The latitudinal speciation gradient in freshwater fishes: higher speciation at higher latitudes in the northern hemisphere

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Abstract

Speciation rates are a key driver of diversity patterns and are often used to explain the latitudinal diversity gradient (LDG). However, latitudinal variation in speciation rates at both assemblage and species levels remains poorly explored in freshwater fishes. This highlights a gap in understanding the mechanisms driving geographic biodiversity gradients in freshwater fishes. Here, we investigated the latitudinal speciation gradient in freshwater fishes, using a comprehensive database of freshwater fish distributions and phylogenetic relationships of Actinopterygian fishes. We estimated speciation rates using three metrics (BAMM, DR, and ClaDS) and evaluated the latitudinal speciation gradient through spatial and phylogenetic regressions at assemblage and species levels. Finally, we analyzed those patterns based on the species assemblage's phylogenetic diversity and structure. Our results show that areas and species with the highest speciation rates were located in the tropics. However, the general assemblage pattern revealed a positive relationship between absolute latitude and speciation rates. This relationship is generally absent in tropical regions below 23.8° and became significant only at higher latitudes, particularly in the Northern Hemisphere. We do not find a significant relationship at the species level, mainly due to the strong influence of hyper-diverse groups like Cichliformes. When Cichliformes were excluded, the relationship between latitude and speciation became significant, aligning with assemblage-level results. The areas whit higher speciation rates also match with areas whit recent radiation and higher clustering in all latitudes. However, septentrional assemblages show high speciation rates, which reflects environmental filtering and dispersal events consistent with the glaciation and deglaciation in the Pleistocene.

1. INTRODUCTION

One of the most striking biodiversity patterns is the remarkable number of species found in tropical regions, contrasting with the lower species richness in temperate regions (Hillebrand, 2004). This well-documented latitudinal diversity gradient (LDG) has been consistently observed across taxa, ranging from microorganisms (Fuhrman et al., 2008) and plants (Jiang et al., 2023) to vertebrates, including marine and freshwater fishes (Miller & Román-Palacios, 2021; Rabosky et al., 2018), amphibians, birds, and mammals (Jenkins et al., 2013). Multiple hypotheses have been posited to explain this gradient, which can be categorized into three main groups: ecological hypotheses, implying factors related to species coexistence and persistence; historical hypotheses, considering the timing of colonization and the duration and extent of tropical environments; and evolutionary hypotheses, which consider the diversification dynamics of studied clades (Mittelbach et al., 2007).

Diversification rates defined as the balance between species origination and extinction rates, should play a pivotal role in shaping the LDG. Indeed, a higher diversification in the tropical region compared to the temperate region would be sufficient to generate the current LDG (Mittelbach et al., 2007). While higher diversification rates in the tropics can explain their exceptional diversity, whether diversification rates in the tropics genuinely surpass those outside this region as well as their consequences for biodiversity gradients, remain relatively less explored compared to ecological and environmental factors (Schluter &

Pennell, 2017). This is mainly because estimating diversification rates is challenging given the time scale at which they occur and the lack of reliable information such as fossils (Morlon, 2014). To overcome these limitations, current methods infer diversification patterns using reconstructed phylogenies, depicting the evolutionary relationships among extant species (Morlon et al., 2024). For example, different metrics have been proposed to estimate tip-level speciation rates, but they provide reliable information only for present-day speciation and have different assumptions that can yield inconsistent results across different metrics (Title & Rabosky, 2019; Vasconcelos et al., 2022).

Despite methodological differences, speciation rate metrics have been useful for assessing the role of speciation in shaping the LDG (Morales-Barbero et al., 2021; Rabosky et al., 2018). Using these metrics, several studies have found that speciation rates are not the primary driver of the LDG. Instead, other factors such as climatic stability and the timing of colonization, have emerged as the key explanatory variables of the LDG (García-Andrade et al., 2023; Miller & Román-Palacios, 2021; Morales-Barbero et al., 2021). Other studies have focused on describing the latitudinal speciation gradient with contrasting results across different scales and taxa (Schluter & Pennell, 2017, Wiens, 2024), varying from positive (Rabosky et al., 2018) to negative (Pulido-Santacruz & Weir, 2016) or no relationship (Economo et al., 2018). Moreover, the majority of studies exploring the latitudinal variation of speciation have been conducted using taxa distributed in open habitats (such as marine and terrestrial ecosystems) or using assemblages as units without considering species-level patterns (Miller & Román-Palacios, 2021). As such, the question of how speciation rates vary across latitude at assemblage and species levels in clades that occupy fragmented habitats that limit connectivity, such as rivers, remains open.

Freshwater habitats, which cover only 0.8% of the Earth's surface (Downing et al., 2006), are characterized by their confinement within terrestrial landscapes forming units delimited by drainage basin boundaries. Inside these basins, the landscape is characterized by drainages that describe an arborescent bifurcation of a mainstem and branches that decrease in size and increase in number as one proceeds up the network (Campbell Grant et al., 2007) and by isolated or connected water surfaces or lakes. The configuration of freshwater habitats limits the dispersion routes available to strictly aquatic organisms to the river branches of the drainage network or maintains organisms isolated in the lake system. Consequently, the freshwater configuration and connectivity profoundly affect the mechanism leading to speciation, extinction, and dispersal (Albert et al., 2020). Furthermore, freshwater habitats are more susceptible to the effects of tectonic movements, which can shape the land's topography, alter river courses and species geographic distributions (Carvajal-Quintero et al., 2019). Such tectonic movements have been linked to the diversification of freshwater taxa, like barrier-displacement allowing dispersal by merging adjacent geographic areas, or barrier-formation resulting in vicariant speciation and heightened extinction risk (Albert et al., 2020).

Actinopterygian fishes comprise ~ 35,000 species (Froese & Pauly, 2024), nearly 50% being freshwater. The similar species richness between marine and freshwater fishes, despite the ample difference in habitable area between both habitats, has incited longstanding interest in understanding the processes driving such pattern. The fragmented nature of freshwater habitats has been suggested as the principal driver of fish species richness through allopatric speciation promoting faster diversification rates compared to those of marine fishes (Tedesco et al., 2017a). However, this idea has been debated as freshwater fish richness appears to be primarily driven by exceptional diversification in confined systems such as lakes (Miller, 2021) and in particular clades (Rabosky, 2020). For example, in Cichlidae, radiation via sympatric speciation have resulted in high diversification rates and ample species richness (Ngoepe et al., 2023). The study of speciation patterns at global scale in Actinopterygian fishes has been facilitated by the development of large calibrated molecular phylogenies (Betancur-R et al., 2015; Rabosky et al., 2018). For marine fishes, a recent study using range maps and a gridded global domain revealed an inverse latitudinal speciation gradient relative to species richness, with higher speciation rates at higher latitudes, a pattern consistent between assemblage and species levels (Rabosky et al., 2018). Similar findings were obtained by Miller & Román-Palacios (2021) for freshwater fishes when evaluating their latitudinal species richness pattern, with higher species richness in tropical basins associated to low speciation rates and low species richness in basins with high speciation rates. This study focused on species richness explanations at the assemblage level using species lists within discrete basins to define their geographic ranges (Tedesco et al., 2017b), but did not consider species-level patterns nor a continuous gridded domain as done by Rabosky et al. (2018) for marine fishes.

Here, we described and evaluated the latitudinal pattern of speciation in freshwater fishes using a comprehensive database of freshwater fish range maps (García-Andrade et al., 2023) and the recent phylogeny of Actinopterygian fishes (Rabosky et al., 2018). We employ two complementary approaches: (1) an assemblage-level analysis considering species sets within a gridded domain as observational units and (2) a species-level analysis considering individual species as observational units. Combining both approaches provide different perspectives on the same pattern and thus a more robust evaluation (Olalla-Tárraga et al., 2010; Ruggiero & Hawkins, 2006). Given the pervasive nature of the hierarchical and bifurcating architecture within dendritic networks across all latitudes, we anticipated a least pronounced pattern in the latitudinal speciation gradient of freshwater fishes compared to the gradient exhibited by marine fishes. In addition, we evaluated the observed speciation gradient based on the phylogenetic diversity and structure patterns of freshwater fish assemblages across the globe. These patterns can provide insights into the evolutionary process of speciation, extinction and dispersal that imprint discernible spatial patterns (Davies & Buckley, 2011) and have been recently evaluated in freshwater fish but focusing on specific regions independently (e.g., North America, Qian et al., 2020; South America, Cassemiro et al., 2023).

2. METHODS

2.1 Geographic and phylogenetic data

Geographical distributions were obtained from García-Andrade et al. (2023), who reconstructed the geographic ranges (i.e., extents of occurrence) of 12,557 freshwater fish species by creating a convex hull for each species based on its occurrence records and overlapping it with the Hydrobasin layer level 8 (see details in García-Andrade et al., 2023). These ranges were projected to the Behrmann equal-area projection. We considered freshwater fishes as those species recorded in either freshwater, brackish or both habitats. Species recorded in freshwater and saline habitats were discarded to avoid including migratory or incidental species in our analyses. This classification was based on a taxonomic review using the FishBase database, accessed via the rfishbase 4.0 package (Boettiger et al., 2012) on January 3, 2024.

Phylogenetic relationships were obtained from a recent species-level phylogeny of Actinopterygian fishes, both freshwater and marine, comprising 31,516 species of ray-finned fishes (Rabosky et al., 2018) from 34,721 known species in 2024 (Froese & Pauly, 2024). This phylogeny was built using a 27-gene multilocus alignment of 11,638 species with genetic data (including 6,407 freshwater species) under a maximum likelihood framework and time-calibrated using fossil data. Species with no genetic data were imputed into this molecular phylogeny based on their taxonomic placement using a stochastic polytomy resolution under constant-rate birth-death process. This imputation was repeated to generate a distribution of 100 all-taxon assembled (ATA) phylogenies comprising 31,516 species of ray-finned fishes.

2.2 Speciation rates

We used three different metrics to calculate tip-level speciation rates (λ), one non-model based and two model-based metrics, to evaluate pattern robustness and avoid artifacts from a given metric. First, we calculated the λDR statistic (Jetz et al., 2012), using the harmonic mean of this metric for each species across the 100 ATA phylogenies with 31,516 species. This metric estimates recent speciation rates based on branch lengths and splitting events without relying on a parameterized model (Title & Rabosky, 2019). Second, we used the model-based estimates of BAMM (Rabosky, 2014), including (i) time-varying rate regimes BAMM (λTV) and (ii) time constant rates (λTC), previously estimated by Rabosky et al. (2018) using only the molecular phylogeny (11,638 tips). The data was available in the Fish Tree of Life website (https://fishtreeoflife.org/). The BAMM method uses a reversible-jump Markov chain model to infer the number and location of diversification-rate shifts across branches of a tree allowing inferring the diversification-rate parameters (speciation and extinction) on each tip of the phylogeny. Third, we calculated tip-level speciation rates using the ClaDS model (λClaDS) (Maliet & Morlon, 2022), a Bayesian approach where diversification rates change only at speciation events. Unlike BAMM, which infers few large shifts in diversification regimes (cohorts), ClaDS infers many small and frequent shifts thus allowing more heterogeneous rates among lineages. We applied the ClaDS model to the molecular phylogeny containing only species with genetic data (11,638 tips) using the recent ClaDS2 model available in jPANDA package in Julia (Maliet & Morlon, 2022), considering the same sampling fraction reported in Rabosky et al. (2018).

After calculating speciation rates for all Actinopterygians fishes, we focused on freshwater species, defined as all those inhabiting freshwater and/or brackish ecosystems. Considering both phylogenies (completed ATA phylogenies and the molecular phylogeny) along with the available geographic ranges, we obtained two final datasets: i) a “complete” dataset with 12,557 species, representing 77% of freshwater fishes with geographic ranges and λDR speciation rate data; and ii) a “molecular” dataset with 5,242 freshwater species from the molecular phylogeny, representing ~ 30% of all freshwater fish species described to date with geographic ranges and λDR, λTC, λTV and λClaDS speciation rate values.

2.3 Assemblage-level analyses

To reconstruct the latitudinal speciation gradient for freshwater fishes at the assemblage level for both datasets, we first created a species' presence-absence matrix using a global grid-cell system of 111 x 111 km resolution overlaid onto the projected geographic ranges using the letsR package (Vilela & Villalobos, 2015). For each grid-cell assemblage of co-occurring species, we computed the mean speciation rate value for each considered metric in four different ways: (i) arithmetic mean, (ii) geometric mean, and their weighted versions: (iii) weighted arithmetic mean and (iv) weighted geometric mean, while also considering their log-transformed versions (Table S1.1). Species weights were determined as the inverses of the number of grid cells in which each species was present (Table S1.1). These metric means were employed to mitigate biases arising from the sensitivity of the arithmetic mean to extreme values and to diminish the influence of widely distributed species (Rabosky et al., 2018).

We assessed the relationship between assemblage speciation rates and latitude applying different models, with the speciation rate as response variable and the absolute latitude as predictor variable to allow fitting linear models. First, we employed ordinary least squares (OLS) to model the global relationship. Then, we considered the presence of spatial autocorrelation and adjusted spatial autoregressive models (SARs). For the global SAR model, we evaluated two neighborhood distance matrices among the grid-cell centroids, 111 km (minimum) and 1000 km (maximum), and three weighting schemes for the distance matrix: row-standardized (W), globally standardized (C), and a variance-stabilizing coding scheme (S) (Kissling & Carl, 2007). We chose the best-fitting SAR model based on AIC values and the highest R^2. SARs models were fitted using the spatialreg package (Pebesma & Bivand, 2023) in R. Then, to model more complex latitude-speciation relationships, we fitted generalized additive models (GAMs) with the mgcv package in R using default settings (Wood, 2017). Finally, we applied segmented OLS models (OLS-seg) to evaluate the presence of breakpoints in the studied relationship corresponding to different slopes for distinct regions of the latitudinal gradient. For this, we used the segmented package for R (Muggeo, 2017) based on the AIC values and the Davies test to determine the presence of a breakpoint. If a breakpoint was identified, separate OLS and SAR models were fitted for each region before and after the breakpoint. SAR models for each region were built using the same configuration as the best-fitting SAR model for the global relationship (i.e., distance matrix and weighting scheme).

2.4 Species-level analyses

To evaluate the latitude-speciation relationship at the species level, we only considered the species present in the molecular phylogeny. We calculated the absolute latitudinal midpoint for each species and related them to their speciation rates using OLS, OLS-seg, and GAM models for each speciation metric. Additionally, we conducted phylogenetic regressions (PGLS) to consider the shared evolutionary history among species and avoid high type 1 error rates on regression coefficients (Revell, 2010). In addition, we ran an ES-SIM model to test the effect of species traits (in this case, absolute latitudinal midpoint) on their speciation rates considering only the λDR metric. This method is robust to model misspecification and phylogenetic pseudo-replication (Harvey & Rabosky, 2018). Additionally, we repeated the species-level analyses excluding the order Cichliformes, which is the most species-rich clade in freshwater fishes and has the highest diversification rates. Doing so allowed us to avoid the potential influence of such a dominant clade and thus a misleading interpretation of the general pattern (Rabosky, 2020). All models in both levels of analysis relating (absolute) latitude and speciation rate were fitted using latitude in meters to conform with the use of projected range maps. However, to facilitate depicting observed patterns and discussing our findings, we report our results with latitude in degrees.

2.5 Assemblage phylogenetic structure

To aid the interpretation of the latitudinal speciation gradient and infer potential underlying processes, we evaluated the phylogenetic structure of freshwater fish assemblages across the globe. We calculated three metrics per grid-cell assemblage using the ATA phylogenies: phylogenetic diversity (PD), residual phylogenetic diversity (rPD), and Net Relatedness Index (NRI). PD considers the shared ancestry among species and is quantified as the total branch length of the tree that connects all species present within an assemblage, which is inherently positively correlated to species richness (Faith, 1992). rPD is the phylogenetic diversity that is not explained solely by species richness and can be used as a proxy of how evolutionary events (i.e., speciation, extinction, and dispersal) have contributed to species assemblages, where areas with low rPD harbor less evolutionary history than expected from their species richness resulting from high *in situ* speciation and few dispersal events, whereas areas with high rPD harbor more evolutionary history than expected from species richness resulting from low *in situ* speciation and frequent dispersal events (Davies & Buckley, 2011). We estimated rPD by extracting the residuals of a locally estimated scatterplot smoothing (LOESS) where species richness was predicted by PD. Finally, NRI measures the standardized effect size of the mean pairwise distance of all species conforming an assemblage relative to random assemblages from a species pool (Webb, 2000). Values of NRI above 1.96 indicate phylogenetic clustering (i.e., more closely related species than expected by chance). Negative values below – 1.96 indicate phylogenetic overdispersion (i.e., more distantly related species than expected by chance), and values between 1.96 and – 1.96 indicate no (i.e., random) phylogenetic structure relative to the species pool (Webb, 2000). We defined the species pool for each grid-cell assemblage as the set of species present in their corresponding biogeographic regions following (Tedesco et al., 2017b): Nearctic, Neotropical, Palearctic, Afrotropical, Australian, Indo-Malay, and Oceania.

3. RESULTS

Both datasets, complete (12,557 species) and molecular (5,242 species), exhibited the same geographic pattern following the classic LDG, with higher species richness in the tropics compared to the temperate regions (Fig. 1 for the molecular set and Fig S1.1 for the complete set). Regarding speciation rates, the average tip-level speciation rate in the complete dataset was λDR = 0.21 species per myr. In the case of the molecular dataset, mean speciation rates showed similar values for λDR and λClaDS metrics (0.21, 0.17; respectively), whereas values for the BAMM-derived metrics were (λTC = 0.23, λTV = 0. 21). Despite the differences among the speciation rate metrics, we found a high correlation (Pearson's r > 0.8) between all the metrics at the species level (Fig. S1.2). Accordingly, in the next sections, we only present results for λDR, λClaDS, and λTC, thus excluding one of the BAMM-derived metrics, λTV, whose results can be found in the extended appendix.

3.1 Latitudinal speciation gradient at the assemblage level

At the assemblage level, mean speciation rate across grid-cells were higher for the BAMM-derived metric (λTC = 0.2) and lower for the λDR (0.13) and λClaDS (0.10). All speciation metrics showed strong geographic structure, with similar peaks of speciation rate values around the tropics (11°-13° south, 11°-12° north) and at northern latitudes (> 45°) (Fig. 1). Despite the tropics exhibiting the highest speciation values, our results consistently revealed an positive latitudinal gradient in which overall speciation rates increased towards higher latitudes outside the tropics, mainly in the northern hemisphere (Fig. 1).

The global OLS model showed a significant positive relationship between absolute latitude and speciation rate using the complete dataset and the λDR metric ($\beta = 0.0004$, $R^2 = 0.0071$, $p < 0.001$; see Table S2.1 for variants of this metric). When considering the molecular dataset, the OLS models also showed a significant positive relationship between latitude and speciation rate for λDR ($\beta = 0.0003$, $R^2 = 0.004$, $p < 0.001$) and λTC ($\beta = 0.0041$, $R^2 = 0.1236$, $p < 0.001$), whereas the relationship was not significant for λClaDS (Fig. 2, Table S2.2). When considering the other variants of the speciation rate metrics, the majority showed similar significant patterns, whereas few metrics showed non-significant relationships (w-λDR-geo, log(λClaDS), log(w-λDR), log(w-λDR-geo)) (Table S2.2).

Spatial regressions consistently showed a significant positive relationship between speciation and latitude, with higher speciation rates at higher latitudes, regardless of the speciation metric. SAR models provided better fit as well as higher explanatory power than OLS models for both the complete (λDR: $\beta = 0.0011$, $R^2=0.5623$, $p < 0.05$) and molecular datasets (λDR: $\beta = 0.0006$, $R^2=0.4889$, $p < 0.05$; λTC: $\beta = 0.0046$, $R^2=0.8548$, $p < 0.05$; and λClaDS: $\beta = 0.0003$, $R^2=0.4978$, $p < 0.05$; Tables S2.1, S2.2.). GAM models, in turn, showed that the relationship between speciation and latitude is spatially complex regardless of the speciation metric (Fig. 2), with all models presenting better fit than OLS models and effective degrees of freedom of the smooth term (edf) higher than 1 (λDR complete: $R^2=0.0414$, edf = 8.25, $p < 0.001$; λDR molecular: $R^2=0.0232$, edf = 8.90, $p < 0.001$; λTC: $R^2=0.2986$, edf = 8.97, $p < 0.001$; λClaDS: $R^2=0.0098$, edf = 8.23, $p < 0.001$; Tables S2.3-S2.4). Similarly, OLS-segmented models provided a better fit than simple OLS models and identified a single breakpoint in the speciation-latitude relationship for most metrics, except for two variants of the ClaDS metric (Table S2.4). The location of these breakpoints along the latitudinal gradient differed among metrics, varying between 23.8º and 50.87º, with the lower latitudinal values for the λClaDS metric variants (23° to 28°) and the higher ones for the λDR for both complete and molecular datasets (29º-50.37º, 32.87–49.52º, respectively), followed by the λTC metric variants (43°- 47°) (Table S2.3, Fig. S2.1-S2.4).

When fitting models separately for each region around the identified breakpoints, in general, the left-side models (i.e., below the breakpoint) showed either negative (in OLS models) or non-significant relationships between speciation and latitude (SAR models). OLS models showed negative relationships for λDR complete (β=-0.0002, R^2=0.0022, $p < 0.001$), λDR molecular (β=-0.0004, R^2=0.0038, $p < 0.001$), and λClaDS (β=-0.0004, R^2=0.0029, $p < 0.001$), whereas it was not significant for λTC. SAR models showed a significant and positive relationship only for the complete dataset (λDR: $\beta = 0.0003$, R^2=0.5622, $p = 0.051$), whereas for the molecular dataset, there was no significant relationship regardless of the speciation metric (Tables S2.5, S2.6). In contrast, both OLS and SAR models fitted to the right-side of (i.e., above) the breakpoint consistently showed significant and positive relationships between speciation and latitude across all speciation metrics (SAR results where λDR complete: $\beta = 0.0101$, R^2=0.5433, $p < 0.001$; λDR molecular: $\beta = 0.0035$, R^2=0.5696, $p < 0.05$; λTC: $\beta = 0.0314$, R^2=0.9113, $p < 0.001$; λClaDS: $\beta = 0.0011$, R^2=0.6138, $p < 0.001$, Tables S2.7, S2.8). Overall, the relationship between speciation and latitude across freshwater fish assemblages holds true only at higher latitudes outside the tropics. Across all models, global and segmented, coefficients showed higher effect sizes and steeper slopes for the BAMM-derived metric λTC, followed by the λDR and the λClaDS metrics.

3.2 Latitudinal speciation gradient at the species-level

At the species level, considering only the molecular dataset, the latitudinal speciation gradient was somewhat similar to the assemblage-level pattern, with species located at the tropics and at northern latitudes exhibiting higher speciation rates than species with midpoints at other latitudes. The highest speciation rates at latitudes > 45º, namely in the northern hemisphere, belonged to species from the Salmoniformes, Perciformes, Clupeiformes, and Cypriniformes which also have marine counterparts (Fig. S3.1), whereas in the tropics these belonged to species of the order Cichliformes. Notably, prominent groups of Ostariophysi (Characiformes and Siluriformes) exhibited similar speciation rates along the latitudinal gradient (Fig. 3, Fig. S3.2).

In contrast to the assemblage-level models, OLS models at the species-level showed significant negative relationships between speciation and latitude for all metrics, but with low coefficients (λDR: β=-0.0008, R^2=0.0009, $p < 0.05$; λTC: β=-0.00011, R^2=0.002, $p < 0.001$; λClaDS: β=-0.00013, R^2=0.0027, $p < 0.001$). GAMs again showed that the relationships are spatially complex (λDR: R^2=0.062, edf = 8.86, $p < 0.001$; λTC: R^2=0.0738, edf = 8.88, $p < 0.001$; λClaDS: R^2=0.0802, edf = 8.9, $p < 0.001$). However, no significant breakpoints were found in the OLS-segmented models (Table S3.1). Finally, PGLS models showed a significant positive relationship only for λDR with high phylogenetic signal ($\beta = 0.000165$, R^2=0.00124, $p < 0.05$; Pagel's $\lambda > 0.75$, Table S3.2, Fig. S3.3).

When conducting the analyses excluding Cichliformes, OLS models showed opposite patterns to those found including the order, with positive and significant for all speciation metrics (λDR: $\beta = 0.00013$, R^2=0.0058, $p < 0.001$; λTC: $\beta = 0.00011$, R^2=0.0054, $p < 0.001$; λClaDS: $\beta = 0.0008$, R^2=0.005, $p < 0.001$).

GAM models also showed complex spatial relationships (λDR: $R^2=0.0432$, edf = 7.68, $p < 0.001$; λTC: $R^2=0.0342$, edf = 7.21, $p < 0.05$; λClaDS: $R^2=0.0444$, edf = 8.49, $p < 0.001$; Table S3.3). In this case, the OLS-segmented did suggest a breakpoint between 41.87º-43.09º for all speciation metrics, with steeper slopes to the right of (higher latitudes) this breakpoint than to the left (lower latitudes) (λDR: β left = 0.0002, β right = 0.047; λTC: β left = 0.0003, β right = 0.0367; λClaDS: β left = 0.0001, β right = 0.032), mirroring the patterns observed in the assemblage-level analysis (Table S3.4). Finally, PGLS models showed significant and positive relationships for λDR (β = 0.0086, $R^2=0.0009$, $p < 0.05$) and λClaDS (β = 0.00062, $R^2=0.00121$, $p < 0.05$; Table S3.4-5, Fig. S3.4).

The ES-SIM analysis did not find a significant correlation between species' latitudinal position and their speciation rates (rho = 0.0225, p = 0.89). However, when excluding the Cichliformes, this correlation turned significant (rho = 0.1421, $p < 0.05$).

3.3 Assemblage phylogenetic structure

As expected, the geographic pattern of phylogenetic diversity (PD) closely followed that of species richness. However, when accounting for the effect of species richness via the residual PD (rPD), a distinct geographic pattern emerged. The lowest rPD values were located in the Afrotropic central region, particularly in Lakes Malawi, Tanganika and Victoria. Negative rPD were also present in some regions from southern North America to South America, western Europe, east Asia, and northern Oceania. Conversely, positive rPD values were present in northern Africa, central India, South Asia, the Malay Archipelago, and northern Siberia (Fig. 4).

Within biogeographic regions, most assemblages showed no phylogenetic structure (non-significant NRI values), with the notable exception of the Neotropical and Nearctic regions. In the Neotropical region, most assemblages across central and northern South America showed positive NRI values, indicating the co-occurrence of closely related species. Still, the highest positive NRI values were found in the Afrotropical region, namely in the assemblages corresponding to Lakes Malawi, Tanganyika, and Victoria. In this region, mainly in the equatorial part, there were several assemblages with negative NRI values, indicating the presence of distantly related species. In the Nearctic region, there was a longitudinal pattern with assemblages showing negative NRI values around the central part of this region, whereas assemblages with positive NRI values were located towards the western and southern parts of this region.

4. DISCUSSION

Here, we evaluated the geographic variation of speciation rates in freshwater fishes on a global scale. To our knowledge, this is the first study to focus exclusively on how speciation rates vary across latitude at both assemblage and species levels in this group of fishes. Overall, our assemblage-level results revealed a positive relationship between latitude and speciation rates, with speciation rates increasing towards higher latitudes mainly in the northern hemisphere, despite the tropics exhibiting the highest single speciation values. Species-level results showed the same relationship, but only when excluding the hyper diverse Cichliformes order.

Our observed assemblage-level pattern aligns with recent studies for various taxa, where speciation rates are positively related with latitude, including birds (Pulido-Santacruz & Weir, 2016), mammals (Morales-Barbero et al., 2021), plants (Igea & Tanentzap, 2020), and marine fishes (Rabosky et al., 2018). Along with these studies, our findings add evidence that the classic LDG does not necessarily result from higher speciation rates in the tropics. In fact, recent studies on freshwater fishes have found that evolutionary time (i.e., the time elapsed since colonization/origination that allows species accumulation) is the primary driver of species richness at the class (Miller & Román-Palacios, 2021) and order level (García-Andrade et al., 2023). In addition, other evolutionary factors such as low extinction rates in the tropics have been found as mainly responsible for the LDG in other vertebrate groups (e.g., mammals, Quintero et al., 2023). Although freshwater fish speciation rates followed an inverse latitudinal gradient relative to species richness somewhat similar to the pattern observed by Rabosky et al. (2018) for marine fishes, there are important differences in the species and assemblage level patterns between these two groups of fishes.

As expected, the assemblage-level pattern is less pronounced than that observed by Rabosky et al. (2018) for marine fishes. This difference between marine and freshwater fish assemblages is mainly due to the higher variation of speciation rates in tropical regions for freshwater compared to marine fishes, with some tropical freshwater assemblages exhibiting the highest single values of speciation. These speciation peaks are found in places with high phylogenetic clustering, indicating the co-occurrence of recently derived and/or closely related lineages within the assemblages (Davies & Buckley, 2011), which is consistent with the findings of Miller & Román-Palacions (2021) were the highest speciation rates are located in recently colonized basins. Assemblages with high phylogenetic clustering and high speciation rates in tropics are mainly found in Africa, especially in the lakes Malawi, Tanganyika, and Victoria, where adaptive radiation driven by ecological opportunities for specialization has resulted in high recent speciation in the Cichliformes group approximately 15 Myr ago (Seehausen, 2015). Another example is the Congo Basin, where hyper-diverse assemblages of Mormyridae (Osteoglossiformes) diversified around 40 Myr (Capobianco & Friedman, 2024). Similar processes have been suggested in South America, namely in La Plata Basin, where the uplift of the Serra do Mar and Serra da Mantiqueira created isolated habitats and opportunities for vicariance, particularly among higher upland species like Hypostomus (Siluriformes) 30 Myr ago (Cassemiro et al., 2023; Cerezer et al., 2023). In the same vein, fragmentation has driven speciation in the western upland Andean regions in groups such as Orestias (Cerezer et al., 2023). Conversely, marine fish do not exhibit such peaks in speciation; indeed, the highest rates of speciation in marine environments only occur outside the tropics (Rabosky et al., 2018). This leads to the identification of a more consistent breakpoint in segmented models for marine fishes, between 27° and 40° latitude (Rabosky et al., 2018), compared to freshwater fishes where the latitudinal position of the breakpoint is broader, ranging between 23.8° and 50°. In general, freshwater fish speciation showed no significant latitudinal pattern below our identified breakpoint (i.e., towards lower latitudes), whereas above the breakpoint (i.e., towards higher latitudes) the relationship between latitude and speciation becomes positive and significant across all Actinopterygians, both marine and freshwater.

In marine fishes, higher speciation outside the tropics has been explained by increased opportunities to transition between different depths (Friedman & Muñoz, 2023) and rapid expansion into new habitats over the last 80 Kya, following repeated glaciation and deglaciation events that wiped out local fauna (Burns et al., 2024; Miller et al., 2018). These events likely fostered speciation through adaptive radiation and ecological opportunities. Indeed, in marine fishes, high-speciation areas have been found in both southern and northern hemispheres, which coupled with morphological evolution provides evidence of radiation in both north and south regions outside the tropics (Burns et al., 2024; Friedman & Muñoz, 2023). Conversely, our findings for freshwater fish revealed that the relationship between latitude and speciation beyond the breakpoint is driven primarily by assemblages located in the northern hemisphere. This result could result from the greater availability of habitat at high latitudes in the northern hemisphere, which comprises 74% of the Earth's landmass (Boggs, 1945). As a result of this geometric constraint, the southern hemisphere has fewer available areas for freshwater fishes, limiting opportunities for dispersal and colonization in southern regions.

Freshwater fish assemblages with high speciation rates in the Nearctic and Palearctic regions, particularly below 45° latitude, showed negative rPD and positive NRI values, indicating recent radiations and phylogenetic clustering. This suggests that these areas served as refugia during glacial periods, where climatic stability and time allowed for speciation and the accumulation of species, as previously proposed for North American fishes (Griffiths, 2015). The high speciation values in these areas could be explained by allopatric speciation mediated by dispersal (Kim et al., 2023) or by adaptive radiation, particularly in lakes that offer greater ecological opportunities (Seehausen, 2015). This is evident in genera such as Coregonus and Salvelinus (Salmoniformes), which underwent rapid adaptive radiation prior to the Last Glacial Maximum (80–90 kya) (Backenstose et al., 2024; De-Kayne et al., 2022), as well as in Cottus (Perciformes) within Lake Baikal (Buser et al., 2024). Adaptative radiation could also have occurred in streams, as observed in North America Cypriniforms that diversify in the benthic-to-pelagic axis (Burress et al., 2017). At even northern latitudes, above 45º, assemblages showed higher speciation rates and positive rPD values, indicating frequent dispersal events and/or environmental filtering (Davies & Buckley, 2011). This pattern is likely due to glaciations and sea-level fluctuations in these areas, which have repeatedly reshaped habitats and driven species to colonize new environments (Griffiths, 2015) following disturbance events (Seehausen & Wagner, 2014). Indeed, species in these assemblages often exhibit high tolerance and dispersal capabilities. For example, species from the order Salmoniformes, which originated in the northern Pacific Ocean, evolved to become anadromous and subsequently migrated to the polar basin and North Atlantic (Dolganov, 2022), as well as Gobiiformes that probably originated in freshwater environments of this region and show multiple transition to marine and euryhaline habitats (Jeon et al., 2021). As such, the northernmost assemblages (above 45º) with higher speciation rates could have resulted from the dispersal of highly radiating lineages from lower latitudes (below 45º).

At the species level, we did not recover a clear latitudinal gradient, although some species did exhibit high (but not the highest) speciation rates in northern latitudes. This discrepancy compared to the assemblage-level pattern may arise from our use of geographic range centroids as proxies for species latitudinal positions, which inherently reduce the spatial variation of observed patterns. This finding also results from the disproportionate influence of specific groups, such as the hyper-diverse and regionally concentrated Cichliformes in tropical zones. Clearly, when we excluded this group, we recovered a significant positive relationship between speciation and latitude similar to the one observed at the assemblage level. This invites us to interpret with caution, without ignoring clade-specific (non-replicated) factors that generate this pattern (Rabosky, 2020). For example, LDG in freshwater fishes exhibits a classic unimodal richness pattern, but this general pattern at the class level emerges from patterns exhibited by individual orders that often display different species richness gradients such as unimodal, bimodal, or multiple peaks (García-Andrade et al., 2023). Although these patterns can be driven by similar processes (García-Andrade et al., 2023), they result in heterogeneous patterns of linage composition that should be further evaluated using, for example, NRI and rPD that can also be useful to identify areas and groups where important evolutionary events occur (Davies & Buckley, 2011). Indeed, a pattern deconstruction approach to evaluate the latitudinal speciation gradient of freshwater fishes at different taxonomic/phylogenetic levels (e.g., orders, García-Andrade et al., 2023) seems necessary to reveal general or idiosyncratic drivers behind such gradient.

In addition to the biases generated by rapid speciation in specific clades, Wallacean and Darwinian shortfalls can directly affect studies of speciation gradients. Indeed, at broad spatial and taxonomic scales, the lack of refinement in the taxonomic delimitation of tropical species could hinder the recognition of recent speciation events, thus potentially underestimating speciation rates for tropical regions (Frateles et al., 2024). However, based on 5,242 species that represent less than half of freshwater fishes, we found a consistent pattern that was similar to the one obtained from ATA phylogenies representing 12,557 species, about 78% of all described species of freshwater Actinopterygii. Another source of bias is the metrics used to calculate speciation rates. Although all our speciation rate metrics were highly correlated, each method has its particular restrictions that can introduce biases into the models. For instance, BAMM uses discrete shifts that tend to underestimate variation, whereas ClaDS and DR may overestimate variation in scenarios where rate heterogeneity is nonexistent (Vasconcelos et al., 2022). These biases in each metric can affect the outcomes and the model accuracy. However, in our case, all metrics produce qualitatively similar patterns that allowed us to compare our results for freshwater fishes to those of marine fishes.

5. CONCLUSION

By integrating assemblage- and species-level approaches, our study revealed an inverse latitudinal gradient of speciation relative to species richness in freshwater fishes, with speciation rates increasing towards higher latitudes, namely in the northern hemisphere, even when the highest rates were located in the tropics. This pattern aligns with similar trends observed in terrestrial vertebrates and marine fishes. Overall, the areas with high speciation rates are congruent with areas of phylogenetic clustering and recent radiation, whereas the northern temperate areas with high speciation show evidence of recent radiation and high clustering as well as dispersal and environmental filtering that further align with glaciation dynamics. These findings suggest the effect of past climatic events and refuge areas as well as biogeographic and evolutionary factors in shaping speciation gradients. Further research should evaluate these (and potentially other) factors to fully understand the drivers behind the geographic variation of speciation rates.

Declarations

SUPPORTING INFORMATION

Additional support information can be found in the Supporting Information section.

Author Contribution

J.H-P., F.V., J.C-Q., and P.T. conceived the idea; J.H-P., A.A., A.B.G-A. and D.V-R. compiled data; J.H-P. and A.A. analyzed the data; J.H-P. wrote the manuscript with help from F.V. and J.C-Q.; All authors reviewed and edited the manuscript.

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Data Availability

All data used in this article has been obtained from the cited references. Processed data and code to repeat the analyses can be accessed from Figshare: https://figshare.com/s/a070ed387a5760098483

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Figures

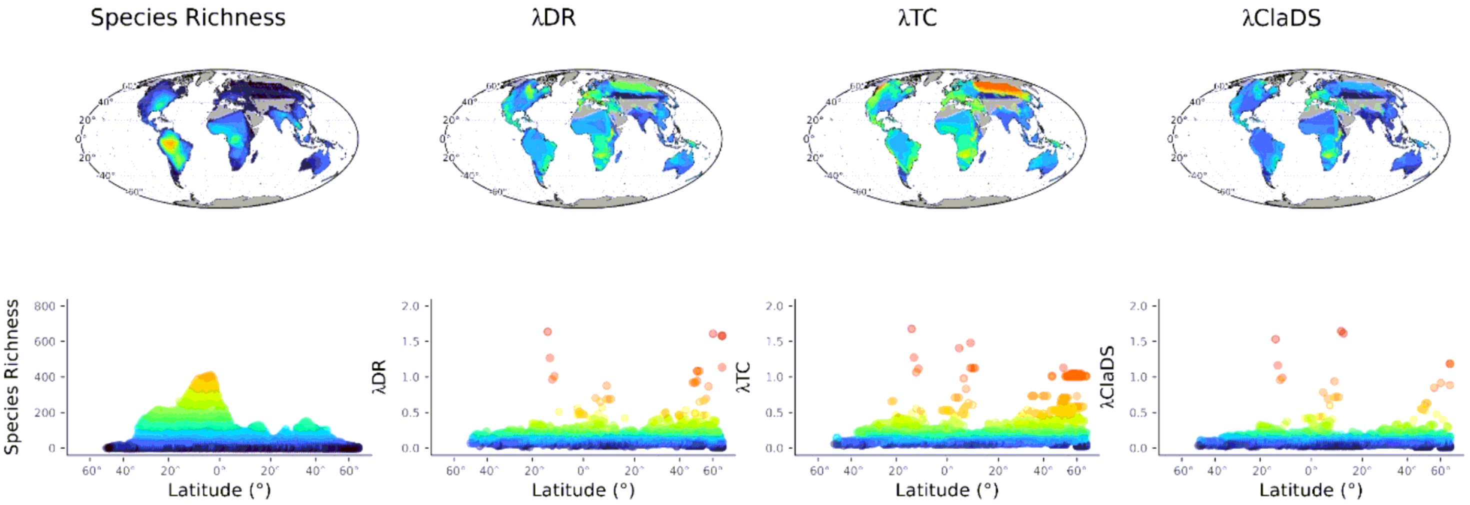

Figure 1

Geographic pattern of species richness and speciation rates under different metrics for the molecular dataset of 5,242 freshwater fish species. The upper row depicts the geographic patterns and the lower row their bivariate representations

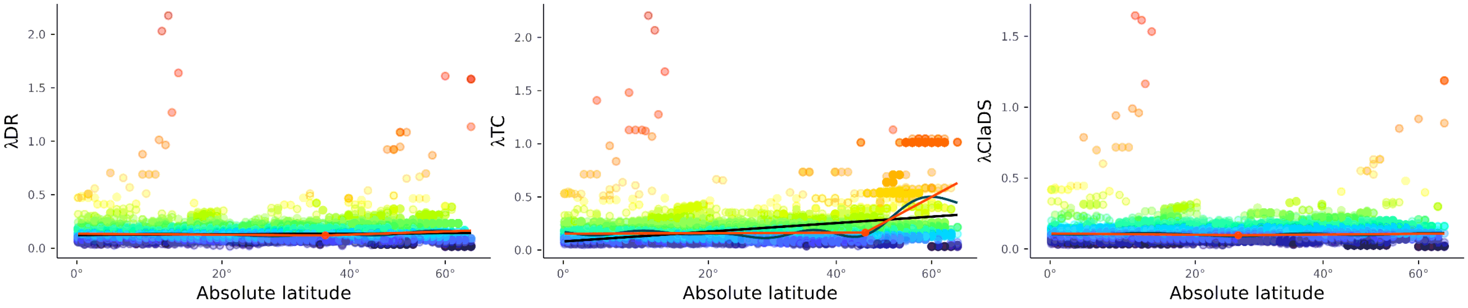

Figure 2

The relationship between speciation rate and the absolute value of latitude using different adjusted models (black - OLS model, red - OLS segmented model, blue - GAM model). All X-axes are in degrees only for illustrative purposes. See Fig. S2-1 to S2-4 for more detailed depictions of the statistical relationships.

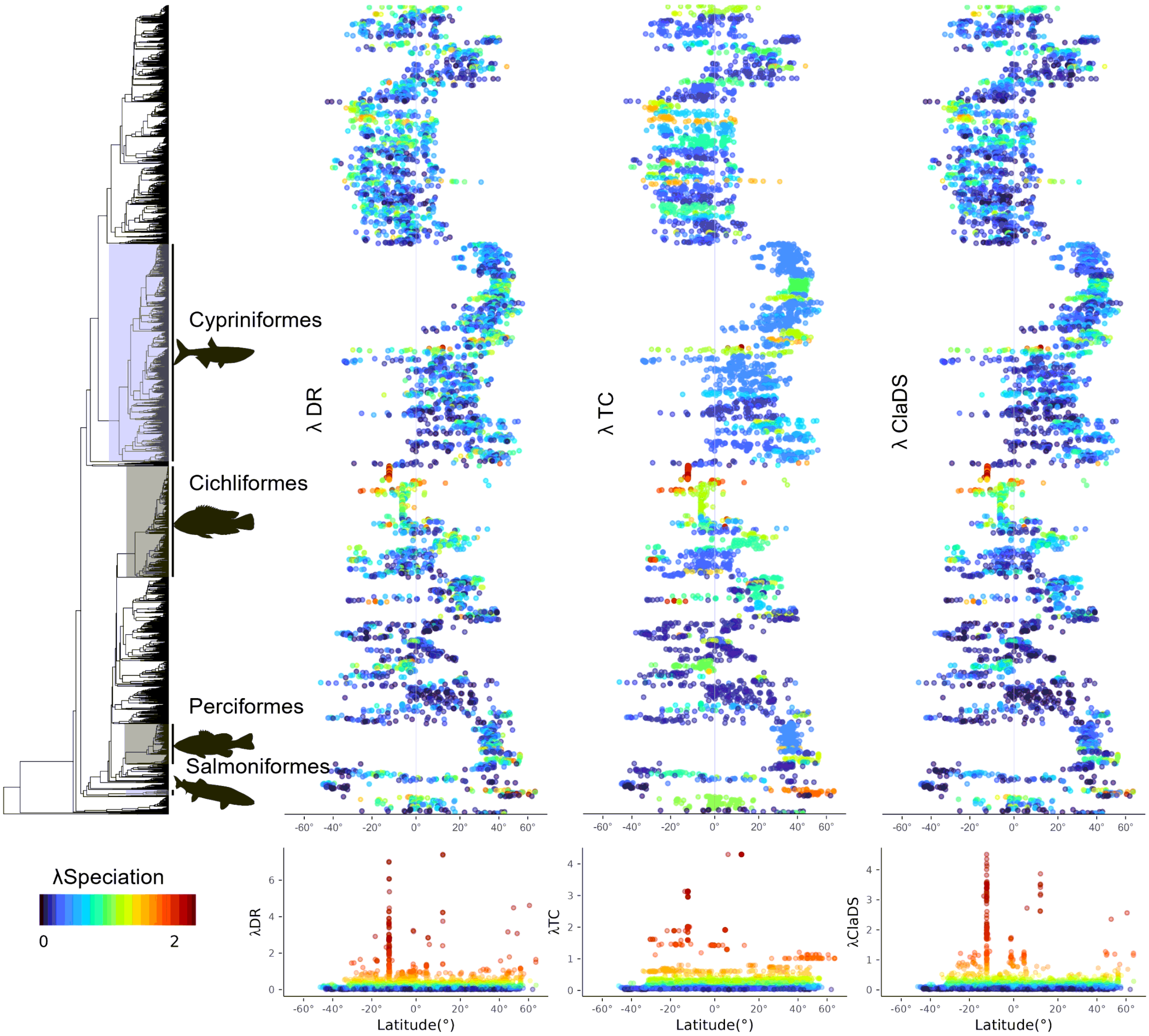

Figure 3

Phylogenetic (top panel) and latitudinal (bottom panel) patterns of tip-level speciation rates. Patterns are shown for λDR, λTC, and λClaDS. Next to the phylogeny, we show the speciation rate per species at their corresponding midpoint along the latitudinal gradient (x-axis). Species have the same colors in both panels.

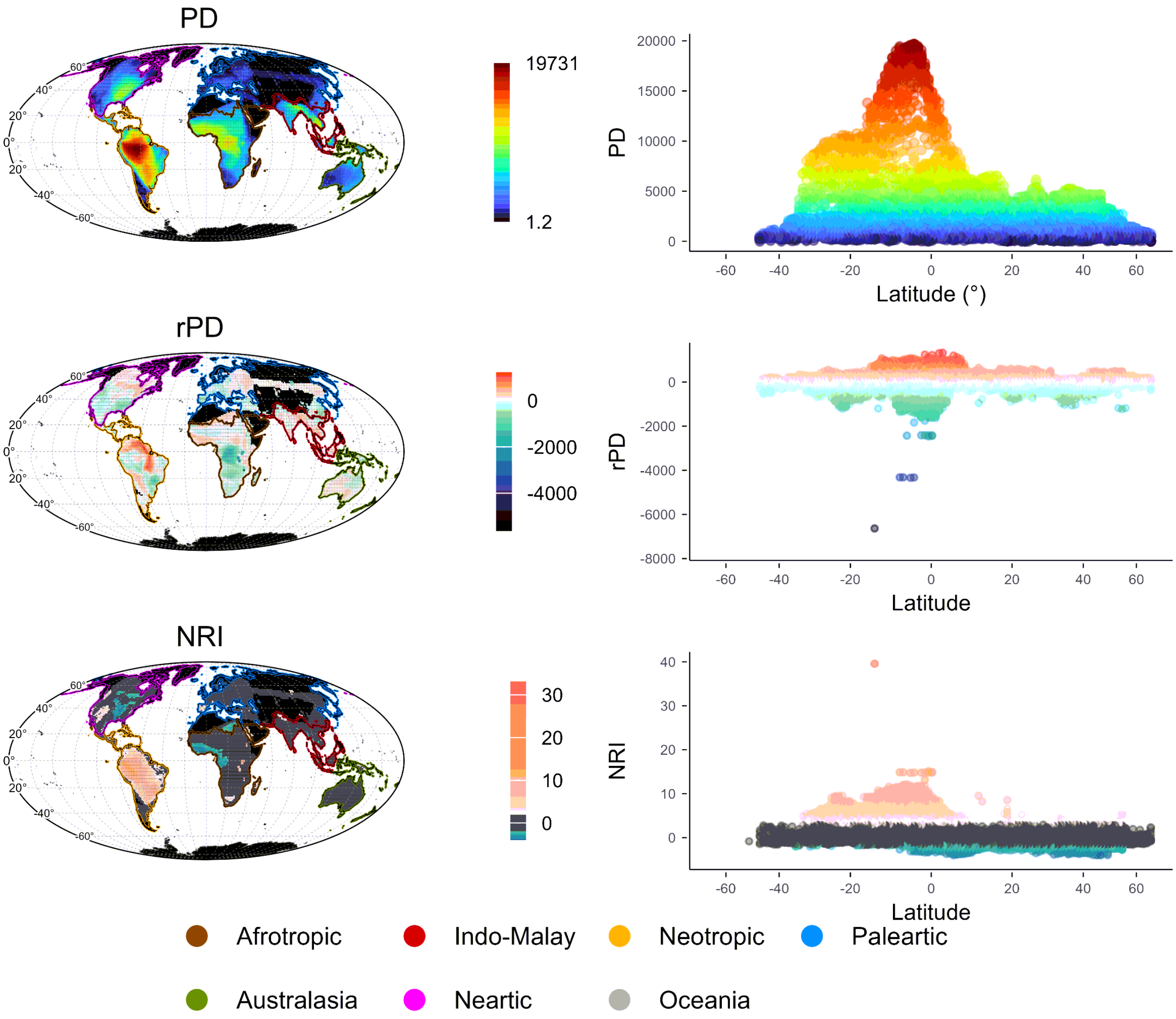

Figure 4

Geographic patterns of phylogenetic diversity (PD), residual phylogenetic diversity (rPD), and (NRI) of freshwater fish assemblages across the globe.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supp1Methods.docx
- Supp2ResultAssamblagelevel.docx
- Supp3ResultSpecieslevel.docx