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Evolutionary time explains the global distribution of freshwater fish diversity

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Abstract:

The latitudinal diversity gradient is Earth's foremost biodiversity pattern, persistent across clades and geologic time. Several recent studies have shown that diversification rates are similar among latitudes, and therefore cannot explain the latitudinal diversity gradient. An alternative explanation is that the tropics were colonized earlier than the temperate zone, allowing more time for speciation to build richness. Here we test the diversification-rate and colonization time hypotheses in freshwater ray-finned fishes, a group comprising nearly a quarter of all living vertebrate species and with a longer evolutionary history than other vertebrates. To build a global timeline for colonization and diversification, we performed ancestral area reconstructions on a time-calibrated phylogeny of all ray-finned fishes using occurrence records from over 3,000 freshwater habitats. We found that diversification rates are not systematically related to latitude, consistent with analyses in other groups. Instead, the timing of colonization to continental regions had 2–5 times more explanatory power for species richness than diversification rates. Earlier colonization explains high richness in the tropics, with the Neotropics in particular supporting the most diverse fauna for the past 100 million years. Most extratropical fish lineages colonized shortly after the end-Cretaceous mass extinction, leaving limited time to build diversity even in places where diversification rates are high. Our results demonstrate that evolutionary time, reflecting colonization and long-term persistence of lineages, is a powerful driver of biodiversity gradients.

Keywords:

Diversification rates, freshwater fishes, latitudinal diversity gradient, species richness, time-for-speciation

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Introduction:

Species richness decreases from the equator to the poles. The latitudinal biodiversity gradient has been called the Earth's first-order biodiversity pattern due to its pervasiveness across groups and geologic time (Hillebrand 2004; Krug et al. 2009). There are only three processes that can directly change regional species richness: in-situ speciation, local extinction, and colonization from other regions (Ricklefs 1987). Therefore, evolutionary hypotheses for the latitudinal diversity gradient ultimately invoke diversification rate differences (involving rates of speciation, extinction or both) and/or differences in the timing or frequency of colonization of the tropics (Mittelbach et al. 2007).

There has been great interest in comparing diversification rates across phylogenies in recent years, due to the confluence of the construction of very large time-calibrated molecular phylogenies (e.g. Jetz et al. 2012; Rabosky et al. 2018) and the increased complexity of models of diversification (e.g. Rabosky 2014; Morlon et al. 2016; Caetano et al. 2018). A growing number of analyses across whole groups are revealing that diversification (and/or speciation) rates are similar among latitudes (Schluter and Pennell 2017; birds: Weir and Schluter 2007, Jetz et al. 2012; mammals: Weir and Schluter 2007, Upham et al. 2019; ants: Economo et al. 2018), or even higher in high latitudes (marine fishes: Rabosky et al. 2018; angiosperms: Igea and Tanentzap 2019). These studies raise the question: if diversification rates do not explain spatial differences in richness then what does? A potential resolution to this question is to compare the relative importance of colonization timing and diversification rates in explaining differences in species richness among regions (Wiens and Donoghue 2004; Wiens 2012; Xing and Ree 2017). For example, marine fishes in the tropics have slow speciation rates (Rabosky et al. 2018), but have achieved high richness because of earlier and more frequent colonization relative to the

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poles (Miller et al. 2018). Although the influence of time on species richness is not a new idea (Wallace 1878; Willis 1922; Stephen and Wiens 2003; Fine and Ree 2006; Jablonski et al. 2006; Mittelbach et al. 2007), relatively few recent studies have simultaneously inferred colonization and diversification history to explain spatial richness patterns (Xing and Ree 2017).

Here we reconstruct the history of colonization and diversification globally to explain the latitudinal diversity gradient in freshwater fishes. Freshwater fishes represent nearly a quarter of all vertebrate species (Cavin 2017) and are major components of ecosystems in both tropical and temperate latitudes. In particular, freshwater fishes have features that are conducive to testing both diversification and colonization hypotheses. First, freshwater fishes represent a wider range of ages than other vertebrates (e.g. birds), with some radiations diversifying during the Mesozoic or earlier (Briggs 2005; Berra 2007) and others only during the most recent glaciation cycles (Seehausen and Wagner 2014). Second, freshwater fishes appear to have high diversification rates in general (Miller et al. 2018), with important groups such as cichlids having especially fast rates (Seehausen and Wagner 2014). Third, freshwater fishes have low dispersal ability due to their restriction to freshwater habitats, and their diversification dynamics likely retain signatures of regional events (Cavin 2017). Our study capitalizes on the aggregation of natural history observations and genetic data over many years (Tedesco et al. 2017; Rabosky et al. 2018), allowing us to make comparisons at larger spatial and taxonomic scales than was feasible in the past.

Results and Discussion:

Diversification and colonization of local faunas: Global richness of freshwater actinopterygian fishes (Lundberg et al. 2000; Leveque et al. 2008; Tedesco et al. 2017) reflects the latitudinal

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diversity gradient found in other major groups (Fig. 1; Spearman's rank correlation between local richness and latitude: rho=-0.27, $p<0.001$). However, latitude alone only explains about 10% of the variance in species richness across basins (Generalized Additive Model [GAM], $r^2=0.092$, $p<0.001$; Fig. 1; Table S1). Longitude alone had similar explanatory power (GAM, $r^2=0.10$, $p<0.001$). Instead, the interaction between longitude and latitude (i.e. geographical position) explains a higher proportion of the variance in species richness ($r^2=0.26$, $p<0.001$). These results appear to reflect the fact that richness is strikingly high in the Neotropics. For example, the Amazon basin alone contains about twice as many known species as the world's second-most rich drainage basin, the Congo River (2,968 vs 1,554 species, respectively; Fig. 2A). This observation prompts a second, related goal in addition to explaining the latitudinal diversity gradient: explaining why richness differs dramatically between realms even at similar latitudes.

Present-day differences in species richness among drainage basins might be explained by either the timing of colonization, rates of diversification (speciation minus extinction rate), or both. We first focused on estimating the individual contribution of diversification rates and colonization times in driving the global patterns of freshwater fish richness. We then compared the relative importance of these two processes.

To examine the role of diversification rates in explaining the spatial distribution of species richness, we first estimated basin-specific rates using occurrence data for 14,947 extant species across 3,119 freshwater drainage basins (Tedesco et al. 2017). Generalized Additive Models were used to test whether diversification rates decrease with increasing latitude. We did not recover a latitudinal trend in diversification rates for freshwater fishes (Fig. 1; Fig. 2B; Table S1). However, we estimated that 34–51% of the variance in species richness among basins is

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explained by differences in diversification rates alone (all $p<0.001$; Table S2). Although we found no systematic tendency for tropical basins to experience greater diversification rates than extratropical basins (Fig. 2B; Table S2), differences in diversification rates have been a relevant driver of spatial differences in species richness in general.

As an alternative to diversification rates, we tested if the timing of colonization could also explain spatial patterns of species richness. For example, if a region was colonized early in the history of a clade, it is expected to contain more species than recently-colonized regions due to greater time for speciation, even if diversification rates are similar among regions (Stephens and Wiens 2003; Wiens and Donoghue 2014). To infer the timing of colonization events to major regions of the world (Tedesco et al. 2017; Fig. 3), we fit a model of range inheritance (Ree and Smith 2008; Matzke 2014) on a time-calibrated molecular phylogeny of all ray-finned fishes (Rabosky et al. 2018). We then traced each species back in time to the arrival of the region(s) it inhabits. Colonization time was summarized as the mean or median regional colonization time of co-occurring species in each basin.

We found that colonization time alone explained 31–32% of the variance in species richness among freshwater basins (GAMs including median or mean colonization time as a predictor of richness with $p<0.001$; Table S2). Furthermore, unlike diversification rates, we found a latitudinal trend in colonization time (Fig. 1). Low-latitudes were colonized earlier by modern fish lineages than higher latitudes on average (median colonization time: $r^2=0.142$, rho=-0.23, $p<0.001$; mean colonization time: $r^2=0.271$, rho=-0.29, $p<0.001$; Figs. 1, 2C; Table S1). The basins found farthest south, in South America and Australasia, represent an exception to this overall pattern (Fig. 2C). These basins contain few species but with ancient colonization times (>180 million years) and appear to be relicts of past Gondwanan connections.

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We found that both diversification rates and the timing of colonization have played an important role in driving the spatial patterns of freshwater fish richness. However, it remains unclear whether or not both processes (diversification and colonization timing) have driven extant diversity across the same set of freshwater basins. For instance, if both diversification and colonization times jointly explain diversity in the most species-rich basins, then one would expect these basins to harbor fast-diversifying lineages that colonized a long time ago. We used spatially explicit regression models to examine the relationship between the timing of colonization and diversification rate across basins. We estimate that colonization times explained 24–42% of variance in diversification rates (all $p<0.001$; Table S3), with diversification rates being higher in recently-colonized basins (rho=-0.13–-0.06, all $p<0.01$; Table S3). This inverse scaling found between diversification rates and colonization times suggests that richness in recently-colonized basins is being primarily driven by diversification rates. Conversely, richness among basins that have been inhabited for a long time is mainly influenced by the relative timing of colonization, allowing longer time scales to build richness through average or slow rates of diversification.

To compare the relative importance of diversification rates and colonization time, we fit GAMs including both variables as predictors of species richness. We found that the present-day distribution of freshwater fish richness across basins, including latitudinal and longitudinal trends, is mainly a consequence of differences in colonization timing and not diversification rates. Specifically, relative to diversification rates, we found that differences in colonization time have contributed 2–5 times more to differences in species richness across basins (based on deviance values for alternative GAM models; Tables S4–S6).

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Altogether, these results show that the latitudinal diversity gradient in freshwater fish diversity is driven by earlier colonization to the tropics rather than faster diversification rates. The freshwater habitats of the Neotropics, Afrotropics, and Indo-Malay regions support many species whose parent clades have been present since the Mesozoic Era (252–66 mya; Fig. 2C). Colonization time also explains the especially high richness of the Amazon River and surrounding basins, with an older median colonization time than that inferred for any African or Southeast Asian basins (Amazon: median colonization time of 159 million years ago; Fig. 2C). In contrast, 95.4% of basins in the Nearctic and Palearctic regions had a median colonization time after the K-T boundary (66 million years ago). Relatively fast diversification rates seem to explain why some temperate basins (e.g. those found 40–50ºN) have higher richness than expected given their recent colonization (Fig. 1). Still, diversification rates have not been fast enough during this short window of time to build richness rivaling that of the tropics. Overall, the much earlier colonization of tropical regions allowed for greater time for speciation to build richness compared to extratropical regions.

Regional diversification and colonization dynamics over time: Given the importance of diversification over long time scales for creating latitudinal and regional diversity differences, we aimed to understand how in-situ diversification has varied over time to build present-day richness. To build a timeline of diversification and lineage accumulation, we used our ancestral area reconstructions to identify the continental region(s) where individual speciation events occurred. We then calculated a rolling per-capita rate of diversification over the past 200 million years for each region (Xing and Ree 2017). We compared diversification dynamics inferred

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using this approach to those from region-specific subtrees made by simply removing all species not found in each region.

Both approaches show that the Neotropics have been the most diverse terrestrial realm for at least the past 100 million years (Fig. 3; Fig. S3). While Neotropical diversification rates are not especially high (Fig. 2B; Fig. S4), they have remained remarkably stable over this time period (Figs. 3, 4; Figs. S3, S4). Our results are consistent with a large body of evidence from fossil and molecular data that the modern Neotropical biota was assembled over long time scales rather than from recent diversification (Hoorn et al. 2010; Wiens et al. 2011; Albert and Reis 2011; Albert et al. 2018; Antonelli et al. 2018). This is in spite of the relatively recent origins of the modern Amazon river (Albert et al. 2018).

The other two major tropical regions (Indo-Malay, Afrotropics) have much more dynamic histories. The Indo-Malayan fauna was nearly as diverse as the Neotropics at the K-T boundary (Fig. 3; Fig. S3). However, diversification rates in the Indo-Malay region declined over the Cenozoic. While Indo-Malayan freshwater habitats are not as well sampled as other regions (Tedesco et al. 2017), simulations using the gamma statistic (Pybus and Harvey 2000) suggest this pattern is not attributable to incomplete sampling alone (Table S8). Slow rates of recent diversification in Southeast Asia conflict with the hypothesis that the region's high diversity is due to repeated allopatry from Pleistocene sea level fluctuations (de Bruyn et al. 2012). The Afrotropics have similar present-day richness to Southeast Asia but show a contrasting temporal pattern. The Afrotropics were less diverse than Southeast Asia during the early-mid Cenozoic (66–34 mya), a pattern also seen in plants (Couvreur 2015). African fishes only recently achieved comparable richness to Southeast Asian fishes due to increasing diversification rates over the past 20 million years. This period saw a number of tectonic changes in Africa, most

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notably collision with the Eurasian plate and the spreading of the East African Rift Valley (Danley et al. 2012; Otero 2015). In contrast with long-term stability of diversification in the Neotropics, diversification dynamics in Africa and Southeast Asia are characterized by periodic changes in rates, contributing to their lower present-day richness.

While major tropical radiations began diversifying during the Mesozoic, most groups in extratropical regions did not arrive until the early Cenozoic (66 mya–present; Figs. 2C, 5). Following the late arrival of freshwater fishes into extratropical regions, diversification rates eventually outpaced those of most tropical areas (Fig. 4; Fig. S4). Interestingly, diversification rates have dropped over the past 20 million years in North America (Fig. 4; Fig. S4; Table S8) but increased in the Palearctic over the same period. One possibility is that uplift of the Colorado Plateau and aridification of the western United States over the past 25 mya slowed diversification rates (Smith et al. 2010; Ross and Matthews 2014). At the same time, rates may have increased in the Palearctic realm in association with orogeny in central Asia (such as the formation of the Qinghai-Tibetan Plateau from 29–18 mya and surrounding mountain ranges more recently; Jing et al. 2013; Xing and Ree 2017), where several unique minnow and catfish lineages are found. In addition, the Palearctic realm includes very young but rapidly diversifying communities found in North African deserts (Fig. 2).

***Clade-specific diversification dynamics*:** The world's freshwater fish communities are primarily dominated by two clades: the Otophysi and the Percomorpha (Fig. 4A). The Otophysi are a monophyletic radiation dating to the early Cretaceous and include minnows (Cypriniformes), catfishes (Siluriformes), tetras (Characiformes), and knifefishes (Gymnotiformes). Almost all species are exclusive to freshwater and are therefore thought to have limited potential for

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dispersal (Chen et al. 2013). In contrast, Percomorpha has marine origins (Carrete Vega and Wiens 2012), with many lineages invading freshwater independently and at different times in geologic history. These lineages include cichlids, guppies, darters, bettas, and many others (Berra 2007). The three major tropical regions (Neotropics, Afrotropics, and Indo-Malay) were colonized by both otophysan and percomorph lineages during the Cretaceous (Fig. 5; Fig. S5), with the most likely region-of-origin for Otophysi in the Neotropics. Thus, both Otophysi and Percomorpha contribute to the early median colonization times for tropical drainage basins (Fig. 2C) despite the older age of the former.

We calculated per-capita rates of diversification for Otophysi and Percomopha to assess how each group has contributed to the dynamic seen for each region as a whole. In spite of the contrasting histories of these clades, their diversification dynamics were congruent in the Neotropics, Indo-Malay, and Nearctic regions (Fig. 4B). In the other three regions, there were interesting contrasts between each clade's dynamics. First, few otophysan lineages reached Australia, so dynamics there are almost entirely driven by percomorphs. Second, the recent increase in African diversification rates are most dramatic in cichlids, which diversified greatly in the East African Rift System (Fig. S5; Danley et al. 2012; Seehausen and Wagner 2014). Third, diversification rates of Palearctic and Nearctic Otophysi are higher than co-occurring percomorphs, though their temporal trends are similar in the Nearctic. These results demonstrate how global richness patterns formed over time from the combined influence of colonization and diversification in two dominant clades.

Tropical conservatism model: Our results for freshwater fishes closely align with the tropical conservatism model (Wiens and Donoghue 2004) and to a lesser extent with the out-of-the-

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tropics model (Jablonski et al. 2006), but with some important caveats. The overall pattern of colonization conforms to the expectations of both models; that is, older tropical clades (e.g. Otophysi) exported nested lineages to extratropical regions (e.g. Nearctic Cyprinidae). However, the out-of-the-tropics model also predicts higher speciation and lower extinction rates in the tropics. A major prediction of the tropical conservatism model is that movement out of the tropics is limited by niche conservatism, or the tendency for related taxa to share similar environmental tolerances (Wiens and Donoghue 2004; Wiens et al. 2006). Although we did not examine niche evolution in this study, some observations suggest that niche conservatism may indeed underlie colonization patterns in freshwater fishes. Many tropical clades have few or no living species that reach temperate latitudes, including Cichlidae, Characiformes, Gymnotiformes, and Cyprinodontiformes. In addition, major radiations found in the Nearctic and Palearctic regions (such as Cypriniformes and Percidae) arrived during a period when the Earth was much warmer overall compared to the present day (Zachos et al. 2008). Warmer global temperatures may have facilitated extratropical colonization during the early Cenozoic, and tolerance to temperate conditions may have evolved after colonization (Chen et al. 2013). We encourage future studies of niche evolution in the context of historical biogeography (Wiens et al. 2006), and we predict that extratropical colonization preceded niche evolution in many groups (Folk et al. 2019).

Environmental and geologic stability of the tropics: The importance of time-for-speciation for explaining richness differences might suggest that the tropics have been more climactically stable over geologic time than higher latitudes, allowing diversification in early-colonizing lineages to proceed over long time scales. Previous work has suggested that Quaternary

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glaciation cycles were important for generating the latitudinal diversity gradient, because lower latitudes were unaffected by glaciation (Bush 1994; Mittelbach et al. 2007). In freshwater fishes, Quaternary climate cycles have left a signature on community composition and richness at high latitudes, suggesting that high latitudes were re-colonized by a subset of species after glaciers receded (Leprieur et al. 2011). Nonetheless, our reconstructions strongly imply that the latitudinal diversity gradient was already established at least by the mid-Cretaceous (Figs. 3, 5). In light of our results, we suggest that Quaternary events affected richness at the basin-level scale (for example, explaining lower richness of northern relative to southern North America; Fig. 2A; Leprieur et al. 2011; Ross and Matthews 2014), and exacerbated, but did not generate, the latitudinal diversity gradient.

An older series of events may have been more important to the formation of the latitudinal diversity gradient in freshwater fishes: the isolation of Laurasia from Gondwana, and the rise and fall of epicontinental seas. Laurasia and Gondwana became separated by the developing Atlantic Ocean over the course of the Jurassic (199.6–145.5 mya), and were separated by the time Otophysi and Percomorpha originated (142.1 and 122.7 mya respectively; Rabosky et al. 2018). Laurasian landmasses were flooded to a much greater extent than Gondwanan landmasses over the Cretaceous (145.5–66 mya; Fig. 5; Ronov 1994). Large epicontinental seas covered central North America and Europe during this time, with North America drying out during the Paleocene and Europe remaining flooded until the Oligocene (Renema et al. 2008).

We tested if past continental flooding left a signal in speciation and extinction rates in the molecular phylogeny. Environmental birth-death models (Condamine et al. 2013) did not support the hypothesis that diversification was impaired by flooding in the Nearctic and Palearctic

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regions, favoring models where diversification varied simply with time (Fig. S6; Tables S9, S10). This result may be expected since the most diverse North American lineages colonized after the disappearance of this epicontinental seaway (Fig. 5; Figs. S5, S6). Most Palearctic diversification during the early Cenozoic likely took place in northern Asia rather than Europe, which was still flooded (Briggs 2005; Leveque et al. 2008). Rather than affecting speciation or extinction rates of modern groups, the flooding of Laurasia and the increasing isolation from Gondwana may have impaired colonization of the landmass by freshwater fishes with poor salt tolerance until the Cenozoic. This later colonization would have reduced the time allowed for diversification, even though diversification rates were high following colonization (Fig. 4B; Figs. S4). In support of this hypothesis, Mesozoic arrivals to Laurasia were groups with recent marine ancestry (Percopsiformes and Centrarchiformes in North America and Salmoniformes in Eurasia), while major Cenozoic arrivals included otophysan groups (cyprinids and cobitids; Fig. 5; Fig. S5).

It is also possible that older lineages went extinct in North America and Eurasia and were later replaced by early Cenozoic arrivals (Wilson and Williams 1992; Chen et al. 2013; Cavin 2017). Such extinctions may not be measurable using molecular phylogenies of living species, especially if entire higher taxa went extinct from these regions. Undetected extinction of older clades would not necessarily conflict with trends reported here for modern groups (Miller and Wiens 2017). In fact, past extinction would be consistent with the importance of time-for-speciation for generating the present-day latitudinal diversity gradient, if young temperate groups had limited time to replace past diversity lost by extinction (Miller and Wiens 2017; Meseguer and Condamine 2019). Understanding the influence of historical extinction on latitudinal diversity patterns will require investigation of the fossil record of freshwater fishes (Cavin 2017).

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Conclusion: We show that the latitudinal diversity gradient in freshwater fishes is driven by earlier colonization to low-latitude regions, extending the timeline of in-situ diversification in the tropics compared to higher latitudes. Freshwater fishes show a general pattern alluded to by Wallace (1878) and becoming increasingly apparent with the construction of large molecular phylogenies. The time-for-speciation effect is a reflection of colonization timing and opportunity, niche conservatism and evolution, environmental stability, and/or past extinction (Stephens and Wiens 2003; Wiens and Donoghue 2004; Jetz and Fine 2012; Miller and Wiens 2017). A priority for future research is determining which of these factors are most important for generating biodiversity patterns, how they interact, and why they vary with latitude.

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Methods:

Occurrence and phylogenetic data: Expert-vetted occurrence records of freshwater actinopterygian species were assembled by Tedesco et al. (2017; see also the Freshwater Biodiversity Data Portal, http://data.freshwaterbiodiversity.edu/). Records were available for 3,119 freshwater drainage basins covering 80% of the Earth's surface (Fig. 2A). We removed non-native records and uncertain records. Altogether, occurrence records from 14,947 species of freshwater fishes were used to estimate richness and diversification rates of basins. All analyses used time-calibrated molecular phylogenies of actinopterygians constructed by Rabosky et al. (2018), which include 11,638 species with genetic data (36% of known species).

Comparing diversification rates and colonization time among basins and regions: To estimate diversification rates for each basin (Figs. 1,2), we used three measures of diversification rates calculated using a megaphylogeny of all ray-finned fishes (Rabosky et al. 2018; see also the R package *fishtree*; Chang et al. 2019, and http://fishtreeoflife.org). First, we used tip-based rates calculated from six different runs of BAMM (Rabosky 2014) using the maximum clade credibility phylogeny of species with genetic data. Second, we used estimates of the DR statistic (Jetz et al. 2012) calculated using phylogenies with all unsampled species grafted using taxonomic constraints. We note that DR tip rates better approximate speciation rates than net diversification rates in comparison to tip rates from BAMM (Title and Rabosky 2019). For tip-based rates, we took the mean of rates among co-occurring species present in each basin. Third, we calculated net diversification rates using the Method-of-Moments estimator (Magallón and Sanderson 2001). Rates for each basin were calculated as the mean rate of all genera present, weighted by the relative local richness of each genus (Miller et al. 2018). Additional details of rate calculations are found in the Extended Methods (Supporting Information).

To estimate colonization timing of major regions (and therefore the amount of time allowed for diversification since colonization; Stephens and Wiens 2003), we fit the dispersal-extinction-cladogenesis model (DEC; Ree and Smith 2008) using the R package *BioGeoBEARS* v.1.1 (Matzke 2014; additional details of reconstructions found in the Extended Methods, Supporting Information). Our analysis included six major continental regions of the world (delimitation following Tedesco et al. 2017): the Neotropics, Afrotropics, Indo-Malay, Australasia, Nearctic, and Palearctic (Fig. 3). Species restricted to marine environments were coded as occurring in a seventh "marine" region. These species inform the timing of colonization of freshwater regions from the marine realm (Fig. S1) but were otherwise not of interest. We used the maximum clade credibility phylogeny including species with genetic data only (Rabosky et al. 2018), because semi-random grafting of unsampled species is inappropriate for comparative methods that model the evolution of traits associated with the tips (Rabosky 2015). We set restrictions on dispersal among regions in concordance with rearranging plate tectonics through time (following Toussaint et al. 2017; Table S7). To count events and incorporate uncertainty in the ancestral area reconstructions in downstream analyses, we simulated 100

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possible biogeographic histories ("stochastic maps") informed by the model fit and phylogeny (Dupin et al. 2016). To summarize the amount of evolutionary time associated with each basin (Fig. 2C), we first traced each species back in time to the colonization of the major region(s) it inhabits; each species was given a mean colonization time across the 100 stochastic maps.

We used alternative Generalized Additive Models (GAMs) to understand the relationships among latitude and longitude, local richness, basin-specific rates of diversification (13 alternative estimates per basin) and colonization time (2 alternative estimates per basin). First, we fit GAMs to examine the change in species richness, net diversification rates, and colonization time with latitude, longitude, and both (i.e. the interaction between longitude and latitude). We fit univariate GAMs between predictor (latitude or longitude) and response variables (species richness, net diversification rates, or colonization time). Models that included the interaction between longitude and latitude as predictors used the smooth term between coordinates for each basin (e.g. s(long,lat) in GAM models). We log-transformed richness and diversification values, and used raw values of colonization times in all models. We analyzed absolute values of latitude in models that did not assume the interaction with longitude. Univariate GAMs were fitted using the gam function in the *mgcv* package in R (base; R Development Core Team 2019; Wood 2011). We also performed Spearman's rank correlation tests (using the "cor.test" function in R) between latitude and either species richness, net diversification rates or colonization times. Second, we analyzed the relationships between basin richness and net diversification alone, and richness and colonization time alone. To account for spatial autocorrelation, we included a smoother term that summarized the interaction between latitude and longitude in each basin (e.g. s(long,lat) in GAM models). Third, we examined the relationship between net diversification rates and colonization time among basins. We also performed Spearman's rank correlation tests between diversification rates and colonization time to quantify the strength and direction of the association between these variables. Fourth, we compared the relative importance of colonization time and diversification rates for explaining richness among basins by including both variables as predictors of richness in GAMs. In total, we analyzed 42 different spatially-explicit GAM models. Our approach, which is based on the comparison of deviance estimates between alternative models, account for the non-independency between colonization times and diversification rates. More details of model fitting and model results can be found in the Extended Methods (Supporting Information) and Tables S1–6.

***Regional diversification dynamics through time*:** Estimating diversification associated with a single region is challenging because regions contain multiple clades of various origin. In addition, common approaches for estimating diversification dynamics of a clade may not capture in-situ diversification dynamics because nested lineages may leave a region and diversify elsewhere, rendering the parent clade paraphyletic. Models that co-estimate diversification and complex colonization dynamics are presently limited to two-region comparisons (Cataeno et al. 2018). We took two approaches to estimating regional diversification in light of these issues. First, we used our ancestral area reconstructions to count splitting events occurring in each

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region during 2-million year time bins across the phylogeny. Following Xing and Ree 2017, we calculated a per-capita in-situ diversification rate for each region as the number of speciation events occurring in the region within the time bin divided by the number of lineages present in the region in the previous time bin. Diversification dynamics were summarized across the 100 stochastic maps to visualize uncertainty in the reconstructions (Fig. 4). Second, we made a subtree for each region by simply removing all species in the phylogeny not found in that region. We then used BAMM v.2.5.0 (Rabosky 2014) to estimate diversification rates through time for each regional phylogeny individually. Unlike our first approach, BAMM can correct for incomplete sampling. However, the first approach may be more appropriate when colonization is frequent, because it can be difficult to estimate the region where speciation took place without ancestral area reconstructions. Both approaches yielded consistent diversification dynamics through time, and in comparison to other regions (Figs. S3, S4).

Diversification declines towards the present were detected in some regions (Figs. 3, 4). To test if these declines could be the result of incomplete sampling, we calculated the gamma statistic and performed the Monte Carlo constant rates test to obtain a corrected p-value (Pybus and Harvey 2000). We calculated gamma separately for each of the clades that comprised most of the diversity of each region (Fig. S5). Each regional radiation was identified using ancestral area reconstructions and was descended from a single colonization of the region (lineages leaving the focal region were removed from clade subtrees).

We tested the hypothesis that past continental flooding left a signature on speciation or extinction rates in the molecular phylogeny. We fit constant, time-dependent, and environmental-dependent models of diversification to regional phylogenies using *RPANDA* v.1.5 (Condamine et al. 2013; Morlon et al. 2016; see Table S9 for model details). Environmental-dependent models related speciation and extinction rates to the area of continent covered by the ocean over time (Fig. 5; Ronov 1994). Continental flooding estimates were available for some landmasses individually (corresponding to the six present-day biogeographic regions) in addition to the estimates for Laurasia and Gondwana used for visualization (Ronov 1994; Fig. 5; Fig. S6). Relative model fits were compared using Akaike weights (Table S9).

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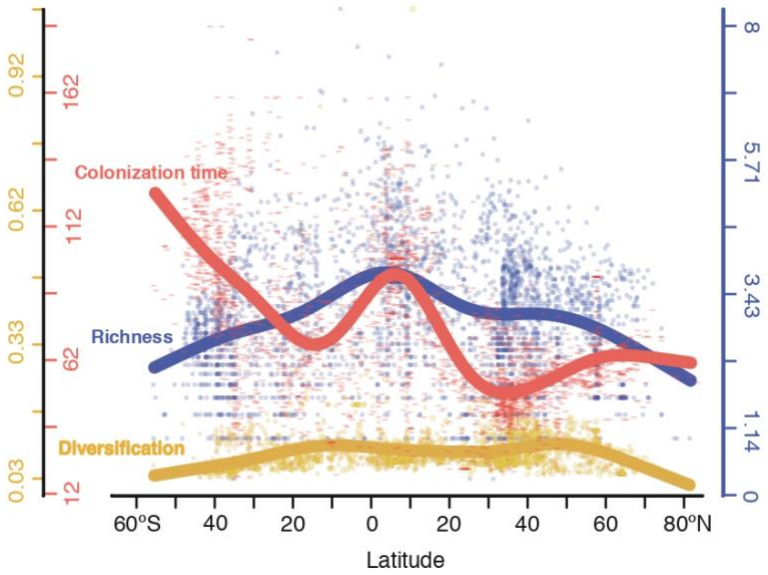

Figure 1: Latitudinal gradients of freshwater fish species richness (blue; number of species), diversification rates (yellow; events/my), and colonization times (red; mya) for individual freshwater drainage basins. Species richness is derived from Tedesco et al. (2017; Fig. 2A). Diversification rates were estimated using BAMM under a time-constant rates model; values represent the mean tip-associated values of species found in each basin (Rabosky et al. 2018; Fig. 2B). Colonization timing of major regions was inferred from ancestral area reconstructions (Matzke 2014); values represent the mean regional colonization time for species in each basin (Fig. 2C).

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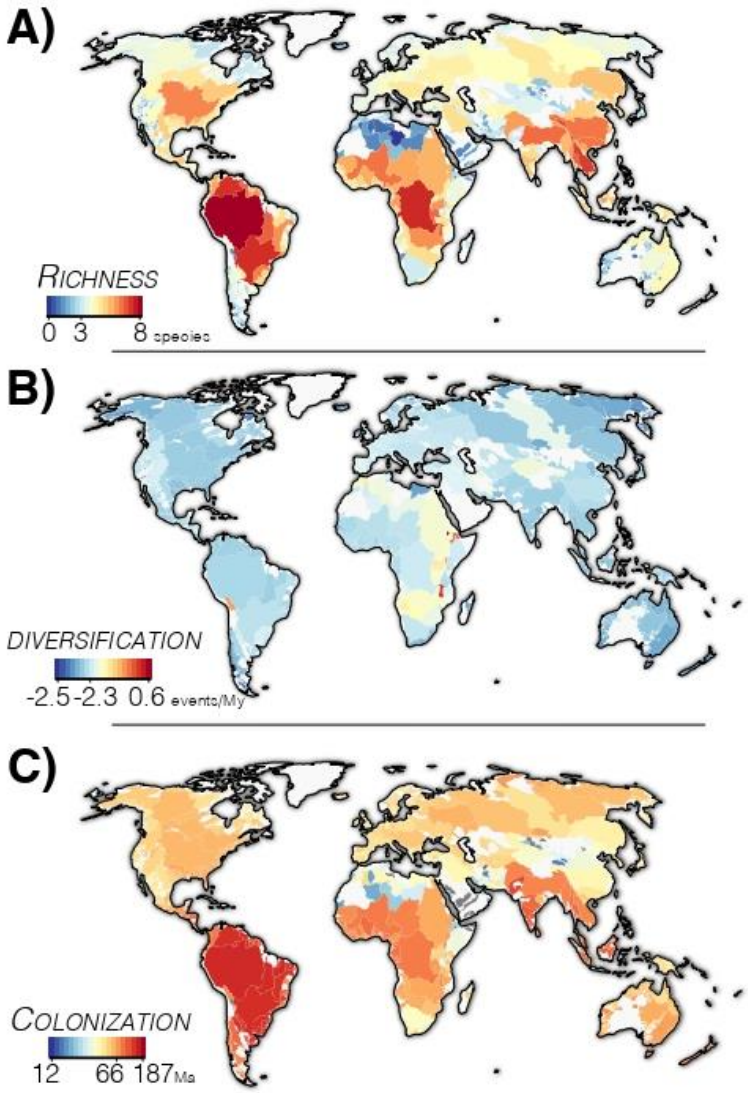

Figure 2: Geographical distribution of global freshwater fish (**A**) richness, (**B**) net diversification rates, and (**C**) mean colonization times of freshwater drainage basins. Species richness of basins is based on occurrences from Tedesco et al. (2017). BAMM-estimated rates of species diversification (Rabosky et al. 2018) calculated under a time-constant rates model (Rabosky et al. 2018) are depicted here. Colonization times of continental regions was inferred from ancestral area reconstructions (Matzke 2014; see Extended Methods). Richness and diversification rates were log-transformed.

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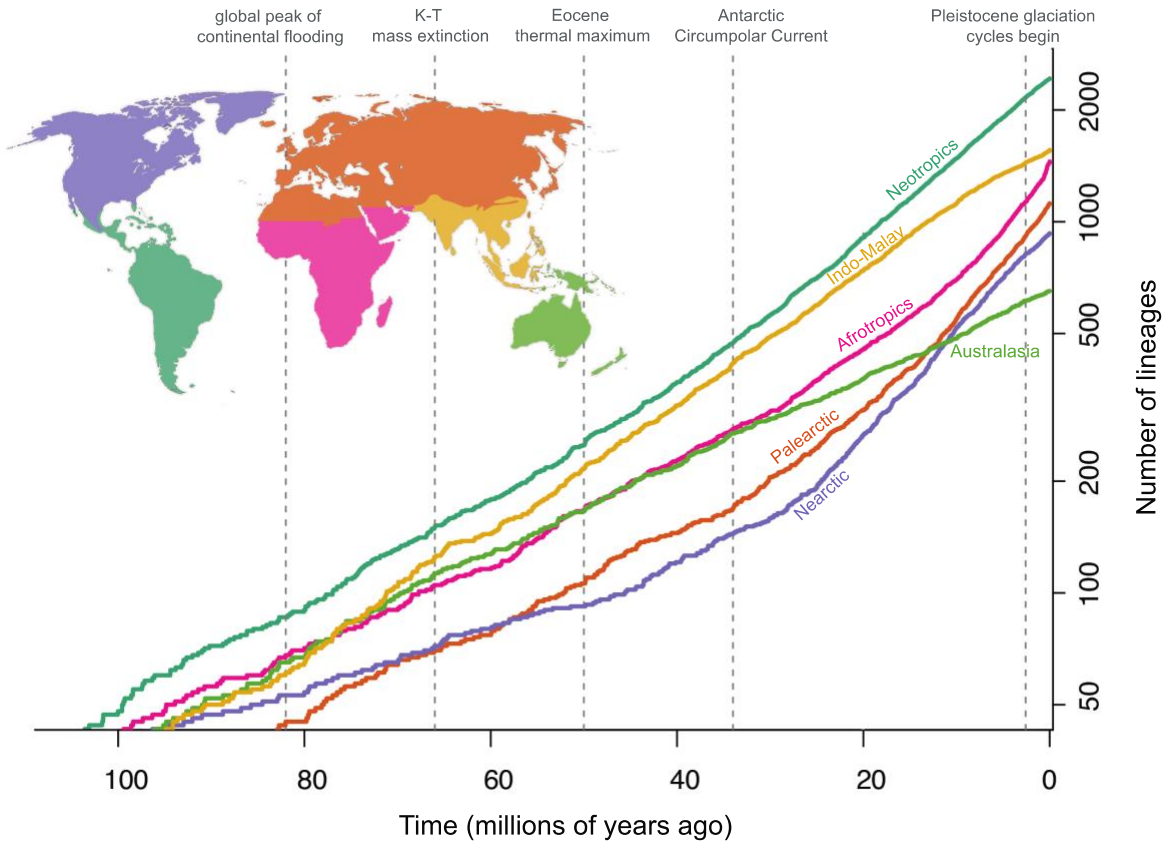

Figure 3: The number of lineages through time for the six major continental regions of the world. Here, temporal dynamics of lineage accumulation were inferred from subtrees for each region, obtained by removing all species from the larger phylogeny not found in that region (occurrences from Tedesco et al. 2017; phylogeny from Rabosky et al. 2018). Important global events are enumerated.

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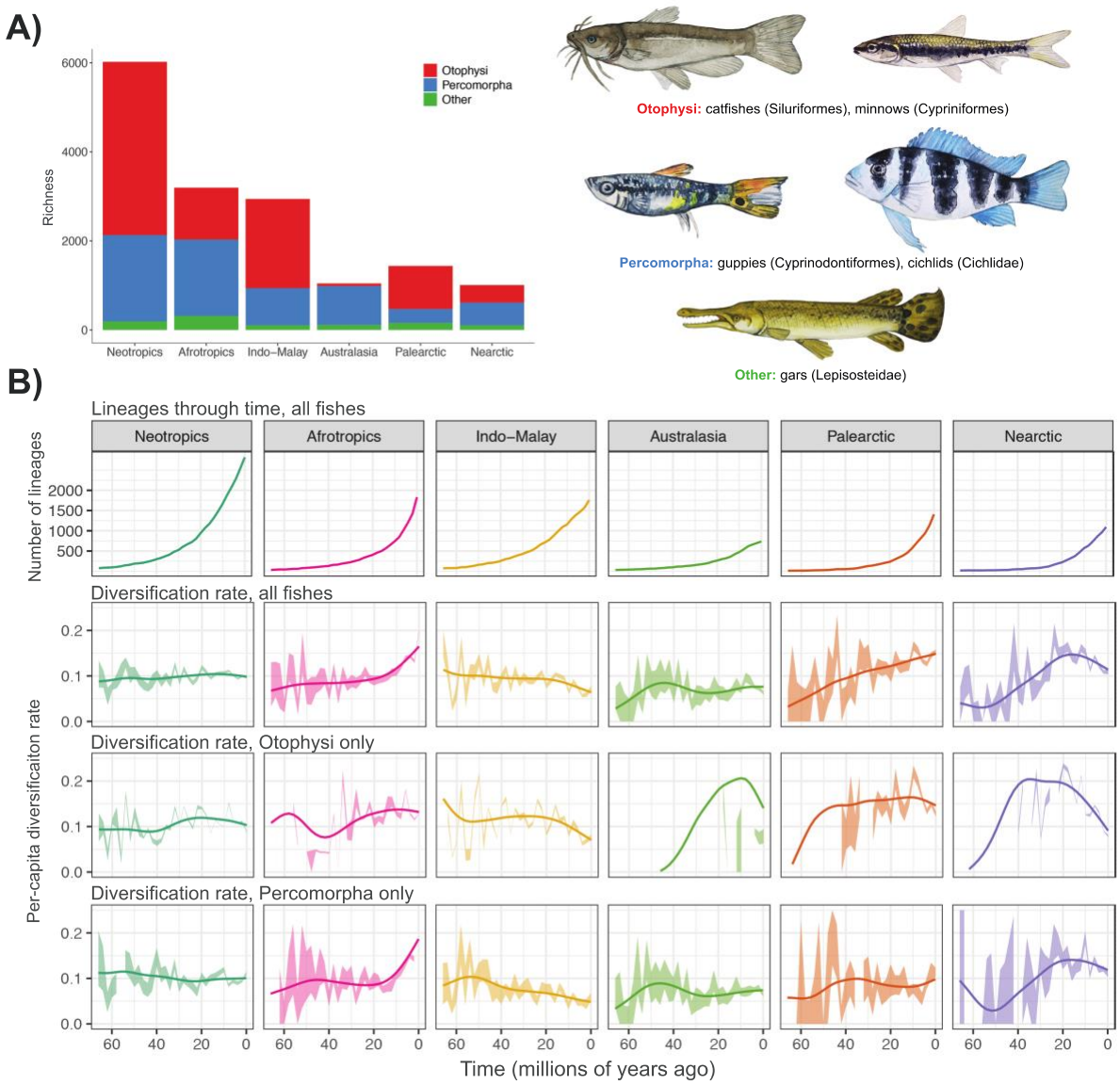

Figure 4: (A): Total regional species richness divided among three groups of ray-finned fishes: Otophysi (red), Percomorpha (blue), and all other clades combined (green). Richness estimates are from Tedesco et al. (2017). Exemplar members of each group are illustrated. **(B):** Lineage accumulation and diversification rates over the Cenozoic (66 mya to present) in each region, estimated by using ancestral area reconstructions (Maztke 2014) on a molecular phylogeny including ~36% of all fish species (Rabosky et al. 2018). Per-capita rates of diversification were estimated using the approach of Xing and Ree (2017). Shaded regions represent the 95% quantile interval of rates among 100 simulated biogeographic histories (Dupin et al. 2016). Paintings by Kathryn Chenard.

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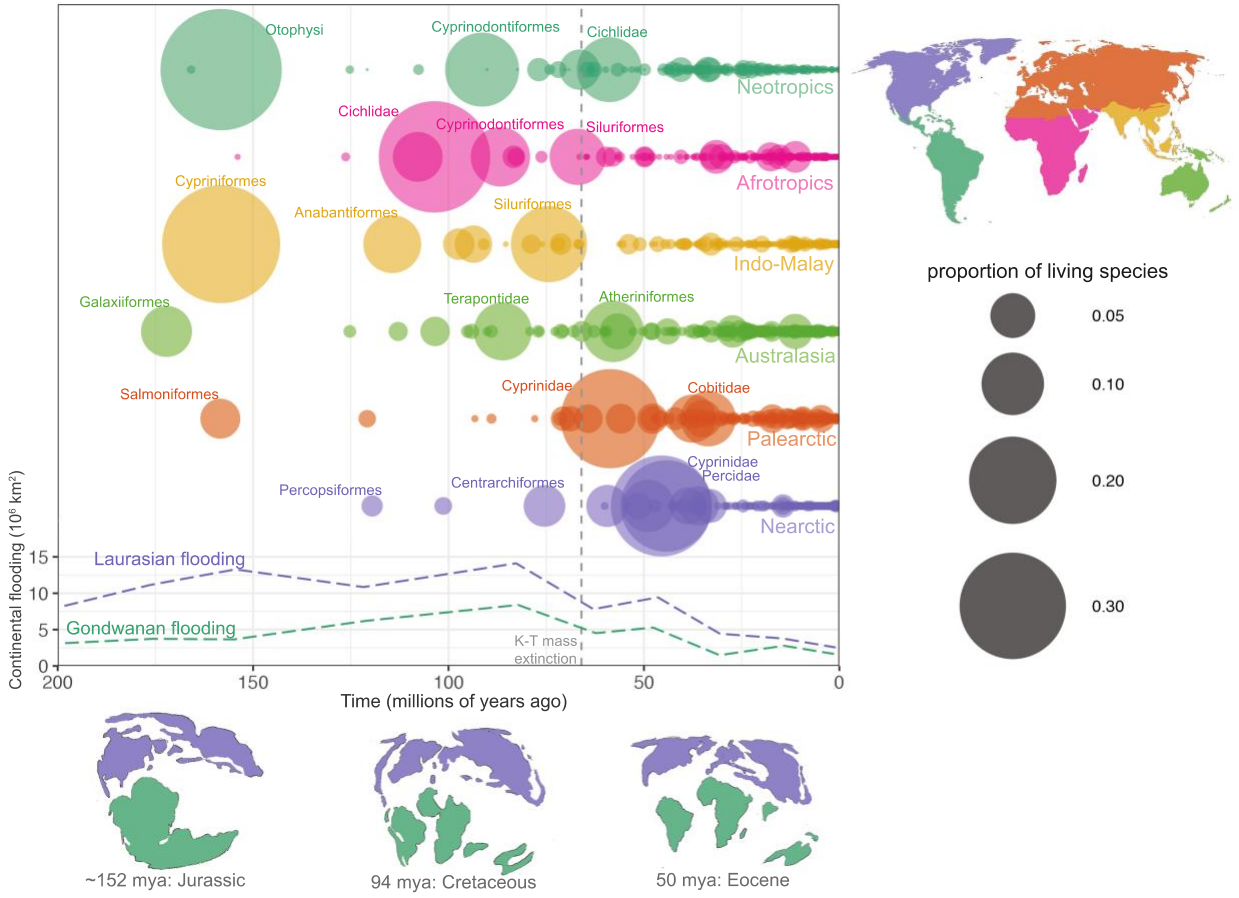

Figure 5. The relative contribution of individual colonization events to present-day species richness in each region. We used stochastic mapping (Dupin et al. 2016) to identify independently colonizing lineages and their living descendants. An example of a simulated biogeographic history is shown here; inferences were similar among the 100 stochastic maps (Fig. S3). Numerically dominant lineages are enumerated, with taxonomy following Rabosky et al. (2018). Continental flooding estimates through time (area in 10^6 km^2 covered by ocean) are from Ronov (1994; see also Fig. S6). Plate tectonic maps were made by C.R. Scotese as part of the PALEOMAP project (http://scotese.com/earth.htm). Earlier colonization events were found to be more species rich than later colonizations (r^2=0.27; $p<0.0001$; Fig. S2).