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Cite this article: García-Andrade AB, Tedesco PA, Carvajal-Quintero JD, Arango A, Villalobos F. 2023 Same process, different patterns: pervasive effect of evolutionary time on species richness in freshwater fishes. *Proc. R. Soc. B* **290**: 20231066.
https://doi.org/10.1098/rspb.2023.1066

Received: 12 May 2023
Accepted: 18 August 2023

Subject Category:
Ecology

Subject Areas:
ecology, evolution

Keywords:
latitudinal diversity gradients, actinopterygii, time-for-speciation hypothesis, temperature seasonality

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Electronic supplementary material is available online at https://doi.org/10.6084/m9.figshare.c.6806599.

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Same process, different patterns: pervasive effect of evolutionary time on species richness in freshwater fishes

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Tropical lands harbour the highest number of species, resulting in the ubiquitous latitudinal diversity gradient (LDG). However, exceptions to this pattern have been observed in some taxa, explained by the interaction between the evolutionary histories and environmental factors that constrain species' physiological and ecological requirements. Here, we applied a deconstruction approach to map the detailed species richness patterns of Actinopterygian freshwater fishes at the class and order levels and to disentangle their drivers using geographical ranges and a phylogeny, comprising 77% (12 557) of all described species. We jointly evaluated seven evolutionary and ecological hypotheses posited to explain the LDG: diversification rate, time for speciation, species–area relationship, environmental heterogeneity, energy, temperature seasonality and past temperature stability. We found distinct diversity gradients across orders, including expected, bimodal and inverse LDGs. Despite these differences, the positive effect of evolutionary time explained patterns for all orders, where species-rich regions are inhabited by older species compared to species-poor regions. Overall, the LDG of each order has been shaped by a unique combination of factors, highlighting the importance of performing a joint evaluation of evolutionary, historical and ecological factors at different taxonomic levels to reach a comprehensive understanding on the causes driving global species richness patterns.

1. Introduction

Worldwide, tropical regions harbour an extraordinary number of species compared with temperate regions, forming the basis for the latitudinal diversity gradient (LDG), one of the best-studied and widely recognized patterns across taxa [1,2]. Nevertheless, this nearly ubiquitous pattern is not followed by some taxa nor consistent across phylogenetic and spatial scales, with inverse LDGs in which diversity peaks are located outside the tropics [3–5]. These inverse LDGs have been explained as resulting from clades' characteristics such as evolutionary history, biogeographical origin, as well as physiological and ecological requirements [3,6].

Hypotheses explaining the causes of LDGs have invoked several mechanisms that can be classified into three non-exclusive categories: diversification rates, time for species accumulation and ecological limits [7]. LDGs can result from geographical variation in diversification rates, with faster speciation and lower extinction in species-rich regions (e.g. tropics) than in species-poor (temperate) regions [8–10]. In addition, species-rich regions can be inhabited by early diverged clades that have had a long time to accumulate species since their

Table 1. Ecological and evolutionary hypotheses assessed within our theoretical model that explain species richness variation of freshwater fishes. In the mechanism column, direct effects on species richness are denoted as D, whereas indirect effects, through speciation rate or evolutionary time, are denoted with I.

hypothesis	mechanism
1. diversification rate [9,10,19]	D: Faster speciation in high-richness regions than in low-richness regions
2. time-for-speciation [8,11,19,20]	D: Species-rich regions harbour clades that colonized or originated early and thus have had more time to accumulate species compared to regions with clades that colonized/originated later
	I: Early colonization/origination time affects speciation through diversity-dependence, with lower speciation rates as diversity increases and ecological opportunities decrease
3. species–area relationship [13,21]	D: Regions with large drainage basins harbour large population sizes and high species richness due to high carrying capacity
	I: Large drainage basins harbour population sizes that favour fast speciation rates
4. environmental heterogeneity [14,22]	D: Regions with large topographical heterogeneity harbour high species richness by allowing species coexistence through species packing
	I: Large topographical heterogeneity causes fast speciation rates due to geographical isolation and ecological specialization
5. energy [2,21]	D: Regions with high energy availability harbour high species richness by supporting large population sizes and carrying capacities
	I: High energy promotes fast speciation rates due to large population sizes, fast metabolic rates and short generation times
6. temperature seasonality [15,16]	D: Regions with low temperature seasonality harbour high species richness by favouring species specialization and thus high coexistence
	I: Low temperature seasonality causes fast speciation rates due to high species specialization and ecological differentiation
7. past temperature stability [17,18]	D: Regions with historically stable temperature harbour high species richness by promoting specialization and coexistence
	I: High past temperature stability favours fast speciation rates through high species specialization because species have sufficient time and resources to become specialized (i.e. narrow niches)
	I: High past temperature stability promotes clade persistence over time, allowing species accumulation

time of colonization/origination in such regions (time-for-speciation hypothesis [11]), retaining their ancestral physiological and ecological requirements that limit their dispersal and establishment into non-ancestral regions (tropical niche conservatism hypothesis [12]). Species richness differences among regions can also be regulated by variations in ecological limits that impose the carrying capacity of such regions and/or affect evolutionary processes [7]. Ecological limits have been related to different aspects of the regions such as available energy [2], area [13], topographical heterogeneity [14], climate seasonality [15,16] and past climate stability [17,18]) (table 1). These ecological and evolutionary mechanisms are not mutually exclusive and their interplay, through both direct and indirect effects, ultimately shapes species richness variation across geography [7,23,24] (figure 1). However, the combined effects of these different drivers on species richness gradients have been poorly explored within and across clades.

Deconstruction approaches, in which a general pattern (e.g. LDG) for a particular taxon is dissected into smaller functional, taxonomic or phylogenetic levels [25], have revealed distinct patterns for such groups, including expected and inverse LDGs. This approach has usually been applied to compare and describe patterns at different taxonomic levels, phylogenetic units (e.g. mammals [26], reptiles [27], marine taxa [28]) or natural history traits (e.g. habitats [29], migratory behaviours [6]). However, why some groups with distinct evolutionary histories or ecologies exhibit similar patterns, such as the LDG, whereas others do not remains elusive.

Similar processes under particular hypotheses have been suggested to explain both expected and inverse LDGs. For example, the diversification rate hypothesis (i.e. fast speciation and low extinction in species-rich regions [8,9]) and the time-for-speciation hypothesis (i.e. longer colonization/origination time allowing greater species accumulation in species-rich regions [11,20]) have been suggested to explain both expected and inverse LDG patterns in tetrapods [3,24]. Although similar processes might lead to different patterns, deviations from the LDG have been explained by differences in ecological and physiological requirements, dispersal capacities and reproductive modes of taxa [3,25,28,30]. Thus, reconciling our understanding on the causes driving different LDG patterns requires a deconstructive approach allowing us to compare among different clades and relate the potential differences with their particular properties such as biogeographic history or ecological traits to infer the underlying drivers and processes.

Freshwater fishes are excellent to test how the interplay of different drivers of species richness shape diversity gradients across taxonomic and phylogenetic scales. These fishes cover a great variety of clades that differ in their numbers of

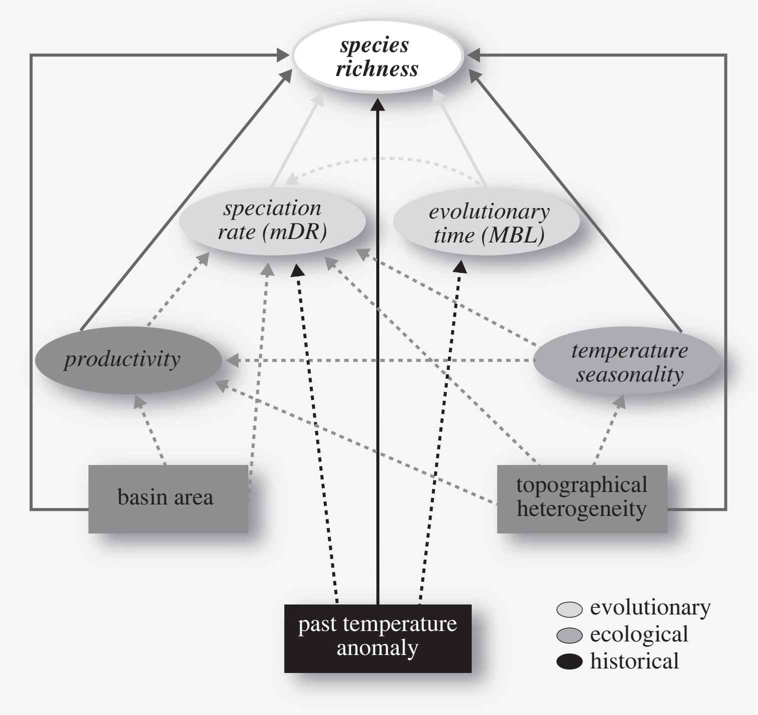

Figure 1. Theoretical model proposed to explain species richness variation of freshwater fishes, in which species richness is shaped by the interplay between the ecological, evolutionary and historical factors through direct and indirect effects. Continuous arrows indicate a direct effect on species richness, whereas dotted arrows denote indirect effects through the evolutionary drivers. mDR, mean speciation rate; MBL, maximum branch length.

species, origination/colonization age and geographical distribution, encompassing approximately 18 535 species worldwide [31]. The geographical diversity gradient of freshwater fishes and its causes have been studied for decades with important contributions in recent years, establishing that this hyperdiverse group shows the LDG in which species-rich drainage basins have larger areas and higher energy availability, and are inhabited by clades with ancient colonization times that have accumulated more species through time than the species-poor basins [19,21,32–34]. However, recent studies in small clades and migratory fishes identified weak, bimodal and inverse gradients, suggesting that each clade's evolutionary history and ecological requirements could play an essential role in the configuration of the global LDG of all freshwater fishes [6,23,35,36]. Indeed, unknown patterns could be hidden under the global LDG of freshwater fishes, and they need to be unravelled to understand the causes driving the diversity of this most diverse group of vertebrates.

Here, we implemented a synthetic approach to reveal the factors driving species richness of freshwater fishes at the global scale while considering the whole class of Actinopterygii and individual orders under a deconstruction approach. We gathered a global dataset of range maps and phylogenetic relationships encompassing 12 557 ray-finned freshwater fishes (approx. 77% of described species; electronic supplementary material, table S1) and developed a structural equation model (SEM) to explain the geographical variation of species richness (figure 1). This SEM allowed us to test the direct and indirect effects among historical, ecological and evolutionary factors based on hypotheses commonly proposed to explain species richness patterns of vertebrates and freshwater fishes (table 1). Finally, through this synthetic framework, we evaluated the effect of the same species richness drivers within each freshwater fish order and the entirety of freshwater fishes on a global scale. Our findings highlight the pervasive effect of evolutionary time as the main driver of geographical species richness patterns of freshwater fishes regardless of their form (expected, bimodal or inverse), also emphasizing the role of current and past temperature variation in explaining these patterns.

2. Methods

To determine the taxonomic arrangement and the number of freshwater fish species worldwide, we used the FishBase database v. 21.06 through the R package rfishbase [37]. Freshwater fishes were determined as all those species recorded in either freshwater, brackish or both habitats. Species registered in freshwater and saline habitats were discarded to avoid including migratory or incidental species in our analyses. Accordingly, we considered 16 248 valid ray-finned freshwater fishes, of which we compiled data on the geographical distribution and phylogenetic relationships for 12 557 species (approx. 77%) encompassing 39 taxonomic orders (electronic supplementary material, table S1). Species phylogenetic relationships were obtained from a set of species-level time-calibrated mega-phylogenies of ray-finned fishes (Actinopterygii), including 31 526 species [38]. These phylogenies assembled genetic information for 11 638 species and placed 19 888 species with no genetic data based on their taxonomy and using a constant-rate birth–death process to resolve polytomies stochastically. To consider phylogenetic uncertainty, we obtained 100 phylogenetic trees from the fish tree of life portal (fishtreeoflife.org).

(a) Geographical distribution of species richness

We reconstructed the geographical ranges of 12557 species following the methodology described in García-Andrade *et al.* [23], using occurrence data from published and open-access databases (electronic supplementary material, table S2). In short, the extent of occurrence was reconstructed by a convex hull overlapped on the Hydrobasin layer level 8 [39]. Then, we constructed detailed species richness maps for all freshwater fishes, as well as 19 individual orders with data for at least 30 species by stacking the species' geographical ranges over an equal-area (Mollweide projection) grid of 110 km of resolution and building a presence–absence matrix using the lets.presab function from R package 'letsR' [40].

(b) Species richness drivers

To evaluate the drivers behind the geographical variation of species richness in freshwater fishes, we tested seven hypotheses proposed to explain species richness patterns of vertebrates, including freshwater fishes (figure 1 and table 1). Our framework is based on an integrative evaluation of ecological, historical and evolutionary factors over species richness to provide a comprehensive understanding of geographical diversity gradients. Under this approach, species richness results from the direct effect of evolutionary drivers that accumulate species (speciation rate and species over time) and the direct and indirect effects of the environment via ecological mechanisms that modulate the dispersal and colonization of species in the present and past, determining the regions' carrying capacity.

(c) Evolutionary drivers

We tested the two main evolutionary hypotheses posed to explain the LDG in different taxa: the diversification rate and the time-for-speciation hypotheses [1,8]. To test the diversification rate hypothesis (table 1), we calculated the speciation rate through the species-level tip DR statistic [41]. Tip DR for each species was calculated as the inverse of the equal splits metric [42] using the evol.distinct function from the R package picante [43] along with the R package ape [44] to manipulate the phylogenetic trees. Then, we calculated mean speciation rate (mDR) as the harmonic mean of tip DR among all species within each grid cell assemblage. In addition, to consider the phylogenetic uncertainty that could have resulted from the imputation procedure to assemble the mega-phylogenies, we calculated the average of the mDR across the pseudo posterior distribution of 100 phylogenetic trees [38].

To test the time-for-speciation hypothesis (table 1), we used the maximum branch length (MBL) as a proxy of evolutionary time. MBL of each grid-cell assemblage was estimated as the age of the species with the longest branch length according to a time-calibrated phylogenetic tree, which represents the divergence time of the oldest species in the assemblage [23,45]. This metric assumes that the MBL of a grid-cell assemblage is relative to the colonization time of such grid cell without considering dispersal and extinction processes. MBL has been previously used to test the role of evolutionary time in species richness, being supported as a good proxy of colonization time on global scales for both aquatic and terrestrial taxa [3,23,45]. For each grid cell, we computed the average MBL across the 100 phylogenetic trees of the pseudo posterior distribution of the extended Actinopterygii mega-phylogeny [38]. MBL and mDR were mapped using the presence–absence matrix.

(d) Evolutionary time sensitivity analyses

The time-for-speciation hypothesis has been tested using several metrics as a proxy of evolutionary time, namely the elapsed time that a clade has had to accumulate species since it originated or colonized a region [11,46]. Previous studies have used the stem and crown age of a clade [24,46] as well as distinct phylogenetic metrics such as mean pairwise distance, mean root distance and MBL as a proxy of time [3,45,47,48]. However, some of these phylogenetic metrics could be biased by involving other evolutionary processes, thus not directly describing the time that a clade has had in a region. To overcome such potential bias, more recent studies have implemented ancestral area reconstruction analyses to directly estimate the colonization time of a clade within a region instead of using a proxy [19,49]. However, ancestral reconstruction at global scales can only estimate colonization time for the broad biogeographic regions that are previously defined, requiring additional regionalizations (and thus reconstructions) for different sets of taxa, including subsets such as those in a deconstruction approach. By contrast, MBL can be used for any set of taxa without requiring different regionalizations and reconstructions that are subject to additional, individual choices, rendering them potentially non-comparable.

To evaluate if MBL was a good proxy of colonization time for freshwater fishes we compared it with the age of first colonization (AFC) [46], using our dataset of 12 557 species and the maximum clade credibility tree from the pseudo posterior distribution of the ray-finned fishes mega-phylogenies [38]. We followed the approach proposed by Miller & Román-Palacios [19] with ancestral area reconstruction analyses using the R package BioGeoBEARS [50,51], considering eight discrete biogeographic regions—Nearctic, Neotropical, Palearctic, Afrotropical, Indo-Malay, Australasia and Oceanic [34], as well as the marine region—to account for lineages that originated in the sea and secondary colonized freshwater habitats, and a time-stratified dispersal model constrained to account for temporal changes of dispersal rates among areas (electronic supplementary material, table S3). Based on this ancestral reconstruction, we calculated the AFC across the presence–absence matrix as the oldest colonization time in each species' grid-cell assemblage. Finally, to determine the relationship between AFC and MBL, and both time metrics against species richness, we performed non-parametric Spearman rank correlations and spatial autoregressive models accounting for the spatial autocorrelation using the R package spdep v.1.1-5 and spatialreg v.1.1-5 [52]. For more details on this sensitivity analysis, see electronic supplementary material.

(e) Ecological drivers

To test the species–area relationship (table 1), we assigned to each grid cell the area of the basin in which they were located. These values were derived from the 30″ HydroSHEDS layer [39]. To test the environmental heterogeneity hypothesis (table 1), we incorporated the topographical heterogeneity index (TH8; www.ipez.es/ModestR/) of each grid cell. The TH8 index describes the topographic differences between a given cell and its eight neighbouring cells, considering elevation, slope and slope aspects [22]. To evaluate the energy hypothesis (table 1), we used the MOD17A3 dataset for 2000–2015 from NASA at resolution of 30 arcsecs [53], downloaded from the Numerical Terradynamic Simulation Group portal (see https://www.umt.edu/). The NASA MOD17A3 dataset representing yearly average net primary productivity has been used to test the energy hypothesis in freshwater fishes [32]. Finally, to test the temperature seasonality hypothesis (table 1), we used the yearly temperature seasonality (BIO4) at a spatial resolution of 10 min from WorldClim v.2 [54] of each grid cell. Both variables were down-sampled to a 110 km grid and projected to Mollweide equal-area.

(f) Historical drivers

To test the past temperature stability hypothesis (table 1), we calculated the past temperature anomaly as the difference between current annual temperature (BIO01) and past annual temperature in the Marine Isotope Stage M2, a glacial event recognized as the most pronounced cooling in the Late Pliocene (approx. 3.3 Ma) [55]. Climate has been highly cyclical in most regions, at least during the last tens of millions of years during the Cenozoic, as a result of periodical changes in the Earth's orbit and axial tilt (Milankovitch oscillations) that affect biodiversity patterns mainly through establishing glacial–interglacial cycles [56]. As such, it is expected that regions with stable climates in the past millions of years would also have been stable over longer periods [1,57]. The current annual temperature layer was downloaded from WorldClim v.2 [54], whereas Late Pliocene annual temperature layer came from the PaleoClim portal [58], both at a spatial resolution of 10 min and then resampled at 110 km and projected onto equal-area Mollweide projection to match with the species richness maps.

(g) Statistical analyses

Following the ecological and evolutionary hypotheses, we proposed a theoretical model to explain species richness variation as a function of direct and indirect effects of causal variables while considering the interplay among these variables (figure 1). To jointly assess these hypothetical relationships, we implemented a piecewise SEM approach that allowed us to evaluate multiple causal hypotheses simultaneously on a response variable accommodating a wide variety of error distributions and random factors, including nesting observation arranged into hierarchical structures, over the traditional SEM [59].

All species richness drivers were standardized to mean zero and standard deviation of one to allow for direct comparison of SEM coefficients. Next, we performed a model selection procedure for each theoretical path equation, testing differences in distances and spatial weight matrices under a spatial autoregressive model (SAR) to find the best parameter combination by Akaike information criterium (AIC). SAR models were run using the R packages spdep v.1.1-5 and spatialreg v.1.1-5 [52]. After finding the best-fitted model for each path equation—the model with the lowest AIC—the complete theoretical SEM was run using the psem function from the R package piecewiseSEM v. 2.0.2 [59]. The goodness of fit of the SEM was assessed using a Fisher's *C* statistic for global model estimation (complete model) based on d-separation tests for local estimations (each individual path), which evaluate if there are important paths (causally dependent) missing in the proposed model. In essence, the Fisher's *C* statistic measures if the hypothesized structured is supported by the data, thus failing to reject (p-value > 0.05) implies no significant missing paths were excluded [59,60]. To run a final SEM, missing paths representing relationships without theoretical foundation were indicated as correlated errors. The final SEM had a p-value higher than 0.05 according to the Fisher's *C* statistic and thus represented the best structured model explaining our data. Finally, we calculated the direct, indirect and total effects using the coefficients estimated in the final SEM. The total effects of variables were calculated as the sum of indirect and direct effects. This procedure was performed independently for the freshwater fishes as a whole and each taxonomic order, performing the model selection procedure for each path and testing the same theoretical model to guarantee that we were using the correct model combination according to each dataset. All analyses were conducted in R [61].

3. Results

(a) Species richness patterns across freshwater fishes

The global LDG of freshwater ray-finned fishes, considering the geographical ranges of approximately 77% of all valid species, showed that the most species-rich regions are in the tropics (figure 2*a,b*), specifically in the Amazon River and La Plata River basins. Other species-rich regions were observed in the Nearctic region in the Mississippi River basin; in the Afrotropics in the Congo and Nile River basins and the Rift Valley Lakes, which include Victoria and Tanganyika lakes, the largest lakes in Africa; and the Indo-Malay regions in the Mekong River basin. Deconstructing the freshwater fish richness pattern, we depicted for the first time the species richness maps of 19 taxonomic orders (electronic supplementary material, figures S1 and S2) identifying expected, bimodal, and inverse LDGs (figure 2). More specifically, 13 of the 19 evaluated orders showed the expected LDG with higher richness in the tropics compared to the temperate region, including the most diverse orders such as Siluriformes (catfishes), Cypriniformes (minnows, carps), Characiformes (characins, tetras), Cichliformes (cichlids) and Cyprinodontiformes (aplocheilids, killifishes, livebearers), together covering more than 80% of all freshwater fish species in the world (electronic supplementary material, figure S1 and table S1). Two orders also had the highest richness in the tropics but showed a bimodal distribution that peaked near mid-latitudes (figure 2*c,d*): Atheriniformes (silversides, rainbowfishes), and Beloniformes (needlefishes, halfbeaks) (electronic supplementary material, figure S3). The order Centrarchiformes also showed a bimodal gradient but with peak richness outside the tropics (figure 2*e,f*). Finally, inverse gradients in which orders showed the highest richness in temperate regions were observed for Perciformes (perches), and the anti-tropical distribution of Galaxiiformes (galaxiids) in the Southern Hemisphere and Salmoniformes (salmon, trouts) in the Northern Hemisphere (figure 2*g–j*).

The geographical variation of species richness across orders showed important differences regardless of the presence of an expected or inverse LDG. For instance, Siluriformes and Characiformes presented the LDG with the highest richness in the Neotropics, whereas Osteoglossiformes showed an LDG with the highest richness in the Afrotropics (electronic supplementary material, figure S1). Other orders showed a bimodal gradient with peaks in the tropics (Atheriniformes; figure 2*c,d*) or at temperate latitudes (Centrarchiformes; figure 2*e,f*). Finally, some groups had an anti-tropical pattern, with a distribution separated by the tropics into disjunct northern and southern regions, such as Perciformes (figure 2*g*) at both hemispheres, Salmoniformes restricted to the Northern Hemisphere (figure 2*i*), and Galaxiiformes (galaxiids) to the Southern Hemisphere (electronic supplementary material, figures S2 and S3).

(b) Species richness drivers of freshwater fishes

The evaluation of our theoretical SEM showed that the global variation in species richness of freshwater fishes was jointly determined by the positive standardized total effect of evolutionary time (0.43), the negative total effect of temperature seasonality along the year (−0.13), a minor but positive total effect (less than 0.05) of speciation rate, basin area, productivity and past temperature anomaly, and topographical heterogeneity on species richness (figure 3; electronic supplementary material, table S5). Regarding orders, the species richness pattern of each order was determined by a unique combination of interacting drivers with different magnitudes of total effect sizes and, in some cases, even directions of the effect. Still, as for all freshwater fishes, the main driver of species richness variation across orders was the

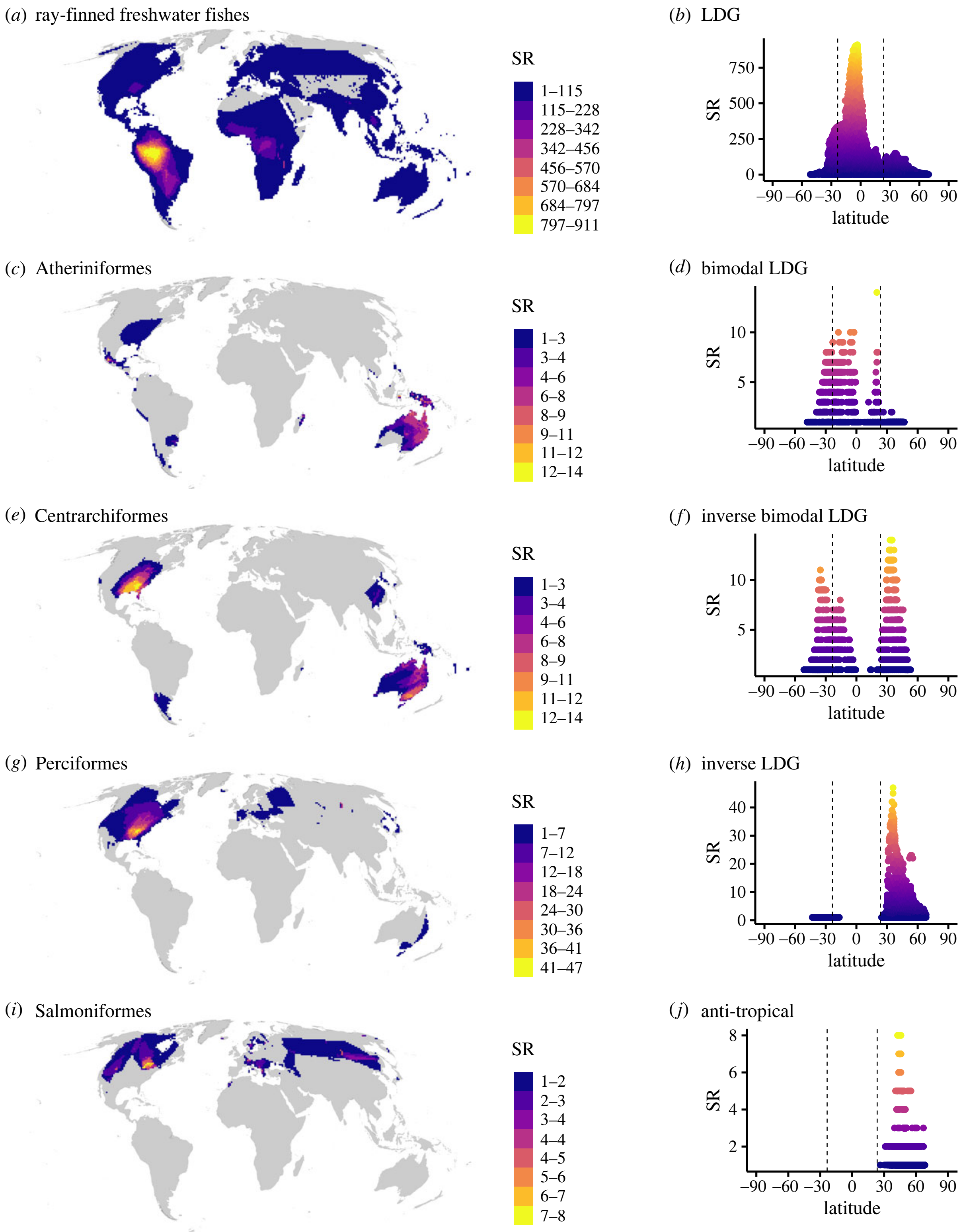

Figure 2. Geographical variation of species richness (SR) and latitudinal diversity gradients (LDG) in select groups of freshwater fishes. The LDG for all freshwater fishes (*a*,*b*), a bimodal pattern peaking inside the tropics for Atheriniformes (*c*,*d*), a bimodal pattern peaking outside the tropics for Centrarchiformes (*e*,*f*), the inverse LDG of Perciformes (*g*,*h*) and Salmoniformes with an anti-tropical distribution restricted to the Northern Hemisphere (*i*,*j*). All maps have an equal grid resolution of 110 km × 110 km on a Mollweide projection. In each scatterplot (*b*,*d*,*f*,*h*,*j*), the coloured points represent species richness matching the map label key at the left, and vertical dashed lines indicate the latitudinal boundaries of the tropical region.

evolutionary time with a positive total effect size (figure 3; electronic supplementary material, figure S4). Similarly, speciation rate had a positive total effect on the richness of 15 orders (0.02–0.51) and a negative total effect for Centrarchiformes (−0.12) (figure 3; electronic supplementary material, figure S4). For temperature seasonality, we found negative total effects on 11 orders (−0.30 to −0.02) and a positive effect for five orders: Perciformes (0.18), Atheriniformes (0.13), Centrarchiformes (0.02), Clupeiformes (0.03) and Pleuronectiformes (0.14). Productivity, basin area, past temperature anomaly and topographical heterogeneity had low or no significant total effect sizes on species richness for all orders, with positive or negative directions but in most cases with total effect sizes lower than 0.1 and −0.1, which

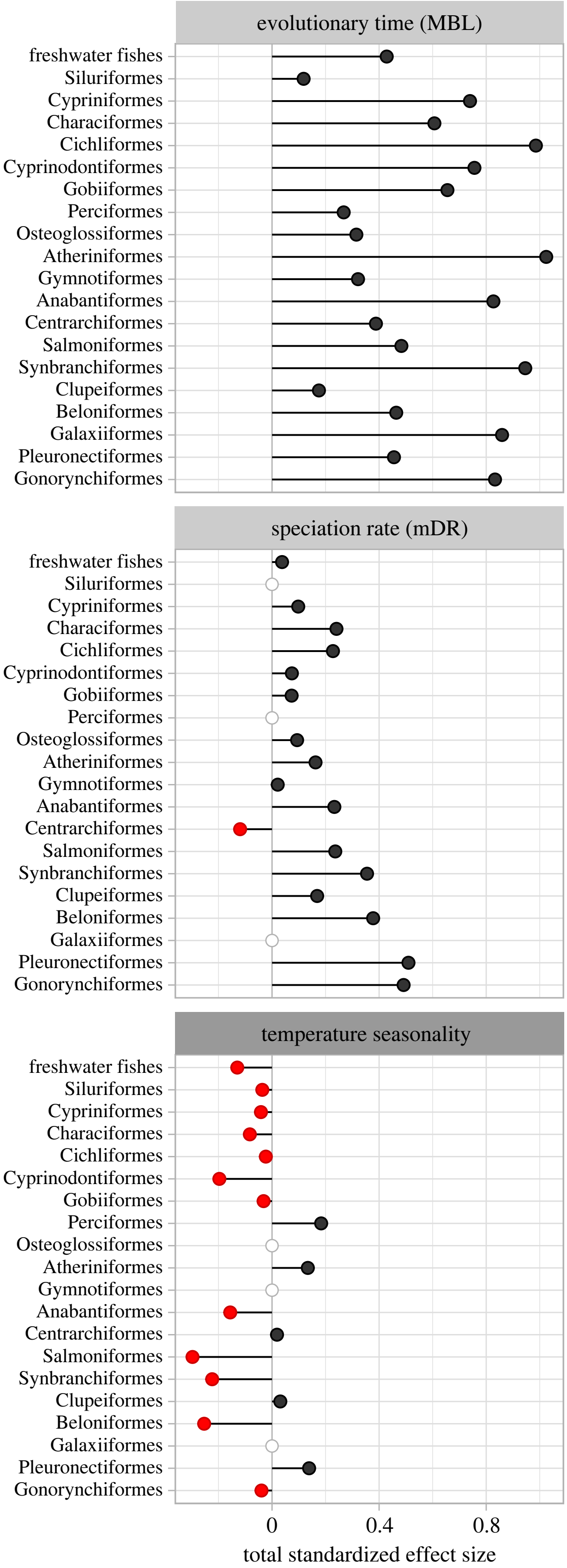

Figure 3. 'Standardized total effect sizes of species richness' drivers for each group evaluated: evolutionary time (MBL), speciation rate (mDR), and temperature seasonality. Black dots indicate positive effects, reds indicate negative effects and white dots indicate non-significant effects. In charts, groups are arranged from most diverse to least. Effect sizes of productivity, basin area and topographical heterogeneity on species richness were omitted in this figure due to their small effects (less than 0.1).

in some cases resulted from indirect effects (electronic supplementary material, table S5).

Our results also corroborated that MBL is a good proxy of evolutionary time, showing a positive and significant relationship with the AFC (Spearman rank correlation; $r = 0.59$, $p < 0.01$). These two variables of evolutionary time also showed a positive and significant relationship when accounting for spatial autocorrelation in the SAR model (electronic supplementary material, figure S6, electronic supplementary material, table S6). Both metrics showed a positive and high correlation with species richness (MBL; $r = 0.81$, $p < 0.01$ and AFC; $r = 0.77$, $p < 0.01$) and a positive effect in the SAR models (electronic supplementary material, figure S6, electronic supplementary material, table S6). Therefore, similar geographical patterns were observed for both metrics, with species in the tropics having diverged and colonized earlier compared to those in temperate regions for all freshwater fishes (electronic supplementary material, figure S7). SEM results for freshwater fishes as a whole using MBL or AFC as a time metric showed similar standardized effect sizes, confirming that evolutionary time was the most important driver of species richness (electronic supplementary material, table S7).

4. Discussion

Geographical diversity gradients can vary across taxonomic levels [4]. For example, the LDG, a nearly ubiquitous pattern across higher taxa, varies even within well-known lineages such as mammals [3,4]. Here, we showed that the LDG of the most diverse group of continental vertebrates, freshwater fishes, varies between the class and order levels as well as among different orders, exhibiting expected, bimodal and inverse LDGs. We present for the first time the detailed global species richness maps for freshwater fishes and the most diverse orders of ray-finned fishes occurring in freshwater and/or brackish habitats. Most importantly, we found that these different LDG patterns are mainly driven by the same process: evolutionary time. Indeed, the time that a clade has had to accumulate species since its origination or colonization of a region is the main factor determining the geographical variation in species richness of freshwater fishes, regardless of the particular form of the observed pattern. The second most important factor driving these different patterns was the speciation rate, which in most orders with expected LDGs—including bimodal that picked inside the tropics—had a positive and direct effect. Additionally, contemporary variables such as temperature seasonality had a negative effect, which can be associated with current ecological processes instead of evolutionary or historical ones. As recently stressed by Marin *et al.* [24], our findings highlight the need to simultaneously evaluate evolutionary and ecological hypotheses within a synthetic framework to disentangle the drivers of species richness patterns.

The most diverse freshwater fish orders showed the expected LDG with the highest richness occurring in the tropics, whereas a few less diverse orders had their highest richness at mid-latitudes and temperate regions. Accordingly, the global LDG pattern in freshwater fishes is shaped by the species-rich orders of ostariophysians such as Siluriformes, Cypriniformes, Characiformes, Gymnotiformes and Gonorynchiformes. These orders group almost 70% of all freshwater fish species, with more than 10 000 described species [62]. Ostariophysians have diversified in the tropics for a long time, sharing a biogeographic and evolutionary history with the development of hydrological basins and continents in these regions [19,63]. Consequently, without a detailed look across taxonomic orders, the LDG pattern ubiquitously observed in highly diverse ostariophysian orders obscured non-expected LDG patterns, as the bimodal and inverse gradients observed in less diverse orders (e.g. Centrarchiformes and Atheriniformes). In species-rich orders, most families are members of the primary division that includes fishes without or with low salinity tolerance (e.g. Siluriformes and Characiformes) that restricts dispersal movements between drainage basins [64]. This isolation effect of the hydrological basins promotes high rates of within-basin speciation [33,65], which explains the high diversity observed in tropical freshwaters [66].

By contrast, some less diverse orders with inverse and bimodal LDGs are classified into the peripheral division, including marine-derived fishes that have recently colonized freshwater habitats (e.g. Atheriniformes) or spent a large portion of their life in the sea (e.g. Salmoniformes). These orders have a high salinity tolerance and dispersal ability [67,68] that favour dispersal between river basins, allowing them to colonize regions inaccessible for strict freshwater fishes after glacial events [69]. Thus, the dispersal capacities of marine-derived fishes could have allowed them to diversify at mid-latitudes and temperate regions (e.g. Atheriniformes and Beloniformes), explaining their distribution, species richness and LDGs [23,68]. For instance, regions such as the Australasia realm are dominated by marine-derived clades belonging to the Galaxiformes and Atheriniformes orders, a pattern explained by its long spatial and temporal isolation that prevented the colonization of primary freshwater clades [70]. However, the role of dispersal ability and salinity in the configuration of LDG at different phylogenetic scales across freshwater fishes should be formally tested.

The deconstruction of the global LDG for freshwater fishes showed expected, bimodal and inverse LDGs across different taxonomic orders. However, all these gradients were mainly explained by the positive effect of evolutionary time on species richness. This means that an older origination or colonization of a clade within a region allows species accumulation. Indeed, evolutionary time had a pervasive effect on the species richness gradients of different freshwater fish orders despite their large differences in diversity, geographical distribution, colonization/origination time, ecological and physiological requirements, as well as dispersal abilities [19,67]. Our findings support the time-for-speciation hypothesis, agreeing with previous studies in terrestrial, flying and aquatic vertebrates in which both expected [19,24,47,71] and inverse LDGs have been explained by the longer establishment of these clades in species-rich regions [11,20,23]. Overall, the positive effect of evolutionary time on species richness is constant across freshwater fish orders. This result supports previous findings (but see [72]) for distinct taxonomic levels, as observed for all freshwater fishes [19], orders like Clupeiformes [47], and families such as Poeciliidae [23], as well as for distinct spatial extents from global [19,47] to continental domains [23], and for distinct spatial grains as drainage basins [19], latitudinal bands [47] and grid-cells [23]. Finally, even though the positive effect of the evolutionary time on species richness seems to be generalized, other evolutionary and ecological drivers also influenced species richness variation of all freshwater fishes and/or individual orders.

Previous studies at global scales in freshwater [19] and marine fishes [38] have shown that species-rich regions are not the product of faster speciation rates. We found support for these previous results for all freshwater fishes and the hyper diverse order Siluriformes where speciation rate had a negligible and non-significant effect, respectively, in determining their species richness variation. These findings do not imply that species origination does not occur or contribute to species richness of these groups, but rather that species accumulate through time instead of through faster origination in particular time periods. In addition, speciation rates may still be important for explaining richness in some regions [33] or taxonomic groups/clades (see below) but not others, with a geographical gradient that could be decoupled from that of species richness at the global scale. Such a decoupled pattern was recently found for marine fishes, where speciation rate showed an inverse LDG with higher rates towards high latitudes characterized by relatively low species richness [38]. Indeed, speciation gradients have gained much attention recently [73–75], but the existence of such gradients deserves further investigation.

In contrast to the class level and the richest order, speciation rate significantly influenced the LDGs of most taxonomic orders, especially those showing an expected LDG. Accordingly, for most freshwater fish orders, species-rich regions result from longer colonization/origination time along with a fast speciation rate, explaining the accumulation of species in the tropics compared to temperate regions. A faster speciation agrees with the diversification hypothesis, suggesting that high speciation in the tropics produced high species richness, with conspicuous groups such as the great radiation of Cichlids in the Americas and Africa [76] and the Characids in the Neotropics [77]. However, contrary to the pervasive positive effect of time, speciation rate had a differential effect depending on the type of LDG, with a negative effect in Centrarchiformes that showed a bimodal inverse LDG and no effect in orders with an inverse LDG or anti-tropical distribution (except for Salmoniformes), which were more influenced by evolutionary time in combination with the effect of ecological limits, mainly through temperature seasonality, but not of faster speciation. Our results support the diversification hypothesis in some groups, but not in others, which coincides with past studies on particular freshwater and reef fishes clades [23,78], suggesting that the role of speciation rate can depend on the scale of analysis, global versus regional [79], taxonomic levels and/or life history [4].

Extinction rates can also affect diversification and thus species richness variation among regions [8]. For instance, the high diversity of Neotropical freshwater fishes has been associated with low extinction rates, suggesting a museum model where diversity increases as species accumulate without going extinct [80]. In our case, the positive effect of evolutionary time along with fast speciation richness on species richness (at least in orders with the LDG) may imply that species-rich regions have had lower extinction rates, compared to higher extinction in species-poor regions, despite low speciation rates that on average result in positive net diversification rates thus favouring species accumulation [8]. However, evaluating the actual effect of extinction on diversity patterns requires a good fossil record that is usually lacking at broad spatial and phylogenetic scales, which is the case for freshwater fishes [81]. Indeed, most studies so far have focused solely on speciation rate as a driver of current diversity patterns (e.g. [38]) mainly because estimating extinction rates from molecular phylogenies is rather uncertain [82]. More data on fossils as well as reliable methods for accurately estimating extinction could eventually confirm or refute our findings, and those of others, on the effect of evolutionary drivers on species richness.

Several different drivers of species richness patterns have been proposed since the description of LDG, from ecological and climatic to historical and evolutionary [8,9,83]. More recently, factors operating over evolutionary time scales, namely speciation, extinction and dispersal, have been recognized as the ultimate processes that change regional species richness [1]. Still, other factors such as climatic conditions and area can influence species richness via their effect on evolutionary factors and directly modulate the dispersal and establishment of species [7,24]. Indeed, in most of the evaluated freshwater fish orders, the annual temperature seasonality was the other most important factor in explaining their species richness patterns through a negative effect. This finding agrees with the climate seasonality hypothesis [15], which posits that regions with lower seasonality throughout the year support larger populations and, thus, higher species richness than more seasonal regions [15,16]. Temperature represents a restrictive factor for ectotherms given its influence on metabolic rates and the availability of resources that determine species' growth, reproduction and establishment [84,85]. In addition, most orders have the highest richness in the tropics, and their distribution could be limited by the low-temperature conditions of temperate seasonal regions that exceed their thermal tolerances.

A negative effect of temperature seasonality in almost all orders suggests that their thermal tolerances play a relevant role in configuring their LDGs, perhaps via phylogenetic niche conservatism [12]. An expected LDG along with a tropical origin of most rich orders [19,47] support the tropical niche conservatism hypothesis in which most lineages are limited to their ancestral tropical conditions [12]. For example, a recent study including marine and freshwater species found an expected LDG in Clupeiformes, supporting that most species are restricted to the tropics with few transitions from tropical to temperate regions [47]. By contrast, fishes that diversified in temperate regions and showed an anti-tropical distribution and inverse LDG (e.g. Perciformes) should have developed survival strategies such as low thermal tolerances and behavioural strategies to cope with cold temperatures [86]. These adaptations to temperate conditions could explain the positive effect of temperature seasonality on species richness outside the tropics and the highest species richness in the north temperate regions. Similarly, the physiological thermal limits of anti-tropical marine fishes were related to their restricted distribution to temperate regions, given their ecological requirements [87]. However, niche conservatism should be further tested to reach a better understanding of the role of contemporary temperature in the distribution and diversification of freshwater fishes.

The small total effect sizes of past temperature anomaly, productivity and basin area (variables frequently advanced to explain species richness in freshwater fishes at global scales [19,21,32,35]) could be explained by differences in the spatial unit and grain size between our study and previous ones (i.e. grid-cells versus drainage basins). Indeed, previous studies support that diversity patterns and the strength of predictors can vary across spatial scales, showing that at coarse grains (e.g. drainage basins), variables such as productivity and basin area can be more important than at fine grains (e.g. grid cells) [79]. Overall, our findings support the role of productivity and basin area in species richness in some orders as well as for all freshwater fishes, but with lower importance considering our finer and standardized sampling units (grid cells) compared with previous studies at the coarser and unstandardized drainage basin grain [19,21].

The dataset used here to disentangle the drivers of the species richness gradient in freshwater fishes, across orders and at the class level (Actinopterygii), covers over two-thirds of the freshwater ray-finned fish species, including information on geographical ranges [34,64] along with phylogenetic relationships from a large phylogenetic tree [38]. Although we used the most comprehensive dataset to date, we recognize that it could have some deficiencies such as low coverage for some groups and regions, potentially generating spurious patterns. For instance, our dataset included species that were imputed to a genetically informed phylogeny [38] with less than half of our total species (4914; approximately 30% of all valid freshwater fishes), which certainly underrepresented some orders (e.g. Beloniformes) and regions. However, we found congruent spatial patterns (electronic supplementary material, figure S8) as well as similar relationships between species richness and the evaluated drivers when comparing results from the complete dataset and the subset of species with genetic information (electronic supplementary material, figure S9). Indeed, Miller & Román-Palacios [19], using only the species with genetic information in the phylogeny of Rabosky *et al*. [38], found similar results to ours at the class level (i.e. the positive effect of evolutionary time on species richness). Overall, the findings from comparing datasets and those of Miller & Román-Palacios [19] support the robustness of our observed patterns and drivers despite limited data coverage.

In sum, our results showed for the first time that in the hyperdiverse freshwater fish group, not all orders follow the LDG, exhibiting also bimodal and inverse gradients. Importantly, our study provides evidence that the LDG in freshwater fishes is most strongly shaped by evolutionary time, consistent with previous findings in flying and terrestrial clades. This factor had a pervasive positive effect on species richness across distinct freshwater fish orders regardless of whether or not their LDGs showed the expected pattern, and for most orders act jointly with fast speciation rate. Current temperature emerged as an important driver of species richness of freshwater fishes, an ectotherm group whose life cycle and establishment are certainly influenced by temperature. Although LDG patterns are mainly explained by evolutionary time, along with speciation rate and current temperature seasonality, species richness in each order is explained by a unique combination of drivers. Furthermore, these drivers vary in their effect sizes and directions, which can be explained as a result of particular traits and histories of fish orders. Finally, our study highlighted the importance of deconstructing global diversity gradients under an integrative approach that allows the simultaneous evaluation of ecological and evolutionary hypotheses to untangle the different drivers responsible for such patterns.

Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. The data generated in this research, as well as the code to perform all the analyses presented, can be accessed at the link: https://doi.org/10.6084/m9.figshare.21122320 [88].

The data are provided in electronic supplementary material [89].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. A.B.G.-A.: conceptualization, data curation, formal analysis, investigation, methodology, project administration, resources, software, validation, visualization, writing—original draft, writing—review and editing; P.A.T.: conceptualization, data curation, funding acquisition, investigation, project administration, resources, supervision, writing—review and editing; J.D.C.-Q.: data curation, funding acquisition, methodology, resources, supervision, validation, writing—review and editing; A.A.: formal analysis, methodology, software, writing—review and editing; F.V.: conceptualization, formal analysis, funding acquisition, investigation, methodology, project administration, supervision, validation, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

Funding. The authors thank the Scientific Cluster BioMimic at Instituto de Ecología A.C. (INECOL) for the supercomputational resources provided at Huitzilin. A.B.G.-A. thanks the Posgrado en Ciencias at INECOL and the Consejo Nacional de Humanidades, Ciencias y Tecnologías (CONAHCYT) for the Doctoral scholarship (#722139). J.D.C.-Q. acknowledges the support of iDiv funded by the German Research Foundation (DFG-FZT 118, 202548816). P.A.T. was supported by the Évolution & Diversité Biologique Laboratory through the LABEX TULIP and CEBA (ANR-10-LABX-41, ANR-10-LABX-25-01). In addition, we thank Elizabeth Miller for her advice (and code) on the ancestral reconstruction analysis.

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