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Existing protected areas provide a poor safety-net for threatened Amazonian fish species

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Abstract

1. Freshwater ecosystems represent less than 0.01% of Earth's surface water but proportionately encompass the most species-rich environment on the planet, including nearly one-third of all vertebrate species. Even though inland continental waters are widely regarded as highly endangered ecosystems, their species assemblages are mostly ignored in conservation plans, largely because spatial patterns of freshwater species remain poorly understood. This is particularly severe throughout the Neotropics, most notably in the Amazon superbasin, where the sheer biotic diversity is coupled with a severe lack of biodiversity knowledge at several levels.
2. Spatial patterns of Neotropical freshwater fishes focusing mainly on the Amazon superbasin were investigated. First, Endemic Amazonian Fish Areas (EAFAs) representing central units for the conservation of continental fishes were delimited. Interpolated maps were then analysed using alternative methodologies to delimit spatial patterns of diversity and endemicity across the Amazon superbasin. Several biogeographical analyses used a comprehensive dataset of species and geographical coordinates of Amazonian fishes.
3. The results reveal well-defined spatial patterns of species richness and endemicity in the Amazonian fish fauna, showing that most protected areas are concentrated in a single bioregion (Amazon lowlands). Those areas are incongruent and insufficient to protect endemic and threatened species, which are mostly distributed in upland regions.
4. Effective conservation of the Amazonian fish fauna should include EAFAs within protected areas, especially those undergoing deforestation and hydropower development pressure and containing a high concentration of threatened species.
5. The following EAFAs should be considered as conservation priorities: Upper Araguaia, Upper Tocantins, Lower Teles Pires/Serra do Cachimbo, Chapada dos Parecis and Upper Marañon. These regions should be urgently protected to avert the loss of important trophic relationships and unique elements of the Amazonian fish fauna.

KEYWORDS
biogeography, conservation priority, deforestation, fish, hydropower, river

1 | INTRODUCTION

Continental waters are thought to be one of the most highly threatened ecosystems on Earth (Abell et al., 2008; Nogueira et al., 2010; Saunders, Meeuwig, & Vincent, 2002), but their biotas are often neglected in conservation policy and biodiversity assessments (Heilpern, 2015). In most cases, freshwater biotas remain too poorly known to be integrated into conservation strategies based on terrestrial environments (Heilpern, 2015). Efficient conservation strategies require detailed knowledge of spatial distributions, which are available for only a few taxonomic groups, most of which are terrestrial vertebrates (Kier et al., 2009). Thus, freshwater habitats are rarely considered conservation priorities, despite their intrinsic importance and urgent need for protection (Amis, Rouget, Lotter, & Day, 2009; Strayer & Dudgeon, 2010).

The taxonomy and geographical distribution of Neotropical fishes are still poorly understood. These circumstances impose severe limitations on our ability to conserve aquatic biodiversity, which increasingly faces a mounting global-scale extinction crisis (Brito, 2010). To transform biodiversity knowledge into conservation actions it is necessary first and foremost to document the taxa and their patterns of diversity (Nogueira et al., 2010). Lack of knowledge about both the diversity and distribution of species has always been considered a problem for allocating and designing protected areas (Brito, 2010; Brooks, Fonseca, & Rodrigues, 2004; Fagan, Fagan, Kennedy, & Unmack, 2005; Gaston & Rodrigues, 2003; Polasky et al., 2000). The current alarming rate of biodiversity loss requires the selection of priority areas and taxa to concentrate limited resources on regions of highest conservation value and with the most pressing need for conservation plans (Brooks et al., 2006; Kier et al., 2009; Margules & Pressey, 2000; Myers, Mittermeier, Mittermeier, da Fonseca, & Kent, 2000; Vane-Wright, Humphries, & Williams, 1991).

An appropriate conservation rationale has rarely been applied to Amazonian fishes and it is only recently that efforts have been dedicated to building predictive scenarios on this topic (Frederico, Olden, & Zuanon, 2016; Jézéquel et al., 2020). The Amazon superbasin contains by far the most species-rich freshwater fish fauna on Earth, with more than 2,700 known species (Dagosta & de Pinna, 2019). Little is known about the patterns of geographical distribution, species richness and endemicity of Amazonian fishes (Dagosta & de Pinna, 2018), which partly explains why this fish fauna continues to be largely ignored by regional-scale conservation plans.

The past few decades have seen some efforts to compartmentalize continents and oceans on the basis of their biotas. However, new reproducible analytical methods began to be applied only recently (Vilhena & Antonelli, 2015). Although this fish fauna has been historically used as a basis for sub-dividing South America into informative biogeographical regions (Abell et al., 2008; Eigenmann, 1909; Géry, 1969; Günther, 1880; Reis et al., 2016; Vari, 1988), none of these segmentation proposals applied robust analytical methods or shared a broad underlying search for patterns of endemism. Identifying endemisms, however, depends on detailed knowledge of the taxonomy, phylogeny and geographical distributions of the taxa concerned. Although some generalizations are possible on the basis of incomplete data, as demonstrated in many valuable insights from previous work, information on Amazonian fishes has only recently accumulated to a degree that ensures quantitative approaches. For the first time, it is possible to delimit areas of endemism for Amazonian fishes (Endemic Amazonian Fish Areas or EAFAs) using analytical tools on the basis of a comprehensive georeferenced dataset. These tools ensure that endemisms can be segregated into various dimensions, including phylogenetic endemism, weight endemism, and frequency of restricted-range taxa. Such fine-grained analyses can potentially generate a new and detailed overview of biodiversity, often revealing unexpected patterns.

This study investigates geographical distributions of Amazonian fishes through different analytical methods, with two main objectives: (i) elucidate the spatial patterns of diversity and endemicity and (ii) identifying EAFAs that can represent the central units of species conservation. Those are critical steps for the establishment of conservation priorities (Britto, 2010; Vane-Wright et al., 1991), particularly in the vast Amazon superbasin. We provide a proposal for bioregionalization of the Amazon superbasin with a qualitative assessment of all existing protected areas and their respective efficacy in setting aside the most highly threatened fish species. The findings presented here can be used to integrate a priority-setting agenda for the Amazonian fish fauna with similar data to those available for terrestrial organisms, resulting in conservation plans based on a more comprehensive fraction of Amazonian biodiversity.

2 | METHODS

2.1 | Data acquisition

A comprehensive dataset comprising 119,394 geographical coordinates from 4,761 Neotropical fish species was assembled using the information available from the taxonomic literature, museum collections and metadata repositories. From that, 80,407 geographical coordinates and 2,726 species were from Amazonian fishes. Customized python scripts with regular expression (i.e. regex) patterns were used to gather coordinates from the literature. Published articles with distribution maps (e.g. classical taxonomic revisions) but lacking listed georeferenced specimens (e.g. classical taxonomic revisions) had coordinates manually captured using georeferenced maps as a proxy. The remaining coordinates were obtained either from museum collections (e.g. AMNH, ANSP, FMNH, LBP, INPA, IvH, MCZ, MNRJ, MPEG, MZUSP, PUJ, USNM; abbreviations in Sabaj, 2016) or from the SpeciesLink repository http://splink.cria.org.br. The latter were obtained mainly for species lacking comprehensive taxonomic reviews or published maps.

2.2 | Data cleaning

After compiling record coordinates, an automated processing routine was applied to identify and remove records with georeferencing problems, especially those obtained from the SpeciesLink repository. The routine included three steps as suggested by Robertson, Visser, and Hui (2016). First, all coordinates lacking locality and/or country information were excluded, as these records are likely to be inexact in metadata repositories. Second, we removed: (i) duplicate coordinates to prevent pseudoreplication; (ii) all coordinates located in the ocean; (iii) coordinates along a meridian or equator (i.e. latitude or longitude exactly zero); (iv) low-precision coordinates (e.g. obtained from a coarse-scale grid); and (v) records with country name mismatches (i.e. in the database vs. inferred from coordinates). Third, species distributions were visually inspected and suspicious records outside species' geographical ranges known from the published literature were removed to ensure data reliability. This task took more than 2 years to complete and was by far the most labour-intensive in the entire process, but it was strictly necessary to minimize inaccurate georeferencing or incorrect identifications in the dataset.

2.3 | Geographical layers and threatened species

Our list of endangered Amazonian fish species followed the reports of the Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, 2018). For those cases where any given species occurred outside Brazil (e.g. Bolivia, Colombia, Venezuela), we adopted the endangered species list from the International Union for Conservation of Nature (IUCN, 2019). Databases for hydroelectric plants, Indigenous territories and Natural Protected Areas were obtained from the Amazon Geo-Referenced Socio-Environmental Information Network, RAISG (2019). Forest loss from 2000 to 2017 followed the updated database provided in Hansen et al. (2013).

2.4 | Taxonomic tree of the Amazonian fish fauna

A species-level tree of the Amazonian fish fauna was built based on the most updated classification of Neotropical fishes summarized in the Catalog of Fishes (van der Laan, Fricke, & Eschmeyer, 2019). Because the Catalog of Fishes classification is limited at the order level, the species taxonomy above this rank adopted the proposal of Nelson, Grande, and Wilson (2016). Branch lengths were set equal to one. Some inferential analyses required an entire bifurcated input tree, hence polytomies were randomly solved assuming all branch lengths to be equal to zero. Although this randomization approach is not ideal, it does not change the calculations of statistical indices relying on measures of branch lengths (e.g. Phylogenetic Diversity and Phylogenetic Endemism, see details below).

2.5 | Biogeographical analysis

All input files for the biogeographical analyses were prepared in R (R Core Team, 2017). In all analyses other than 'Species Richness Interpolation (i)', the interpolation delimitation mask was the Amazon ichthyo-hydrological basin contour (see Figure 1 in Dagosta & de Pinna, 2017). In the 'Species Richness Interpolation (i)' analysis, the delimitation mask was the entire Neotropical region.

2.5.1 | Bioregionalization of the Amazonian fish fauna

Bioregionalization of the Amazonian fish fauna was performed using the Infomap Bioregions software (Edler, Guedes, Zizka, Rosvall, & Antonelli, 2017). This software uses an adaptive resolution of grid cells, especially when sampling effort is uneven, to generate bipartite networks (i.e. species vs. grid cells) that are clustered using the Infomap clustering algorithm. The analysis applied the following settings: maximum cell size = 8°, minimum cell size = 1°, maximum cell capacity = 300 and minimum cell capacity = 40.

2.5.2 | Species richness interpolation

The species richness interpolation (SRI) analysis was performed to map fish species richness using the spline interpolation algorithm. The estimates were inferred based on the Biodinamica software (Oliveira, Soares-Filho, Leitão, & Rodrigues, 2019) and used in three separate analyses:

1. Species richness interpolation using the entire dataset (including all Amazonian fish species together with those occurring outside the basin) in a mask including the Neotropical region (hexagon size of sample unit 1.5; raster resolution 0.5; minimum of one sample per hexagon; remaining parameter as per default settings of the software). Analyses were done at the level of species, genus and family.
2. Distribution overlap of Amazonian endemics (*sensu* Dagosta & de Pinna, 2019). The list of Amazonian species with occurrences restricted to a single Amazonian sub-drainage was taken from Dagosta and de Pinna (2019) and used as the parameter to determine endemic species. Only species from that list were maintained in the spline interpolation analysis (hexagon size of sampling unit 1.5; raster resolution 0.2; minimum of one sample per hexagon; remaining parameters as per default settings of the program).
3. Distribution overlap of endemics with smaller distribution polygons. This analysis uses measurements of the area of species occurrence. A polygon of occurrence area was generated for each Amazonian fish species in the database, using an R code (R Core Team, 2017) and the results were used to calculate the average occurrence area of all Amazonian species (also including coordinates outside the basin). Species with 10% or less of that average were defined as endemics (hexagon size of sampling unit 1.5; raster resolution 0.2; minimum of one sample per hexagon; remaining parameters as per default settings of the program). The estimates were inferred using the spline interpolator.

2.5.3 | EnDemisM

EnDemisM (NDM) is a method that optimizes species spatial distributions on grid cells adopting an endemism index. This analysis was implemented in the program NDM/VNDM (Goloboff, 2005) with the following settings: grid cell of 1° size, grid origin $X = -106.059$/$Y = 26.132$, matrix comprised of 65 rows × 108 columns. The sets were saved with scores above 2.0 and five or more endemic species. The number of endemic species was used because fish faunas include many narrowly distributed species. Using two endemic species results in several hundred areas covering nearly the entire Amazon basin, thus failing the main goal of identifying regions where endemic species are concentrated. The search was repeated 10 times, retaining overlapping areas only if 90% of all species within each of them were unique. Only grids containing species records were included in the analysis. The consensus flexible areas of endemism criterion included areas that share at least 1% of their endemic species, aiming to coalesce areas of endemism as much as possible. The remaining parameters follow the program defaults. Only Amazonian fish species (also including coordinates outside the basin) of the dataset were used.

2.5.4 | Geographic Interpolation of Endemism

Geographic Interpolation of Endemism (GIE) is a method that delimits areas of endemism through a kernel interpolation of multiple centroids estimated independently from multiple species distributions (Oliveira, Brescovit, & Santos, 2015). EAFAs were inferred from the spatial kernel density of centroids (i.e. kernel index) given the distance-based groups of species distribution ranges (Oliveira et al., 2015). Here, species were classified into nine groups, with the main criteria being Euclidean distances between their centroids and the farthest coordinate points: (i) up to 50 km; (ii) 51–100 km; (iii) 101–150 km; (iv) 151–200 km; (v) 201–250 km; (vi) 251–300 km; (vii) 301–400 km; (viii) 401–500 km; and (ix) 501–700 km. The analysis applied the following inputs: minimum number of endemic species = 2; no weight in consensus; minimum of one sample per sampling unit; and raster resolution of 0.4. Only Amazonian fish species (also including coordinates outside the basin) in the dataset were used.

2.5.5 | Weight Endemism Index

The Weight Endemism Index (WEI) combines endemicity and species richness (Williams & Humphries, 1994), and is calculated as the inverse function of species ranges, so pools of species that occur over smaller ranges are given higher scores (Kier & Barthlott, 2001; Oliveira, Vasconcelos, & Santos, 2017). The overall WEI was calculated per cell by summing over all independently calculated WEI values for each species within 1.5 hexagon grid cells assuming at least one sample and an output raster resolution of 0.2. EAFAs were inferred by the spline interpolator under the Biodinamica software default parameters (Oliveira et al., 2019). Only Amazonian fish species (also including coordinates outside the basin) in the dataset were used.

2.5.6 | Phylogenetic diversity

The phylogenetic diversity (PD) method defines the phylogenetic diversity of a group of species within an area as the sum of all branches of a given tree that span all members of that group (Faith, 1992). The estimates were inferred using the spline interpolator under the default parameters of the Biodinamica software (Oliveira et al., 2019): hexagon size of sampling unit 1.5; raster resolution 0.2; and at least one sample per hexagon. Only Amazonian fish species (also including coordinates outside the basin) in the dataset were used.

2.5.7 | Phylogenetic endemism

Phylogenetic endemism (PE) aims to identify areas encompassing a higher concentration of endemic lineages by integrating PD and WEI calculations into a novel analytical framework (Rosauer, Laffan, Crisp, Donnellan, & Cook, 2009). It describes the extent to which unique phylogenetic lineages are constrained into restricted geographical areas. The PE was calculated assuming as input the taxonomic tree and 1.5 hexagon grid cells assuming at least one sample and an output raster resolution of 0.2. This was inferred using the spline interpolator under the Biodinamica software default parameters (Oliveira et al., 2019). Only Amazonian fish species (also including coordinates outside the basin) of the dataset were used.

2.5.8 | Sampling effort

Overall sampling effort in terms of ichthyofaunal inventories across the Amazon superbasin was estimated using kernel interpolation in the Biodinamica software (Oliveira et al., 2019) assuming an area of influence of 10 km and a cell size output raster of 0.05. Only Amazonian fish species (also including coordinates outside the basin) in the dataset were used.

3 | RESULTS

3.1 | Definition of Amazonian bioregions based on fishes

Infomap Bioregions defined 24 bioregions in South America (Figure 1), 10 of which are included within the Amazon superbasin (Figure 2) as described below in more detail.

FIGURE 1 Bioregionalization of the South American fish fauna using bipartite networks and infomap clustering algorithms. The map shows 24 bioregions, 10 of which are included in the Amazon superbasin

3.1.1 | Amazon lowlands

This is the largest bioregion within the Amazon superbasin, and comprises mostly lowland rivers with few geographical elements acting as vicariant barriers to its fish fauna, high species richness and low numbers of endemic species. This Amazon region comprises whitewater rivers with nutrient-rich, high sediment loads. The main river tributaries are the Rio Madeira, Rio Purus, Rio Juruá, Rio Jutaí, Rio Japurá, Rio Napo, Rio Putumayo and part of the Rio Branco and Rio Marañón. The Amazonas lowlands delimited herein closely resembles the Amazon-only Lowland (*sensu* Dagosta & de Pinna, 2019), except for the inclusion of the Rio Amazonas and Rio Orinoco mouths.

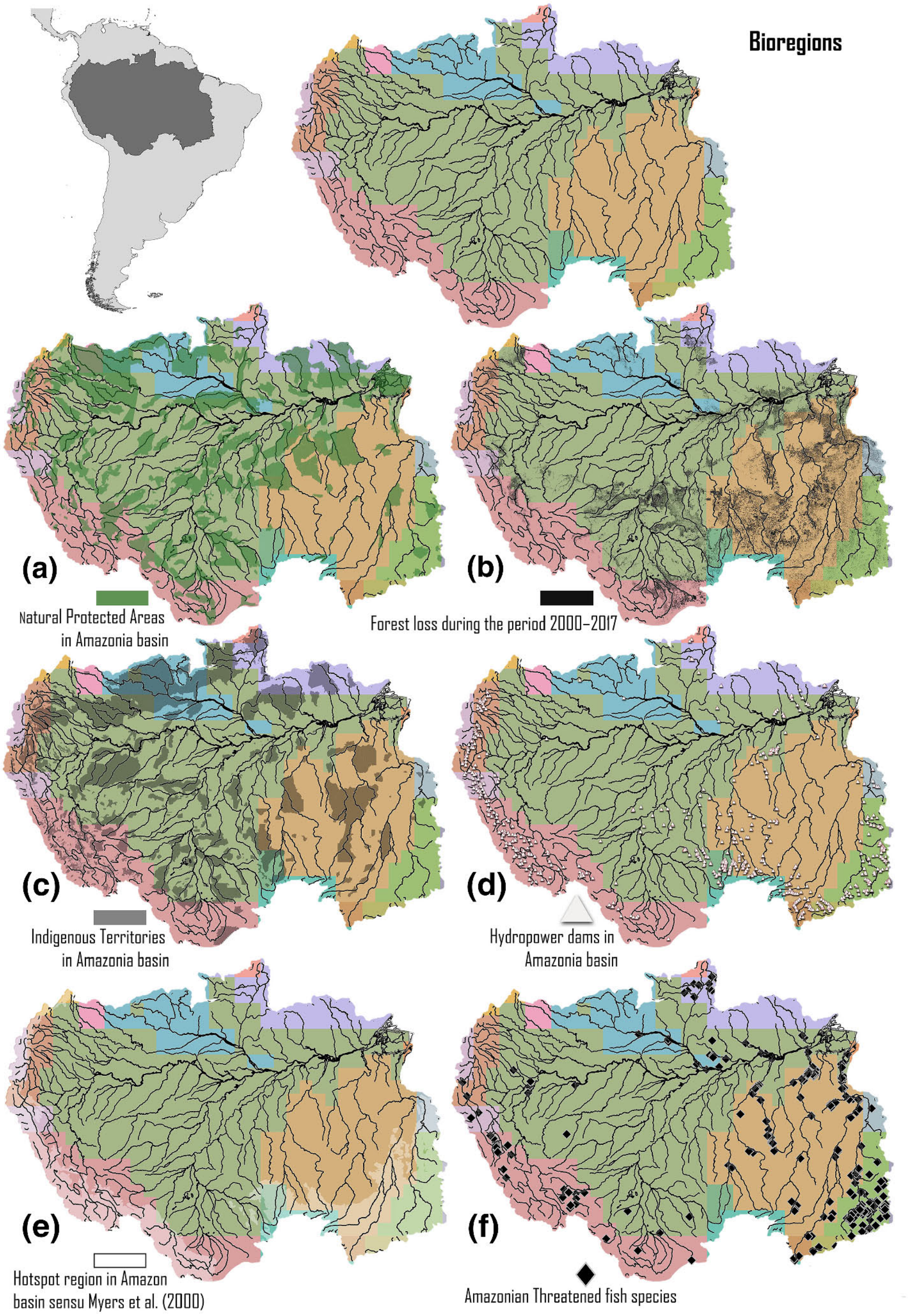


FIGURE 2 Bioregions of the Amazonian fish fauna. The two largest bioregions are the Amazonian lowlands (green) and the Brazilian Shield (pale orange), respectively. Most of the protected areas and Indigenous territories are located within the Amazonian lowlands. The fish fauna in the Brazilian Shield, including the Upper Tocantins (see Figure 1) contains numerous threatened species and has been strongly affected by forest loss and hydroelectric dams. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; (d) hydropower dams (including planned and under construction); (e) hotspot region, *sensu* Myers et al. (2000); localities of threatened fish species, *sensu* ICMBio (2018) and IUCN (2019); and (f) localities of threatened fish species, *sensu* ICMBio (2018) and IUCN (2019)

3.1.2 | Central Blackwater Amazon

This bioregion comprises the Rio Negro basin and the Upper Orinoco and several of its right-margin tributaries, and includes acidic blackwater rivers draining the central region of the Amazon superbasin. Although water acidity limits the distribution of several lineages typical of whitewaters, this region is extremely rich in a vast number of endemics belonging to many different lineages, mainly in the families Characidae and Loricariidae (e.g. *Aulixidens eugeniae*, *Bryconamericus singularis*, *Bryconops humeralis*, *Acestridium dichromum*, *Acestridium martini*, *Hemiancistrus subviridis*, *Hypostomus rhantos*, *Pseudolithoxus anthrax*, *Pseudolithoxus dumus* and *Laetacara fulvipinnis*).

3.1.3 | Western Amazon Piedmont

This province analogously corresponds to the ecoregion bearing the same name in Abell et al. (2008). It is located along the eastern slopes of the Andean Cordilleras, a montane region consisting of torrential watercourses forming the Rio Japurá, Rio Putumayo, Rio Napo and Rio Marañón. This bioregion is rich in rheophilic lineages, including several representatives of the genera *Astroblepus*, *Chaetostoma*, *Panaqolus*, *Bryconamericus*, *Creagrutus*, *Hemibrycon* and *Knodus*, among others. It is also considered one of the global biodiversity hotspots, *sensu* Myers et al. (2000) (Figure 2e).

3.1.4 | Andean Foothills

This bioregion is placed within the Andean foothills drained by the Rio Ucayali, with its eastern border drained by the headwaters of the Rio Juruá, Rio Purus and Rio Madeira. The relatively young age of the Andes results in heavily silted whitewaters and torrential water-courses inhabited by many rheophilic species adapted to fast-flowing and highly oxygenated waters. This bioregion resembles the region bearing a similar name in Dagosta and de Pinna (2019), and is part of the 'Amazonas High Andes' ecoregion proposed by Abell et al. (2008). This bioregion is considered one of the world's biodiversity hotspots, *sensu* Myers et al. (2000) (Figure 2e).

3.1.5 | Guianan Shield

This bioregion is drained by rivers flowing from the Guianan Shield, including part of the Orinoco, several Atlantic Guianese systems and the headwaters of the Rio Trombetas, Rio Paru, Rio Jari and part of the Rio Branco. Rivers in this bioregion are mostly clearwater, carrying low sediment and nutrient loads and exhibiting numerous rapids and/or waterfalls. The bioregion contains several endemic species (e.g. *Caenotropus maculosus*, *Hemigrammus boesemani*, *Petulanos plicatus*, *Prochilodus rubrotaeniatus*, *Doras carinatus*, *Hypostomus hemiurus*, *Parotocinclus britskii*, *Apistogramma ortmanni* and *Guianacara dacrya*), and many rheophilic species. This province resembles a region bearing a similar name in Dagosta and de Pinna (2019), except for some tributaries of the Orinoco system located in the Guianan Shield.

3.1.6 | Brazilian Shield

This is the second largest bioregion within the Amazon superbasin, and spans the uplands of the ancient crystalline basement of the Brazilian Shield. This bioregion is drained by clearwater rivers with low sediment and nutrient loads, and often containing rapids and/or waterfalls. The bioregion contains many independently evolving species (e.g. *Jupiaba apenima*, *Melanocharacidium auroradiatum*, *Hemiodus vorderwinkleri*, *Hyphessobrycon vilmae*, *Hyphessobrycon pinnistriatus*, *Hypomasticus julii*, *Aspidoras microgalaeus*, *Hopliancistrus tricornis*, *Pterygoplichthys joselimaianus* and *Cichla melaniae*). This bioregion resembles a region bearing a similar name in Dagosta and de Pinna (2019). Part of its geographical range (e.g. headwaters of the Rio Juruena, Rio Teles Pires, Rio Xingu and Upper-middle Rio Araguaia) is considered one of the world's biodiversity hotspots, *sensu* Myers et al. (2000) (Figure 2e).

3.1.7 | Chapada dos Parecis

This bioregion is placed within an elevated geomorphological formation located in the western portions of the Brazilian Shield, including the headwaters of the Rio Machado, Rio Guaporé, Rio Paraguay and most of the Rio Juruena. This bioregion is characterized by (i) high levels of endemicity, especially within the Characidae and Loricariidae, (ii) rare cases of closely related coexisting lineages, (iii) low species diversity and (iv) rare occurrences of broadly distributed species. It resembles a region bearing a similar name in Dagosta and de Pinna (2019). This bioregion straddles the boundary of the Cerrado hotspot, and is considered one of the world's biodiversity hotspots, *sensu* Myers et al. (2000) (Figure 2e).

3.1.8 | Upper Tocantins

This bioregion ranges from the upstream region of the Rio Vermelho mouth up to the headwaters of the Rio Tocantins along the watershed divide in the Upper Paraná in the Central Brazilian Plateau. To the north east, its geography includes part of the Rio Parnaíba in the Nascentes do Rio Parnaíba National Park. The rivers in this bioregion are mostly clearwater, with low sediment and nutrient loads and several endemics (e.g. *Astyanax courensis*, *A. unitaeniatus*, *Creagrutus britskii*, *C. saxatilis*, *Hemigrammus ataktos*, *H. tocantinsi*, *Hyphessobrycon balbus*, *H. stegemanni*, *Moenkhausia tergimacula*, *Ancistrus cryptophthalmus*, *Aspidoras albater* and *Xyliphius anachoretes*). This bioregion is located in the Cerrado hotspot periphery, and is considered one of the world's biodiversity hotspots, *sensu* Myers et al. (2000) (Figure 2e).

3.1.9 | Upper Araguaia

This is one of the smallest bioregions and comprises the region upstream from Barra do Garças up to the headwaters of the Rio Araguaia and Paraguay (Correntes and Taquari drainages). The Upper Rio Araguaia drainage contains clearwaters, with high species richness and endemicity (e.g. *Astyanax joaovitori*, *Cyphocharax boiadeiro*, *Hyphessobrycon eilyos*, *H. langeanii*, *H. weitzmanorum*, *Aspidoras velites*, *A. taurus*, *Cnesterodon septentrionalis*, *Melanorivulus litteratus* and *Simpsonichthys cholopteryx*). This bioregion is located in the Cerrado hotspot perimeter, and is considered one of the world's biodiversity hotspots, *sensu* Myers et al. (2000) (Figure 2e).

3.1.10 | Tepui

Like the Upper Araguaia bioregion, this is one of the smallest bioregions, spanning the areas of the Rio Caroni (Orinoco basin), Rio Cuyuni and Rio Mazaruni (Essequibo basin), and Rio Branco. Its rivers are sediment and nutrient poor and include several rapids and/or waterfalls draining blocks of precambrian quartz arenite sandstone. This bioregion includes many rheophilic endemic species (e.g. *Apareiodon agmatos*, *Brycon coquenani*, *Derhamia hoffmannorum*, *Lebiasina unitaeniata*, *Lebiasina yuruaniensis*, *Neblinichthys brevibracchium*, *N. echinasus*, *N. roraima*, *Paulasquama callis*, *Akawaio penak* and *Laimosemion torrenticola*).

3.2 | Taxonomic tree of Amazonian fishes

To correlate the different evolutionary lineages with the proposed bioregions the consensus taxonomic tree of Amazonian fishes was associated with those areas. The Amazonas lowlands bioregion is represented at all positions in the tree (which is evident from the predominantly green tree branches in Figure 3). Only a few small lineages are restricted to a single biogeographical region. Individual lineages mostly occur across several bioregions, again demonstrating the reticulating and complex biogeographical history of the Amazon basin.

3.3 | Species richness interpolation

Northern South America contains the highest diversity of freshwater fish on any continent. The area indicated by the warmer colours in Figure 4 corresponds to the Amazon–Orinoco–Guyana region (AOG), which is covered by lowland wetlands and upland rainforest both of which are drained by many major rivers, including the Amazon itself (Reis et al., 2016; van der Sleen & Albert, 2018). The inclusion of parts of the Trans-Andean region in the warmest area is attributed to the extrapolation of the spline interpolator. Diversity decreases at family, genus and species levels when moving away from either the Central Amazonian region or the Orinoco main channel. Figures 4 and 5 further show that species richness decreases at higher elevations, a macroecological pattern that is frequently observed (cf. Albert, Petry, & Reis, 2011; Dagosta & de Pinna, 2017, 2019; Lima & Ribeiro, 2011). This pattern is most evident in the Andean range, but also observed in the Guianan and Brazilian Shields.

As expected, the Patagonian and north-eastern Brazilian regions were estimated to be markedly species poor. More surprising, however, is the relatively low diversity observed in the Lower Paraná basin. Species richness can be influenced by systematic sampling bias, so that intensively sampled areas near large urban centres in South America such as Rio de Janeiro and São Paulo usually record a large number of species. The apparently low diversity of the Lower Paraná, Middle São Francisco, Middle Paraguay and Middle Tocantins regions can be attributed, at least in part, to the limited number of records in

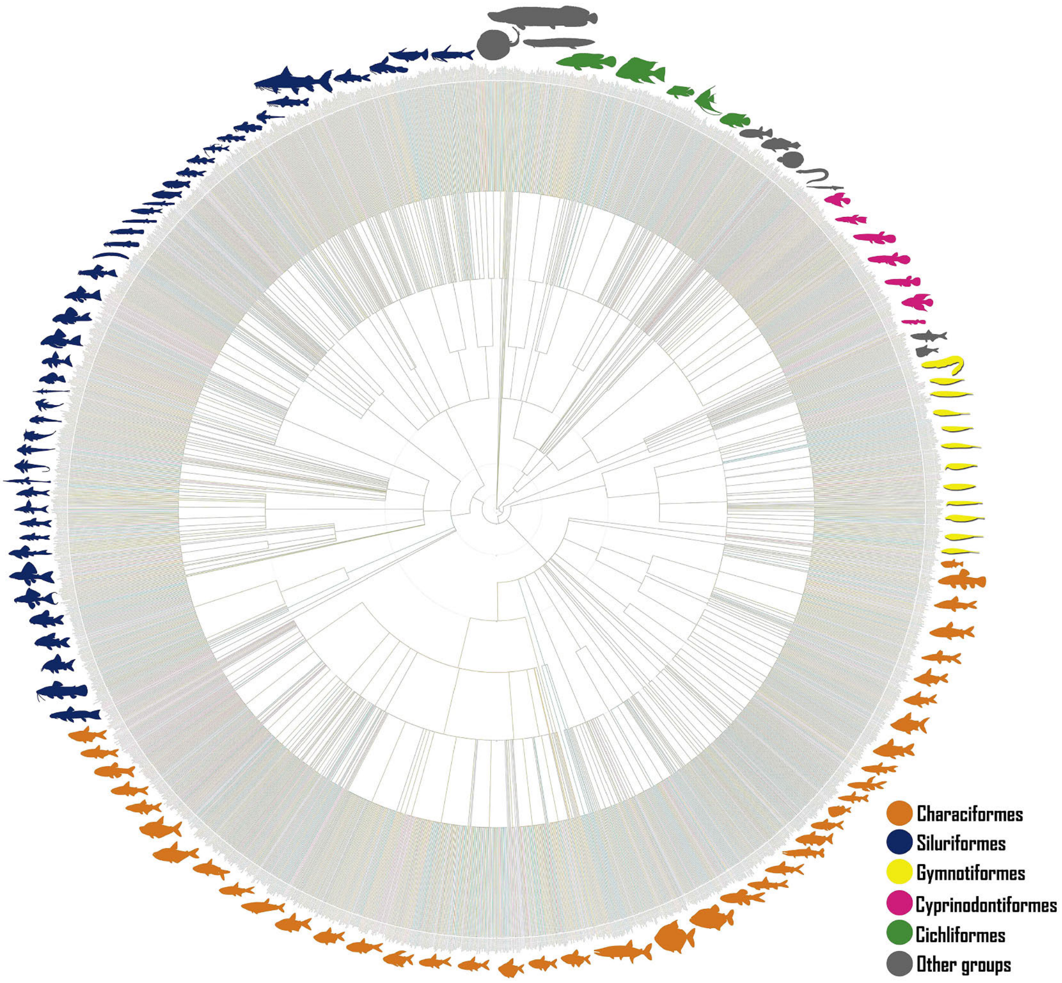


FIGURE 3 Species-level taxonomic tree of 2,726 species of Amazonian fishes built using the current classification of van der Laan et al. (2019) and Nelson et al. (2016). Branch colours match the bioregions defined in Figures 1 and 2

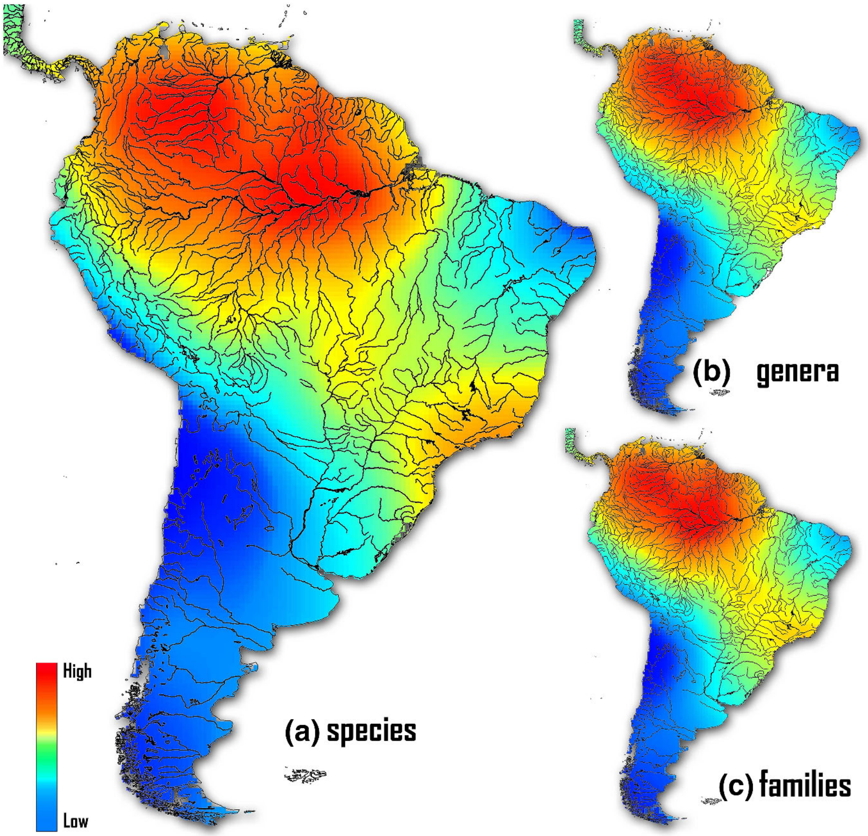


FIGURE 4 The richness of South American fish lineages estimated by spline interpolation: (a) species; (b) genera; and (c) families. Warmer colours indicate regions of higher species diversity, while colder colours indicate regions of lower species diversity

the surveyed databases. Despite potential sampling biases, we can clearly claim that the AOG is the most species-rich region in South America.

Fish species richness patterns documented here are almost identical to those in other groups of Neotropical vertebrates (cf. Jenkins, Pimm, & Joppa, 2013; Teixeira, 2017). All tetrapod taxa have their largest diversity, often at a global scale, in the AOG region. Regions such as north-eastern and southern Brazil are species poor both for fish and for terrestrial vertebrates. The region corresponding to the Atlantic Forest is highly diverse, although to a lesser extent than the AOG. South American fishes and tetrapod taxa are consistent in showing that the most species-rich areas are incongruent with those of restricted-range species richness. The most important difference between the patterns of diversity in South American fishes and terrestrial vertebrates is that the former are most diverse along the main channels of the Rio Amazonas and Rio Orinoco, whereas the latter show their highest diversity in the Amazonian region near the Andes (Jenkins et al., 2013; Jetz, Thomas, Joy, Hartmann, & Mooers, 2012; Peres & Janson, 1999; Silva et al., 2019).

3.4 | Spatial patterns of endemicity

Five approaches were used to identify geographical areas with narrowly-distributed species assemblages (see further details in Methods): (i) distributional overlap of Amazonian fish endemics, *sensu* Dagosta and de Pinna (2019); (ii) distributional overlap of endemics with smaller distribution polygons; (iii) endemicity estimates by NDM; (iv) endemicity estimates by GIE; and (v) endemicity estimates by WEI.

3.4.1 | Distributional overlap of Amazonian endemic fish species *sensu* Dagosta and de Pinna (2019)

The largest concentration of narrowly distributed species is located in the Brazilian Shield and eastern Amazon, especially in the Upper Tocantins, Lower Tapajós–Xingu and Upper Tapajós (Figure 6). We further point out the high concentration of endemic species in the Beni-Madre de Dios and the Rio Negro basins. The results highlight the fast rate of forest loss in the Lower Tapajós–Xingu region and the large number of hydroelectric dams that have either been built or planned for the Upper Tocantins (Lees, Peres, Fearnside, Schneider, & Zuanon, 2016), a region containing the highest concentration of endemic fishes across the entire Amazon basin.

3.4.2 | Distributional overlap of endemic fish species with smaller distribution polygons

The results are similar to those described above. The main difference is the greater importance of the Rio Negro basin as an aquatic biodiversity hotspot, including many endemic species,

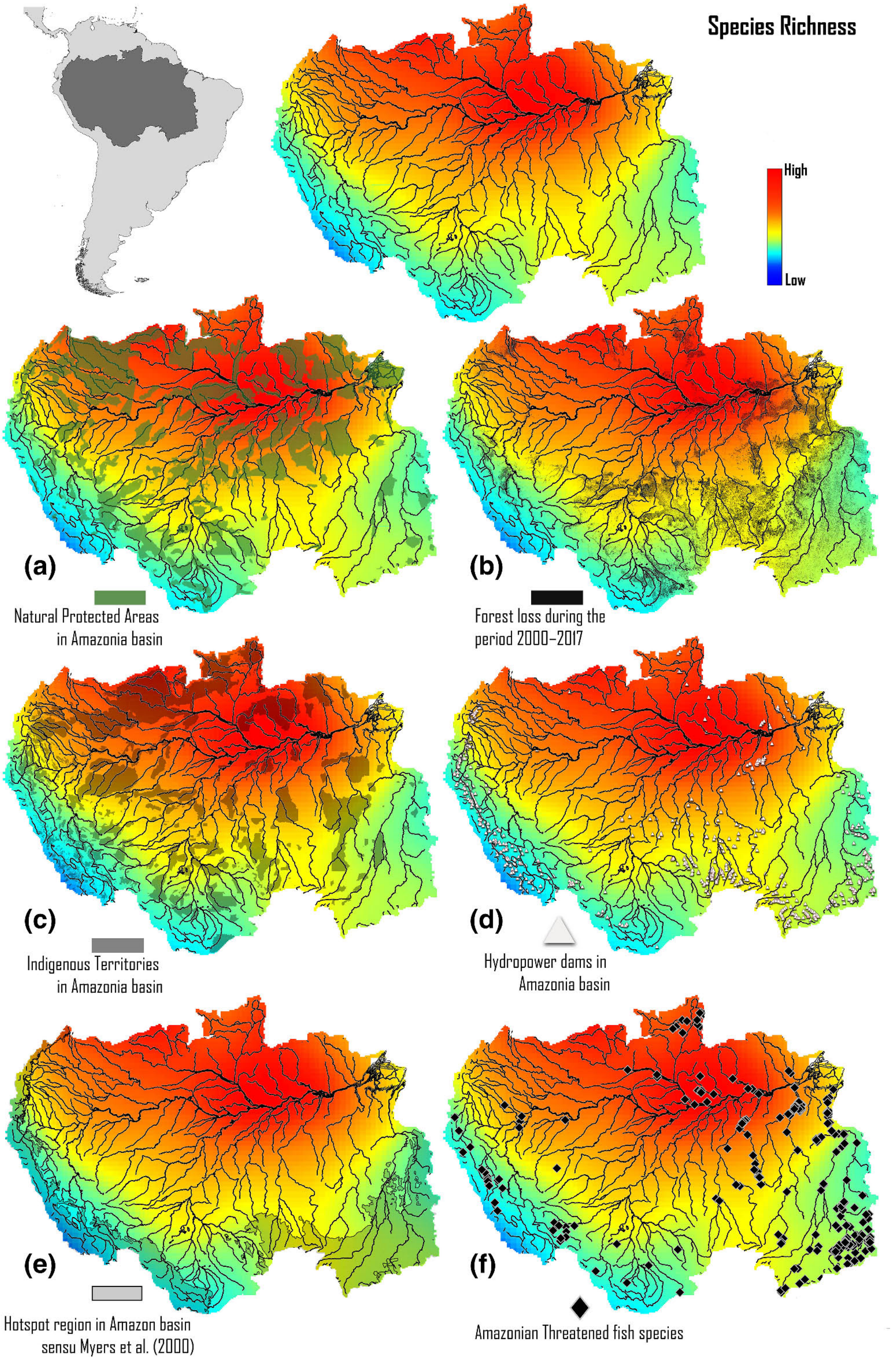


FIGURE 5 Fish species richness in the Amazon superbasin estimated by spline interpolation based on Figure 4a. National Protected Areas and Indigenous territories encompass most regions of elevated species richness in the Amazonian lowlands. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; (d) hydropower dams (including planned and under construction); (e) hotspot region, *sensu* Myers et al. (2000); and (f) localities of threatened fish species, *sensu* ICMBio (2018) and IUCN (2019)

together with the Lower Tapajós–Xingu region (Figure 7). Another difference is the headwater regions of the Rio Japurá, Rio Putumayo, Rio Napo and Rio Marañón, an area including high concentrations of geographically restricted species. One reason for this observed pattern is that the spatial ranges are considered to be independent from the basin profiles. The headwaters of the Western Amazon Piedmont are all in proximity to one another, thereby often undergoing dispersal across neighbouring stream capture events, resulting in contiguous distributions between adjacent drainages. As the previous analysis considers as input only those species restricted to a single sub-drainage, those species occurring across the region were not incorporated, explaining why the region had not been previously detected as a potential area of high concentration of endemic species.

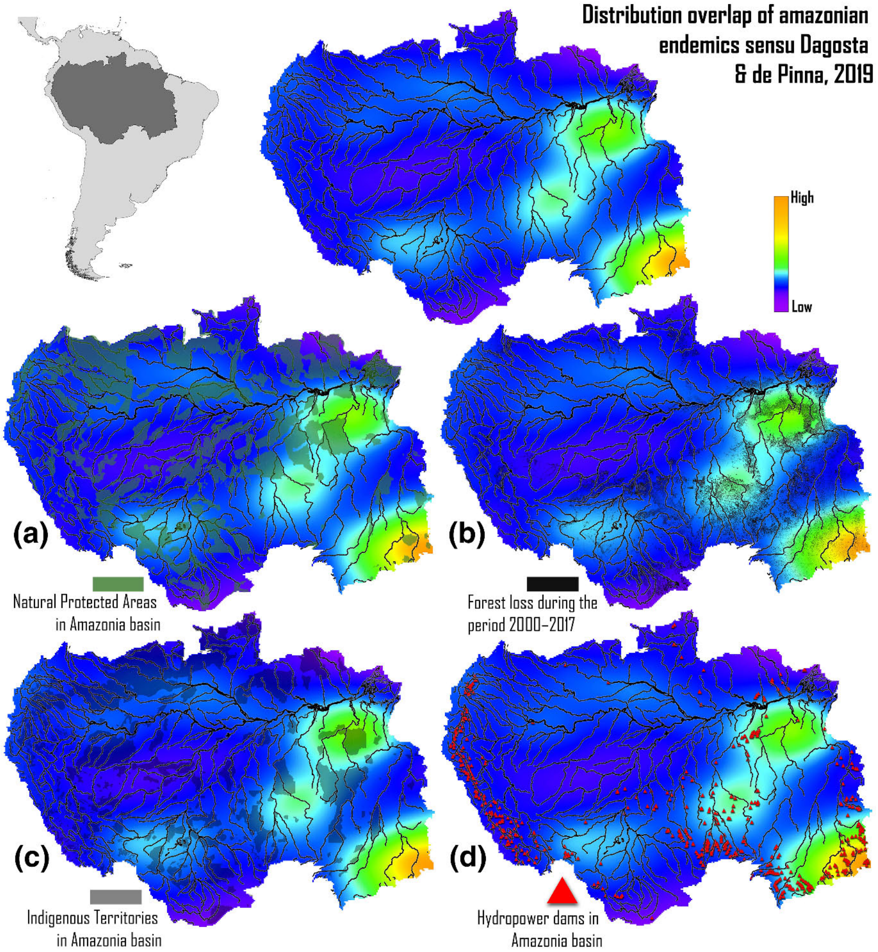


FIGURE 6 Species richness map of restricted-range fish species (i.e. distribution overlap of Amazonian endemics, *sensu* Dagosta & de Pinna, 2019). Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; and (d) hydropower dams (including planned and under construction)

3.4.3 | EnDemisM (NDM)

The NDM analysis identified 58 regions of geographically restricted species distributions (Figure 8), but this number was then reduced to 21 regions by flexible consensus. Consensus areas are similar to those above, except for the inclusion of two small regions: the Rio Roosevelt and the Upper Rio Tocantins, both of which are defined as retaining high concentrations of endemic species.

3.4.4 | Geographic Interpolation of Endemism (GIE)

The result of the GIE analysis is qualitatively similar to those presented above (Figure 9). The Lower Xingu, Lower Tapajós and the Western Amazon Piedmont are the regions with the greatest concentration of endemics.

3.4.5 | Weight Endemism Index (WEI)

The WEI geography diverges from the patterns presented so far (Figure 10) because this method considers not only the classic definition of endemic species (i.e. taxa with restricted occurrence), but also their range-size rarity (i.e. a measure of endemicity). Therefore, regions with many exclusive species such as the Andean Cordilleras and the Brazilian Shield yield higher WEI values. The analysis corroborates the Amazonian Uplands as areas of a high concentration of endemicity, a pattern even more clearly evident than that derived from other methods. The overall WEI map shows that endemic fishes are concentrated in highland areas, especially along the boundaries of neighbouring basins. Conversely, lowland regions are dark blue (low concentration of endemics) in the shape of a triangle within the Western Amazon, matching the Amazon-only Lowland as described by Dagosta and de Pinna (2019).

3.5 | Phylogenetic diversity (PD)

The PD results show that the highest diversity of fish lineages is located in the central region of the Amazon, precisely at the fluvial intersection of the three major water geochemistry types across the basin: white, clear and black (Figure 11). Another factor contributing to the higher estimates of lineage richness in the region is perhaps its historical biogeography. This region is located in the ancient

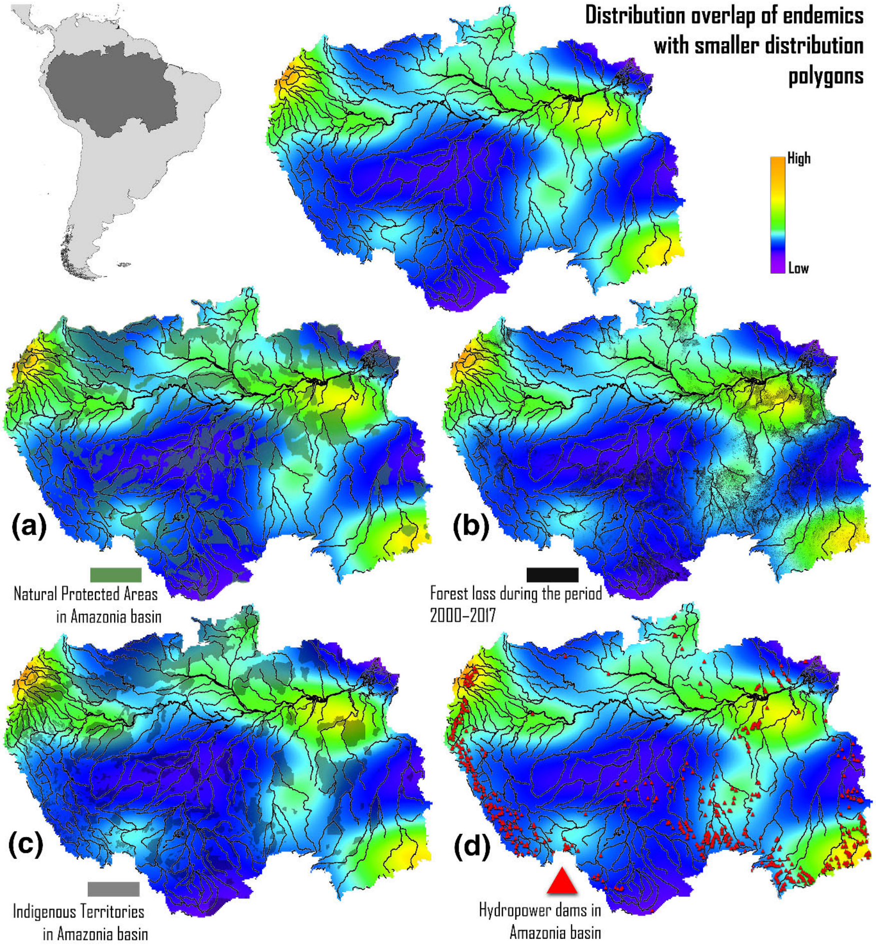


FIGURE 7 Species richness interpolated map of restricted-range fish species (i.e. distribution overlap of Amazonian endemics with smaller distribution polygons). Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; and (d) hydropower dams (including planned and under construction)

East–West Amazon divide, the Purus Arch, and therefore comprises lineages historically related to both sides of this ancient Amazonian divide. It also includes portions draining lowlands and highlands, with distinct historical and ecological fish assemblages derived from both the lowlands and the ancient shields. An example is the region of Itaituba in the Lower Tapajós, where typical lowland species such as *Brachyplatystoma* and *Colossoma* coexist with shield lineages like *Ancistomus*, *Sartor* and *Leporacanthicus*. The evolutionary significance of the Central Amazon is further evidenced by the fact that it spans 18 of the 20 biogeographical patterns identified by Dagosta and de Pinna (2019). Naturally, the region is also one of the most heavily sampled in the entire Amazon, which may incur some sampling artefacts. However, this historical collecting bias is only a matter of detail and the evidence remains sufficiently strong to consider the Central Amazon as the phylogenetically richest region of the superbasin.

3.6 | Phylogenetic endemism (PE)

The PE results are similar to those above, except for the presence of the Andean foothills, especially the headwaters of the Rio Ucayali (Figure 12). PE searches not only for geographical concentrations of phylogenetic diversity, but also spatially restricted phylogenetic nodes (Rosauer & Jetz, 2014). As such, the Andean foothills are highly significant here because of the occurrence of lineages that are both exclusive and narrowly distributed, such as Astroblepidae, *Attonitus*, *Astyanacinus*, many Hoplomyzontines and *Orestias*.

3.7 | Sampling effort

The most heavily sampled areas across the entire Amazon are those near the urban centre of Manaus (Figures 13 and 14), the Amazon's largest city, owing to its long history of ichthyofaunal inventories. Other well-sampled regions are associated with the main channels of large rivers or near other large cities or research institutions. Lowlands are relatively well sampled, especially the main channels of the Rio Amazonas and Rio Madeira. Sampling gaps are mostly located in protected areas, including formally designated Indigenous territories. This situation poses a perplexing problem because part of the fish fauna is certainly protected (*de jure* if not *de facto*) within forest reserves, but its overall composition remains unknown. Additional sampling effort in those regions becomes necessary so that the full composition of the protected

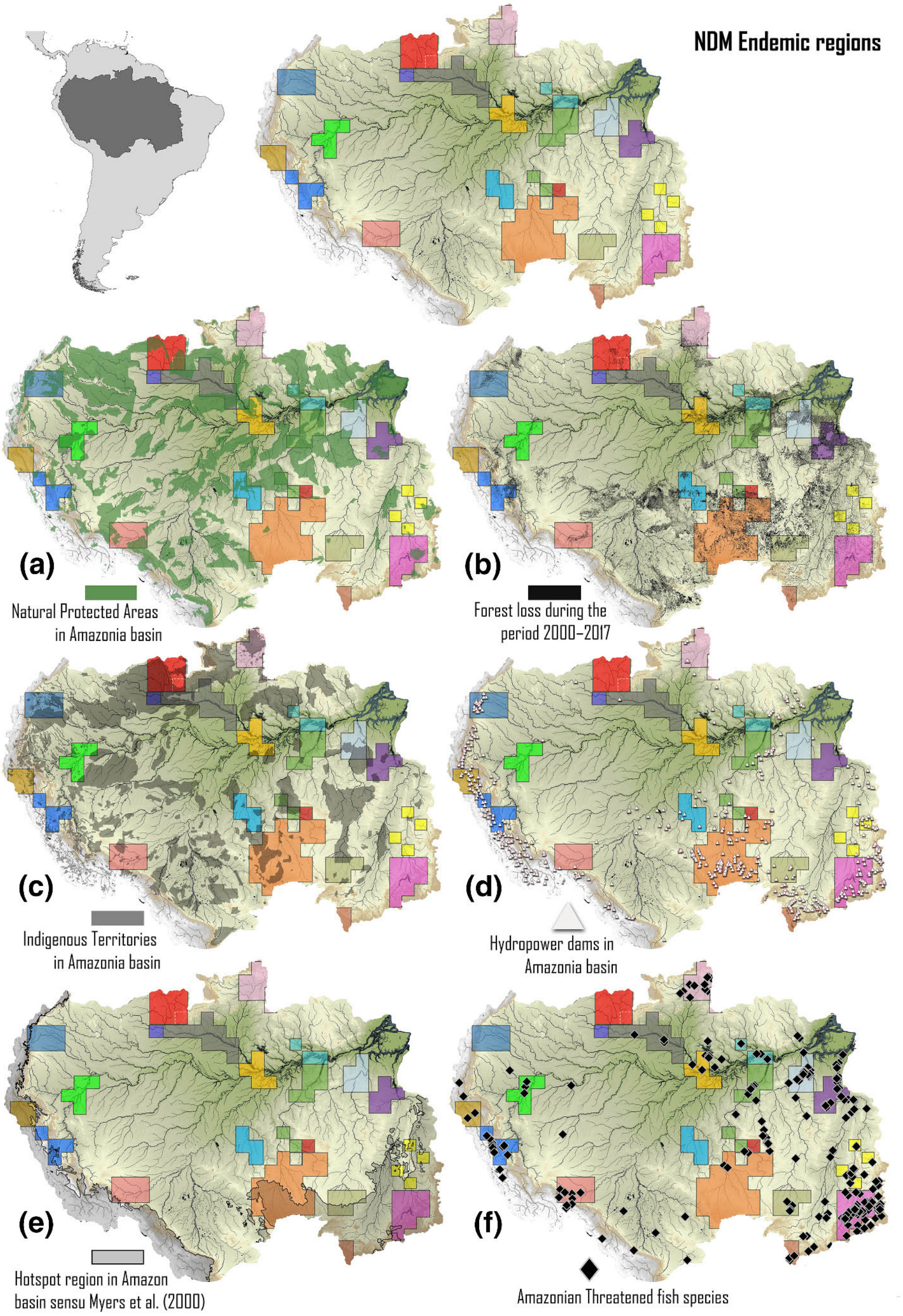


FIGURE 8 Endemism analysis map. Coloured blocks represent 21 areas resulting from the flexible consensus (sharing at least 1% of their endemic species). Overlapping layers: (a) Natural Protected Areas; (b) forest loss during the period 2000–2017; (c) Indigenous territories; (d) hydropower dams (including planned and under construction); (e) hotspot region, *sensu* Myers et al. (2000); and (f) localities of threatened fish species, *sensu* ICMBio (2018) and IUCN (2019)

fish fauna can be documented, thereby ensuring a better understanding of the unprotected complement.

Eight main regions recognized here have rarely been sampled (Figure 14) and therefore require immediate attention in future inventories. The Calha Norte region of northern Pará is particularly noteworthy and remains virtually unknown to ichthyologists, partly because fluvial access to its headwater regions is hindered by many rapids and waterfalls. Perhaps the most alarming result is that some Amazonian regions that have been barely sampled are being rapidly deforested into other land uses, such as the Xingu–Araguaia, Roosevelt–Machado and Upper Mamoré regions (Figure 14).

4 | DISCUSSION

Simple measurements are clearly insufficient to convey complex geographical patterns of biodiversity (Vane-Wright et al., 1991; Whittaker, 1972). Mapping Amazonian fish diversity thus requires different complementary methods. The highly consistent set of results uncovered here using different methods is remarkable and demonstrates that some patterns of diversification are expressed at multiple layers of the diversity spectrum.

The first approach used here to understand the distribution of the Amazonian fish fauna relied on the segmentation of

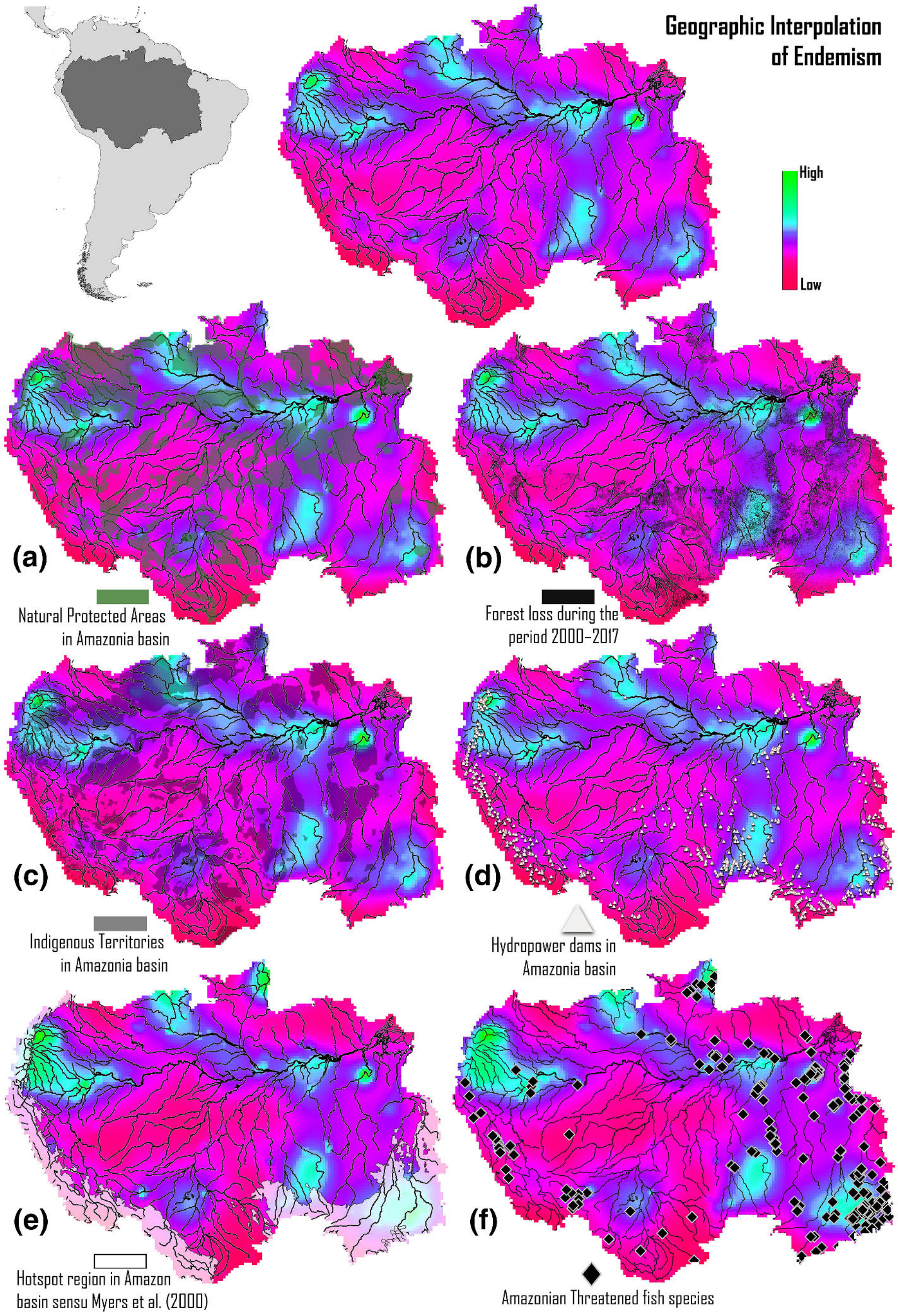


FIGURE 9 Geographic Interpolation of Endemism map. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; (d) hydropower dams (including planned and under construction); (e) hotspot region, *sensu* Myers et al. (2000); and (f) localities of threatened fish species, *sensu* ICMBio (2018) and IUCN (2019)

biogeographical regions (=bioregions). Regions delimited in these terms comprise geographically distinct assemblages of species and communities based on the spatial distribution of their taxa. Those units constitute a cornerstone of ecology, biogeography, evolution and conservation biology (Vilhena & Antonelli, 2015). They can be used as operational units in analyses attempting to understand the biogeographical processes responsible for biotic diversification (e.g. dispersal, vicariance, speciation and extinction) and ancestral-range reconstructions. They allow estimations of how the geographical ranges of lineages in a phylogeny have evolved over time (Goldberg, Lancaster, & Ree, 2011; Matzke, 2014; Silvestro, Schnitzler, & Zizka, 2011; Vilhena & Antonelli, 2015). In addition, bioregions have important advantages in conservation biology compared with strategies focused on single taxa (Vilhena & Antonelli, 2015). Conservation efforts are better directed towards protecting large portions of different bioregions rather than focusing on a particular lineage.

Some of the bioregions identified (Figures 1 and 2) are analogous to some of those in previous studies. For example, the Upper Paraná, Guianas and Northeast areas in Vari (1988) provide a nearly identical match to the patterns found here, but many differences also exist. Günther (1880) and Eigenmann (1909) proposed a broad regionalization of South America, in line with the inevitably very limited knowledge on distributions of aquatic organisms at that time. The more recent proposals of Géry (1969) and Vari (1988) are more complex, based on comparatively more accurate knowledge about the South American fish fauna, and their delimitations transcend the physical

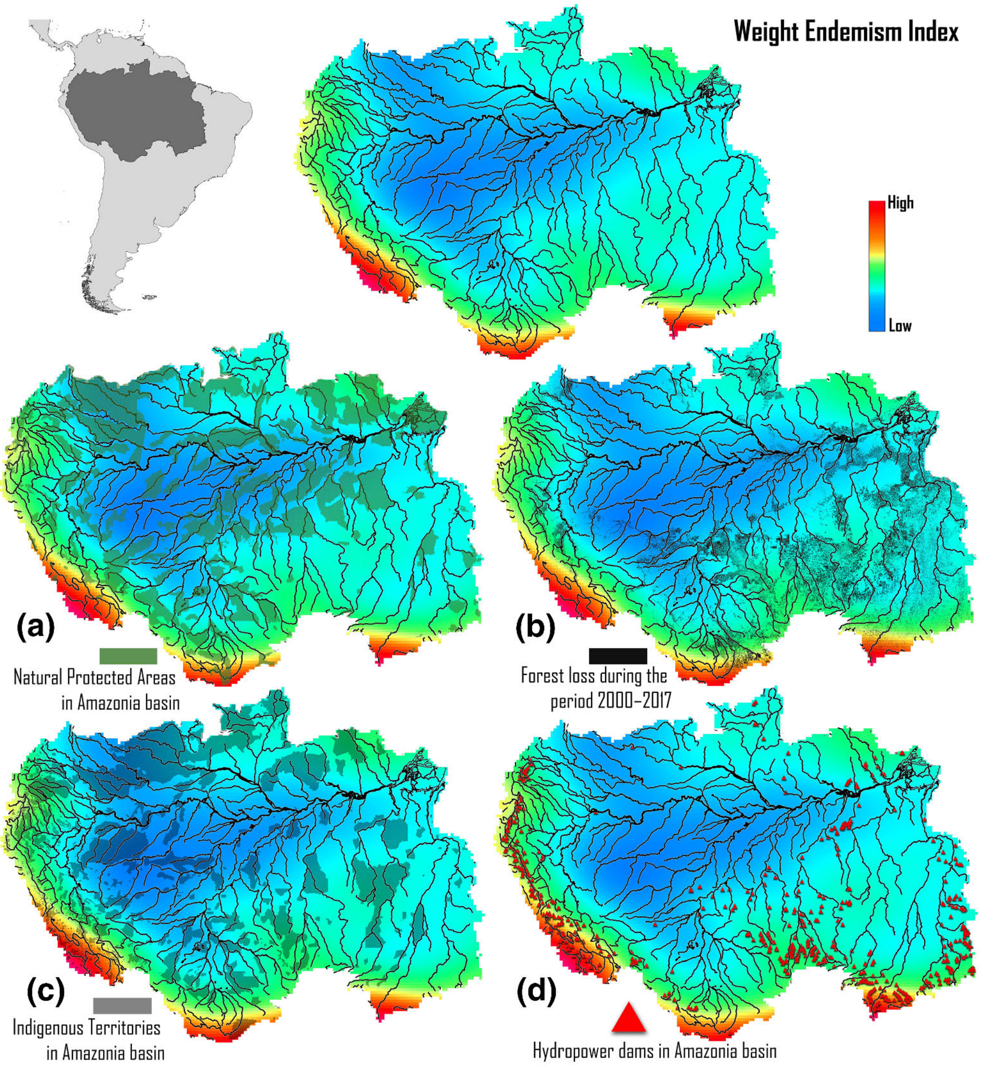


FIGURE 10 Weight endemism index map. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; and (d) hydropower dams (including planned and under construction)

limits of hydrographic basins. The proposal of Abell et al. (2008) is considerably more intricate but mostly comprises regions delimited by basin profiles, an assumption that is not supported by our analyses. In fact, present-day drainage morphologies have recently been shown to relate poorly to the history of their respective taxa (see Dagosta & de Pinna, 2017).

Regions that concentrate spatial congruence of species are often referred to in the literature as areas of endemism (Carine, Humphries, Guma, Reyes-Betancort, & Santos Guerra, 2009; Cracraft, 1985; Haffer, 1985; Linder, 2001; Morrone, 1994; Platnick, 1991). These are hypotheses that can be refuted by new distributional data or by records of additional taxa. The manner in which such areas are delimited herein differs from some previous studies in being agnostic about the reasons for the origin and distributions of their taxa (i.e. spatial congruence does not necessarily imply a single biogeographical history, neither in temporal nor in diversification processes). Such an approach stems from evidence that congruent distributions of freshwater fishes are a consequence of different historical connectivity of watercourses through time (Dagosta & de Pinna, 2017, 2019). Therefore, we used the term Endemic Amazonian Fish Area, defined as a region with a large degree of overlap in distributions of restricted-range species, without assuming historical association by default.

Beyond the incontrovertible existence of such areas in a general sense, uncertainty remains on how best to identify and delimit these regions. The five different analyses conducted here resulted in marked levels of congruence in EAFA delimitations (Figure 15), despite their divergent methodologies and databases. Only a few sectors were not present in all five analyses, and most of those were represented in three or four of them. This is a clear demonstration that the singularity of EAFAs found here is supported by strong empirical evidence.

4.1 | Mismatches in bioregionalization of Amazonian fish and other taxa

The bioregions of freshwater fishes in South America, particularly in Amazonia, are not congruent with those delimited for terrestrial organisms (cf. Cabrera & Yepes, 1940; Cracraft, 1985; Edler et al., 2017; Fittkau, 1969; Kuschel, 1969; Olson et al., 2001).

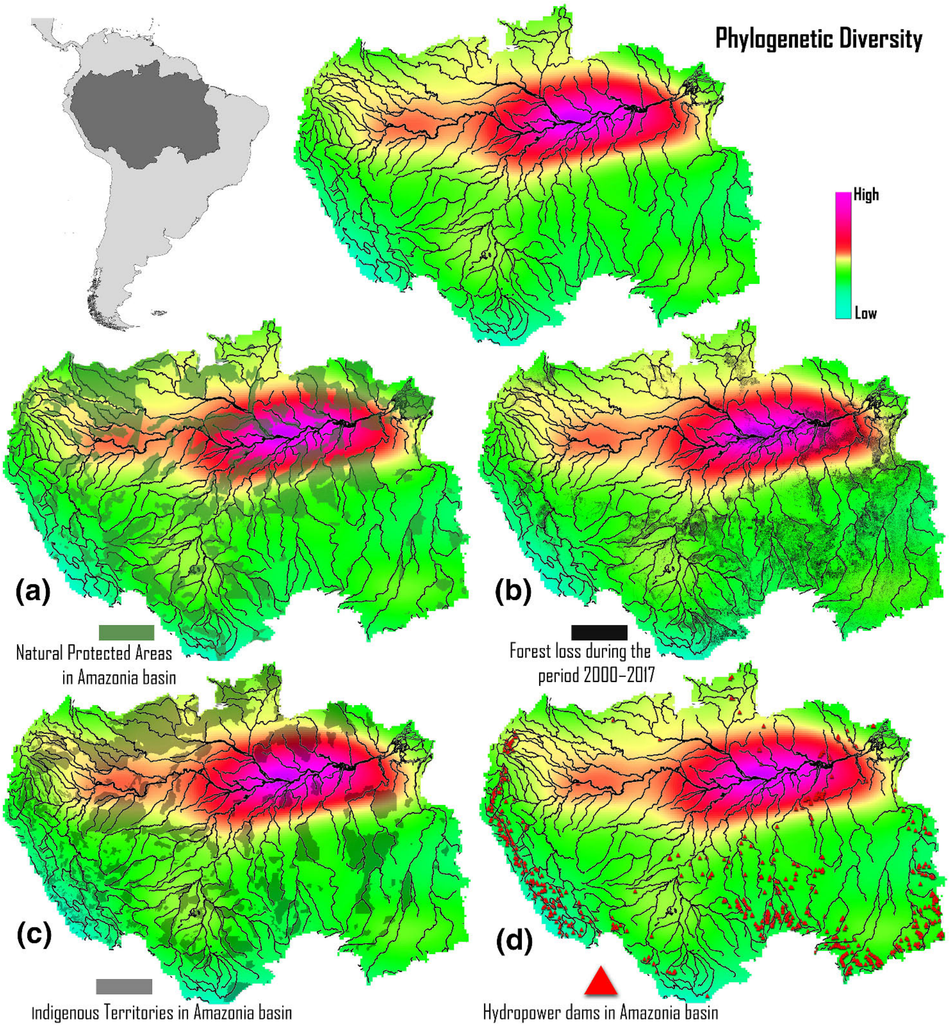


FIGURE 11 Phylogenetic diversity map. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; and (d) hydropower dams (including planned and under construction)

Although the biogeographical history of aquatic and terrestrial environments may be causally and temporally connected, the patterns found here indicate that processes responsible for the diversification of aquatic organisms are predominantly distinct from those acting on terrestrial biotas. As pointed out by Dagosta and de Pinna (2019), barriers for terrestrial animals are corridors for fishes and vice versa, except in special cases. Of all Amazonian regionalization maps proposed to date, Morrone (2014) is the most complete and the only one bearing some resemblance to what we propose here.

4.2 | Existing protected areas do not safeguard the Amazonian fish fauna

Although regions of remarkable diversity of Amazonian fishes are relatively well represented within existing protected areas (PAs) (Figures 5a,c and 11a,c), results from the bioregionalization map reveal a disastrous mismatch for Amazonian fishes: existing Amazonian PAs clearly fail to represent several biogeographical regions. The vast majority of PAs are concentrated in a single region (Amazon lowlands; Figure 2a), which does not contain most endemic and/or endangered species (Figures 2f and 15a). These species ranges often coincide with steeper gradients of fluvial discharge and largely overlap priority areas earmarked for hydropower infrastructure, and are therefore expected to eventually experience massive deforestation pressure (Figure 15b, d). For example, the Upper Tocantins bioregion combines several aggravating factors: (i) dozens of endemic and threatened species; (ii) minimal representation within Natural Protected Areas; and (iii) dozens of hydroelectric dams that are either planned or under construction. The situation is even worse in the Upper Araguaia bioregion, which is heavily populated with planned hydropower dams and lacks even a single PA (cf. Latrubesse et al., 2019). The Chapada dos Parecis bioregion is also severely threatened with numerous planned (and already built) dams in the headwaters of the Rio Juruena and the absence of any PA; although there are some Indigenous territories, they have already been heavily deforested. Clearly, the Brazilian Shield is both the bioregion most affected by deforestation and the least represented by PAs.

Our analysis clearly demonstrates that a reconfiguration of Amazonian PAs is necessary to account for under-protected bioregions of the aquatic biota, a conclusion consistent with spatial modelling by Frederico, Zuanon, and de Marco (2018). This is a key conservation planning imperative to be implemented if the loss of a large portion of unique elements of the Amazonian fish fauna is to be prevented.

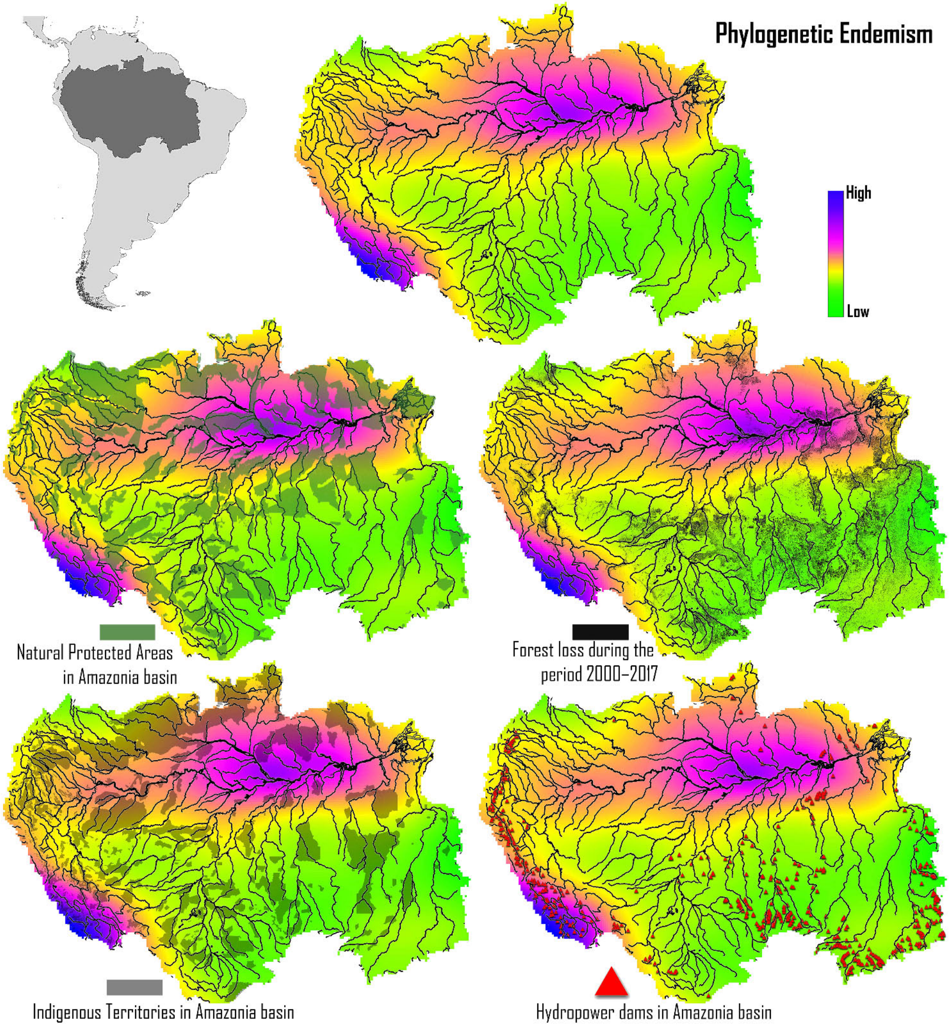


FIGURE 12 Phylogenetic endemism map. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; and (d) hydropower dams (including planned and under construction)

The present bioregional delimitation of the Amazon basin remains spatially too coarse for conservation planning. Fine-scale identification of hotspots would enable the delimitation of the largest concentrations of restricted-range species. All other factors being equal, species occupying smaller geographical ranges are assumed to be exposed to higher extinction risk (Lee & Jetz, 2011; Runge, Tulloch, Hammill, Possingham, & Fuller, 2015; Tagliacollo, Camelier, Zanata, & Reis, 2019).

Deficits in the knowledge of both taxonomic and spatial distribution (Linnean and Wallacean shortfalls) for Amazonian fishes have historically resulted in their negligible contribution to PA design and allocation, which are by default primarily based on other elements of the biota. The bulk of Amazonian fish diversity, both at the species level and above, is already included within existing PAs, because of the incidental overlap in patterns of diversity between aquatic and terrestrial vertebrates (Figure 5a). However, such an apparently positive outlook is misleading. Species richness and phylogenetic diversity indices, taken in isolation, are insufficient to support conservation planning. Conservation priorities should thus be focused on those species at greatest extinction risk, rather than necessarily where the greatest diversity is currently located (Arthington, Dulvy, Gladstone, & Winfield, 2016).

A comparison of Amazonian biogeographical regions (Figure 2) and EAFAs (Figure 15) shows that protected areas are not efficiently distributed to safeguard the long-term heterogeneity of the fish fauna. The most highly threatened freshwater species are generally small-bodied habitat specialists, exhibit low dispersal capability and are often geographically isolated or persist in fragmented habitats (Arthington et al., 2016; McKinney, 1997). This is exactly the functional group of species included within EAFAs, which are not at present protected by existing nature and Indigenous territories. The latter are concentrated in lowland regions of the Amazon, and largely fail to overlap the EAFAs identified here.

4.3 | Hydropower development areas are the most species-rich in endemic and threatened fish

This is a perilous congruence across the Amazon basin. Regions of maximum overlap of species with narrow geographical distributions (Figures 6–10) contain the greatest number of planned, under-construction or existing hydroelectric dams. Amazonian highlands have the greatest hydropower potential because of their steep slopes

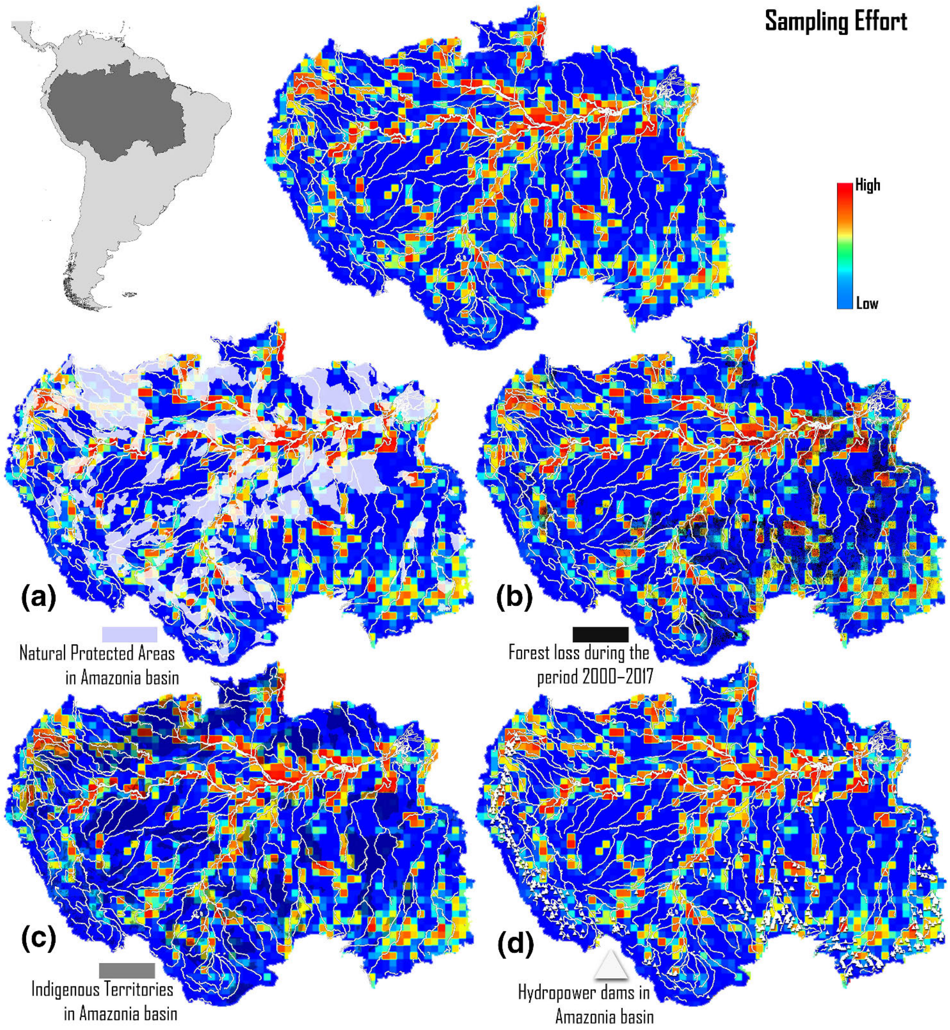


FIGURE 13 Sampling effort in the Amazon superbasin showing the concentration of sampling near urban centres and/or research institutions and major sampling gaps in natural and Indigenous parks. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; and (d) hydropower dams (including planned and under construction)

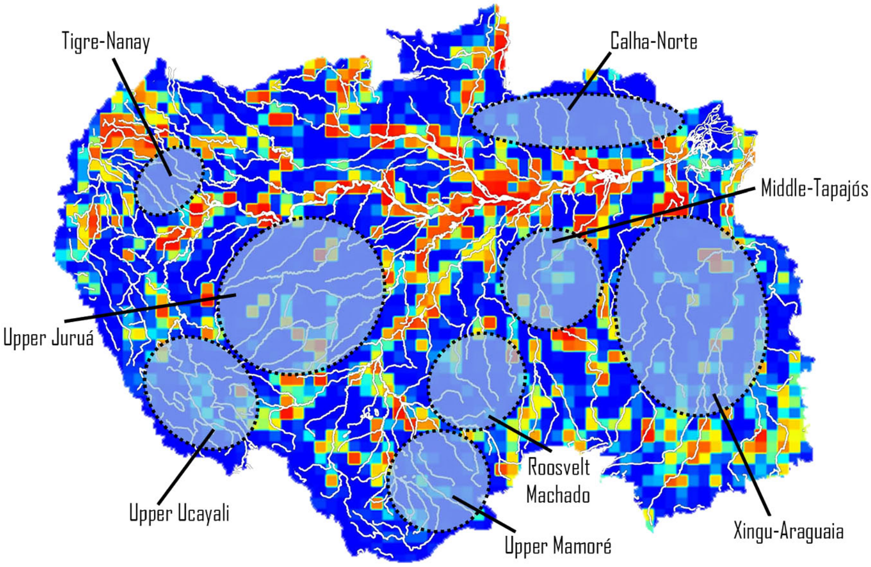
and fast discharge. Those same biophysical properties also result in peculiar conditions favouring higher levels of fish endemism (Winemiller et al., 2016). Amazonian uplands are nutrient-poor, less prone to hydrogeological changes and less dynamic than those of lowland areas. These characteristics result in lower species diversity, fewer cases of sympatry and, most importantly for conservation, large concentrations of restricted-range species (cf. Albert et al., 2011; Dagosta & de Pinna, 2017, 2019; Lima & Ribeiro, 2011). These are the reasons why so many EAFAs are located in upland regions of Amazonia.

Habitat loss has been recognized as one of the main threats to Earth's biodiversity (Brooks et al., 2002; Groom, Meffe, & Carroll, 2006; Hanski, 2011). In the case of Amazonian fishes, this is an important factor, with habitat loss as the primary driver of nearly all threatened species (ICMBio, 2018; IUCN, 2019). The drivers of habitat loss for the aquatic biota are primarily deforestation and dams. The degree of concentration of EAFAs exactly in the region most affected by Amazonian deforestation is alarming (Figures 6–10 and 15b), and this is aggravated by the concentration of planned hydroelectric dams in the same regions (Figures 6–10 and 15d). EAFAs, therefore, fit the concept of biodiversity hotspots (*sensu* Myers et al., 2000), in that they both harbour exceptional concentrations of endemic species and are undergoing exceptional habitat loss.

Many South American nations have invested heavily in hydropower development as a response to growing energy needs (Hrbek, Meliciano, Zuanon, & Farias, 2018). Although this energy source has been hailed as 'clean' and sustainable, the relentless development of hydropower infrastructure results in wide-ranging environmental stress, often including the complete obliteration of rheophilic habitats (Castello & Macedo, 2016; Clausen & York, 2008; Hrbek et al., 2018; Reis et al., 2016). Those habitats are particularly rich in Amazonian endemic fish species and their concentration converges with the delimitation of a large proportion of EAFAs.

Upland Amazonian regions contain myriad high-energy lotic habitats with a complex substrate matrix comprising rock boulders, submerged plateaus, caves, cracks and crevices with lodged tree branches, all of which form a highly diverse set of niches (Hrbek et al., 2018). Dam building causes profound and permanent changes to such habitats, converting them from lotic to lentic, and resulting in the extinction of specialized rheophilic flora and fauna (Fitzgerald et al., 2018; Lees et al., 2016; Lujan & Conway, 2015; Winemiller et al., 2016). The extirpation of the rheophilic fish fauna in areas

FIGURE 14 Amazonian regions in priority need of additional sampling effort for fishes



affected by hydroelectric enterprises leads to the extinction of those lineages (Hrbek et al., 2018; Reis et al., 2016).

4.4 | The most species-rich areas and clades are incongruent with endemism or threats

Figures 4 and 10 show that the Central Amazon is the most species-rich region within the superbasin, a result diverging from previous studies (Oberdorff et al., 2019). Such divergence most likely results from the finer analytical scale used here. The Central Amazon concentrates the highest diversity of fish species in terms of ecological niches and evolutionary ages. It includes the convergence of upland and lowland regions, three different water types (white, clear and black) and the geomorphological formation of the Purus Arch, which once served as an effective barrier between eastern and western Amazonas. Our results show that species diversity decreases away from the core of Central Amazonia. The westward decline can be explained by the predominantly whitewater lowland systems, and to the east this is mainly due to tidal effects (see Jégu & Keith, 1999; Lima & Ribeiro, 2011). The Central Amazon is the most species-rich region in the Amazon basin, but it should not necessarily be considered an immediate conservation priority as there are few imminent threats and few threatened and narrowly distributed species (Figure 5).

4.5 | Conservation plans should consider the highly heterogeneous Amazonian fish fauna

The conservation status of the South American fish fauna is significantly better than those of other freshwater regions worldwide (Reis et al., 2016). Within the continent, the Amazonian basin is more extensively protected in terms of total area than several other major river basins, especially compared with headwater systems located in upland regions of the shields. Nonetheless, our endemicity analyses demonstrate that the distribution of the entire Amazonian fish fauna is highly heterogeneous. Amazonian EAFAs are the most threatened areas in the Amazon superbasin, and are exposed to continuing agricultural frontier expansion, deforestation and hydropower development. EAFAs should thus be urgently included within conservation plans, otherwise Amazonian biodiversity may soon lose important trophic relationships and unique elements of its fish fauna.

4.6 | What to conserve and where?

Data on the biodiversity patterns of Amazonian fishes need to be effectively integrated into the delimitation of conservation priority areas, with a focus on the protection of restricted-range species. Those species have several extinction-prone traits. They often represent trophically specialized lineages (e.g. rheophilic species), are often intolerant to anthropogenic change and have limited dispersal ability and small populations (Helfman, 2007; McKinney, 1997). Small ranges, in particular, mean fewer source areas to maintain adjacent populations or replenish any area after extirpation. This trait has been identified as the single best predictor of extinction risk across several temporal and spatial scales (McKinney, 1997).

Delimitation of protected areas should urgently include different EAFAs to ensure that narrowly distributed and threatened species are set aside. We suggest that regions included in EAFAs should receive priority for conservation status (Figure 15), especially those: (i) lacking nearby protected areas; (ii) with a concentration of threatened species; (iii) undergoing deforestation pressure; and (iv) impaired or targeted by dam construction. Under such criteria, the following areas are considered as conservation priorities for Amazonian fishes: (i) Upper Araguaia; (ii) Upper Tocantins; (iii) Lower Teles Pires/Serra do Cachimbo; (iv) Chapada dos Parecis; and (v) Upper Marañon.

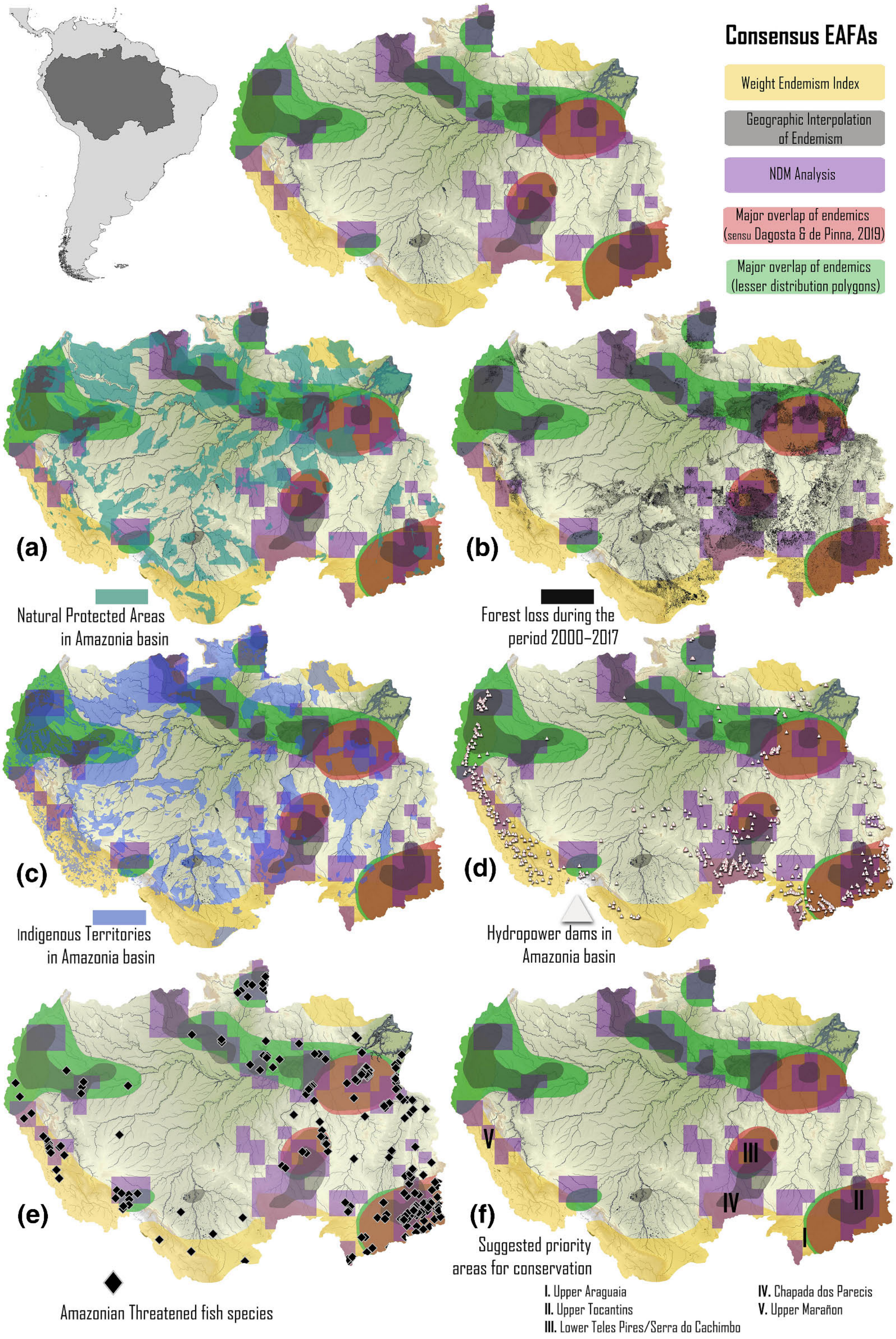


FIGURE 15 Overlap of five independent analytical estimates of spatial patterns of species endemicity across the Amazon superbasin. Results show five EAFAs (Endemic Amazonian Fish Areas) priority areas for conservation: (i) Upper Araguaia; (ii) Upper Tocantins; (iii) Lower Teles Pires/Serra do Cachimbo; (iv) Chapada dos Parecis; and (v) Upper Marañon. Overlapping layers: (a) Natural Protected Areas; (b) forest loss 2000–2017; (c) Indigenous territories; (d) hydropower dams (including planned and under construction); (e) hotspot region, *sensu* Myers et al. (2000); and (f) suggested priority areas for conservation

The immensity of the Amazon domain that is often far removed from large urban centres (and thus from headquarters of regulatory agencies) results in protected areas that exist on paper only. Most of those PAs lack an adequate administrative basis, are severely understaffed and cannot enforce environmental legislation (Peres & Terborgh, 1995). The only physical evidence of their existence is official signs posted at wide intervals. Some measure of scepticism is inevitable about their effectiveness in retaining fish diversity (see Dudley, Harrison, Kettunen, Madgwick, & Mauerhofer, 2016; Hermoso, Abell, Linke, & Boon, 2016; Raghavan, Das, Bijukumar, & Dahanukar, 2016). Despite such apparent situation of neglect, our data show that deforestation pressure is indeed lower within PAs than in adjacent unprotected areas. Therefore, the creation of new PAs in ichthyologically critical areas, while not fully ensuring their preservation, does indeed minimize the rate of population losses, warranting some degree of protection to aquatic biodiversity.

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