effect of dispersal limitation in shaping current fish assemblages in the Amazon River basin.

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CONFLICT OF INTEREST

There is no conflict of interest among co-authors.

DATA AVAILABILITY STATEMENT

Species present in each sub-drainage (Jézéquel, Tedesco, Bigorne, et al., 2020) and the global scale phylogeny (Rabosky, 2020; Rabosky et al., 2018) are available through [https://figshare.com/articles/datas et/A_database_of_freshwater_fish_species_of_the_Amazon_Basin/9923762](https://figshare.com/articles/datas_et/A_database_of_freshwater_fish_species_of_the_Amazon_Basin/9923762) and <https://fishtreeoflife.org/>, respectively. Phylogenetic metrics and their standardized effect size (ses), together with all environmental and historical predictors included in the statistical models are available for each of the 97 sub-drainages in supplementary materials (Tables S1.2, S1.3 and S2.5) and online at Zenodo <https://doi.org/10.5281/zenodo.5589763> (Salgueiro et al., 2021).

ORCID

Lais Salgueiro  <https://orcid.org/0000-0003-4338-1942>

Fernanda A. S. Cassemiro  <https://orcid.org/0000-0003-3015-5524>

Thierry Oberdorff  <https://orcid.org/0000-0001-7323-2599>

Murilo S. Dias  <http://orcid.org/0000-0002-7213-5284>

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BIOSKETCH

Laís Salgueiro is an ecologist mostly interested in the study of biogeography and phylogenetic patterns of freshwater organisms. This work is a result of her Master degree at the Universidade de Brasília.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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