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Diversification of Neotropical Freshwater Fishes

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Abstract

Neotropical freshwater fishes (NFFs) constitute the most diverse continental vertebrate fauna on Earth, with more than 6,200 named species compressed into an aquatic footprint <0.5% the total regional land-surface area and representing the greatest phenotypic disparity and functional diversity of any continental ichthyofauna. Data from the fossil record and time-calibrated molecular phylogenies indicate that most higher taxa (e.g., genera, families) diversified relatively continuously through the Cenozoic, across broad geographic ranges of the South American platform. Biodiversity data for most NFF clades support a model of continental radiation rather than adaptive radiation, in which speciation occurs mainly in allopatry, and speciation and adaptation are largely decoupled. These radiations occurred under the perennial influence of river capture and sea-level oscillations, which episodically fragmented and merged portions of adjacent river networks. The future of the NFF fauna into the Anthropocene is uncertain, facing numerous threats at local, regional, and continental scales.

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1. INTRODUCTION

1.1. Neotropical Freshwater Fishes

Species: a single evolutionary lineage with a characteristic and limited range of genetic and phenotypic variation and finite geographic and temporal ranges

Diversity: phenotypic or genetic differences among different species usually characterized by non-normal (e.g., skewed, hollow-curve) statistical distributions

Phenotypic disparity: quantitative measure of trait differences among species in a morphospace

Functional diversity: phenotypic differences among species that have physiological or ecological consequences related to habitat utilization, trophic (or feeding) specialization, and reproduction

The humid Neotropics is home to the greatest concentration of biodiversity on Earth for many groups of organisms (Antonelli et al. 2018b), and this is especially true for continental or freshwater fishes (Albert et al. 2011c, Reis et al. 2016). Neotropical freshwater fishes (NFFs) constitute a taxonomically distinct fauna that extends across 85° of latitude, from the arid uplands of the Central Mexican Plateau (∼30°N) to the subpolar forests of southern Argentina (∼55°S). As of this writing, systematic ichthyologists have described approximately 6,080 NFF species inhabiting diverse aquatic habitats, ecoregions, and climate zones (Dagosta & de Pinna 2019). From torrential headwaters cascading off the Andean cordilleras and upland (>250–300 m elevation) cratonic shields, to the murky waters of large lowland (<250–300 m elevation) river channels, floodplains, and swamps, NFF taxa thrive in astonishing abundance and diversity.

The center of diversity for the NFF fauna is the humid, lowland river basins of northern South America, a region known as Greater Amazonia. This vast region, composed of the Amazon and Orinoco basins and smaller coastal basins of the Guianas, drains hundreds of thousands of kilometers of streams and small rivers (stream orders 1–5) that flow under a closed forest canopy and tens of thousands of kilometers of large rivers (stream orders 6–10) that meander across broad, sunlit floodplains. Waterways of Greater Amazonia constitute the largest volume of aquatic habitat of any region of comparable size on Earth, as measured, for example, by total catchment area (7.05 million km^2 or 2.72 million mi^2), stream/river surface area (93,800 km^2 or 36,200 mi^2, approximately 12% of the global total), or average annual water discharge volume (256,000 m^3/s for the Amazon and Orinoco rivers combined, about 17% of the global total) (Allen & Pavelsky 2018). Near its mouth the Amazon divides into several broad channels, each approximately 10–12 km across, such that the opposite banks disappear over the horizon to a human observer. Here the Amazon discharges a volume of water into the Atlantic so great that it accounts for approximately one-sixth to one-fifth of all the Earth's river water flow, depending on the year.

The relatively high volume of freshwater habitat in Neotropical watersheds is, however, miniscule in comparison to that of the hydrosphere as a whole, representing less than 0.0001% of the world's total water supply. Freshwater ecosystems in general have exceptionally high vertebrate species density, as compared with marine or terrestrial ecosystems, and Neotropical freshwaters represent some of the highest vertebrate species densities on Earth when assessed at large (>10^3 km^2) spatial scales (Bloom et al. 2013, Worm & Tittensor 2018).

The NFF fauna is still very much in a pioneering stage of discovery, with dozens of new NFF species being described each year (Birindelli & Sidlauskas 2018, Dagosta & de Pinna 2018). The current pace of discovery indicates the actual NFF diversity exceeds 9,000 species, meaning as much as one-third of the species in the wild have yet to be described (Reis et al. 2016). The rich NFF fauna therefore constitutes a sizeable fraction of all known vertebrate diversity, including approximately 2 in 5 (40%) of all obligate freshwater fishes (14,760 species total), 1 in 6 (17%) of all fishes (35,150 species total), and approximately 1 in 13 (8%) of all vertebrates (∼68,000 species) on Earth (fish species richness data from Fricke et al. 2019).

1.2. Biodiversity Dimensions

Species richness, measured as the number of independent evolutionary lineages, is only one of many important biodiversity measures (Magurran 2013). NFF species also encompass the greatest amount of phenotypic disparity and functional diversity of any continental ichthyofauna (Su et al. 2019). NFF species display a bewildering array of organismal phenotypes (Roxo et al. 2017) (**Figure 1**) and trophic specializations (Lujan et al. 2011, Evans et al. 2019). Many NFF taxa

(Caption appears on following page)

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Figure 1 (*Figure appears on preceding page*)

A sample of phenotypic disparity observed among Neotropical freshwater fishes. Image displays 78 fish species commonly collected from the Fitzcarrald region of the southwestern Amazon in Peru (Albert et al. 2011b). Note the diversity in many aspects of the integrated phenotype, including body size; shape and coloration; and proportions of the head, mouth, body, and fins. These species also exhibit disparity in traits that are not visually apparent, including morphological and physiological specializations of sensory and behavioral systems associated with feeding, locomotion, dispersal, habitat utilization, courtship, and mating. Photos and montage provided by Tiago Carvalho.

possess ecophysiological, behavioral, and life-history traits and tolerances that promote coexistence in species-rich local assemblages (Winemiller et al. 2015, Arbour & López-Fernández 2016, Evans et al. 2018, Burns & Sidlauskas 2019), which in turn may enhance evolutionary diversification (Albert et al. 2011b,c). Specialized phenotypes are present in sexual communication systems, including sensory cues and courtship displays that reduce the chances for species to hybridize in sympatry, and therefore further promote species richness (Crampton et al. 2016). Studies of communication systems in different NFF taxa have focused on those using chemosensory (Lujan & Conway 2015), visual (Hauser et al. 2017), auditory (Mélotte et al. 2019), and electrical (Crampton 2019) cues.

The phenotypic and ecological disparity of NFF species enables them to inhabit almost all the aquatic habitats of the region, from torrential mountain rivers to subterranean caves and aquifers. Some of the most diverse clades include small-bodied and brightly colored characins, cyprinodonts, and cichlid fishes with spectacular courtship and mating behaviors. Neotropical freshwater fishes also include endemic radiations of marine-derived stingrays, flatfishes, and pufferfishes that inhabit sandy margins of large lowland rivers and freshwater-derived ariid (hard-head) catfishes that inhabit brackish and marine saltmarshes, mangroves, and mudflats. The fauna has specialized algivorous (algae-scraping) loricariid catfishes and folivorous (leaf-eating) serrasalmine piranhas in tumultuous rapids of large rivers, two-meter goliath catfishes (*Brachyplatystoma* spp.) that migrate thousands of kilometers across the transcontinental Amazon river from the Amazon estuary to spawning grounds in the Andean piedmont, parasitic vampire catfishes (*Vandellia* spp.) that suck blood from the gills of larger pimelodid catfishes (and occasionally become lodged in the skin and even urethras of unfortunate humans), and cave-like catfishes and electric fishes with almost no eyes or pigments in the murky depths (up to 100 m) of the Amazon river channel and its larger tributaries.

Despite their extraordinary diversity and ecological importance, NFFs have only recently been included in larger discussions of Neotropical biodiversity (Hoorn et al. 2010, Antonelli et al. 2018a, Musher et al. 2019). Yet ecological and evolutionary processes often differ markedly between obligate aquatic and terrestrial taxa due to the different ways in which these groups use habitats and respond to geographic and climatic changes (Antonelli et al. 2018b). Studies of evolutionary morphology NFF taxa include sexual dimorphism (Pastana et al. 2017 Evans et al. 2018), ecomorphology (Burress et al. 2016; Evans et al. 2017a, 2019; Roxo et al. 2017), and convergent evolution (Gallant et al. 2014, Burress et al. 2018a, Wendt et al. 2019). Quantitative morphometric and multilocus genetic data sets have now been developed to study geographic variation within and among closely related species in many NFF taxa (e.g., Crampton et al. 2016, Melo et al. 2016a, Silva et al. 2016, Craig et al. 2017, Burress et al. 2018b). These data sets have been used to delimit species boundaries (e.g., Bagley et al. 2015, Roxo et al. 2015, Faria-Pereira et al. 2019, Serrano et al. 2019) and to identify cryptic and pseudocryptic species (e.g., Melo et al. 2016b). The emerging use of genome-wide markers [e.g., ultraconserved elements (UCEs), restriction site–associated DNA sequencing (RADseq) markers] has expanded the number of loci used in phylogenetic and phylogeographic analyses of NFF taxa into the thousands (Chakrabarty et al. 2017, Alda et al. 2018, Roxo et al. 2019).

Evolutionary diversification: increase in species richness of clade due to an excess of speciation over extinction

Variation: phenotypic or genetic differences within species subject to the action of natural selection and genetic drift, usually characterized by a normal statistical distribution
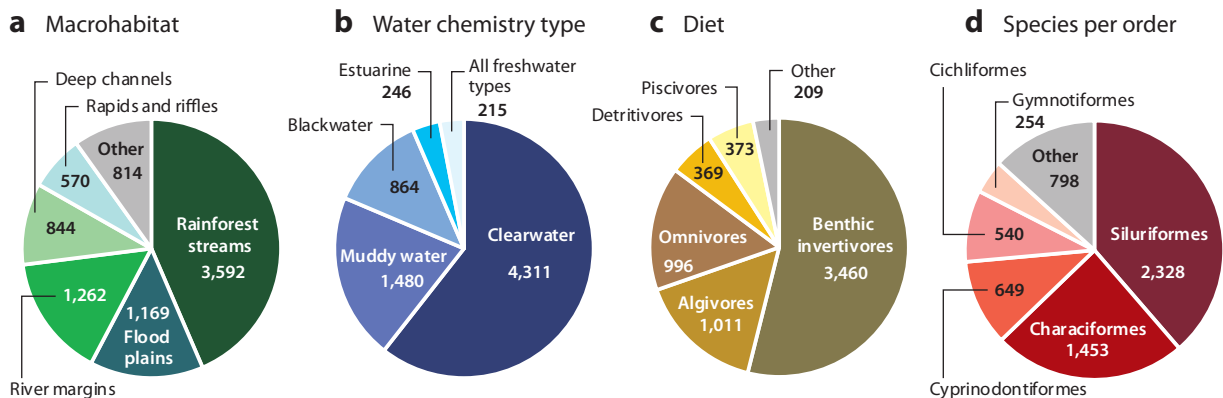
Figure 2

Summaries of ecological and taxonomic diversity for Neotropical freshwater fish (NFF) species. Data for 6,200 species collated from multiple sources; see references in van der Sleen & Albert (2017) and Dagosta & de Pinna (2019). (*a*) Species per modal adult macrohabitat type (assessed at the >100 m scale). Other macrohabitat types include acidic blackwaters, sandy beaches, and coastal rivers/estuaries. (*b*) Species per major water chemistry type. Clearwaters are sediment poor and slightly acidic (pH = 6.1–6.7), muddy waters are sediment rich and neutral (pH = 6.8–7.0), blackwaters are sediment poor and very acidic (pH = 3.5–6.0), and estuarine waters are freshwater or brackish and neutral pH; generalists may inhabit all inland freshwater types. (*c*) Species per modal adult diet type. Other major diet types include planktivores, folivores, and frugivores. (*d*) Species per taxonomic order. The other category includes 35 orders. Note that species numbers in the habitat and diet categories sum to more than total NFF species richness because some species are eurytopic (inhabiting more than one habitat type) or eurytrophic (consuming food items in more than one diet category).

2. WHY SO MANY FISHES?

Differences in species richness and other biodiversity measures among higher taxa have long been a focus of the *Annual Review of Ecology, Evolution, and Systematics*; (e.g., Wiens & Graham 2005, Seehausen & Wagner 2014, Linder & Verboom 2015, Svenning et al. 2015). Contemporary research in Neotropical ichthyology uses concepts and data from all three interrelated fields of ecology, systematics, and evolution to help understand and explain differences in the species richness of higher taxa (Malabarba et al. 1998, Albert et al. 2011b, Reis et al. 2016, Dagosta & de Pinna 2018).

2.1. Ecology

Recent advances in NFF ecology have focused on the roles of morphological and physiological traits in habitat utilization and trophic ecology (Crampton 2011, Lujan et al. 2013, Fitzgerald et al. 2017, Rodrigues-Filho et al. 2018). The major aquatic macrohabitat and trophic categories summarized in **Figure 2** predict many functional and physiological phenotypes observed in NFF taxa (e.g., Val & de Almeida-Val 2012, Lujan et al. 2013, Winemiller et al. 2015, Santos et al. 2019). This section reviews several prominent ecological hypotheses for high NFF diversity from the perspective of community ecology, focusing on environmental properties that contribute to habitat heterogeneity and organismal properties underlying habitat and trophic specializations. The role of organismal movements (i.e., dispersion) in the processes of NFF diversification are discussed in Section 2.3.

2.1.1. Local assemblages. Some hypotheses for exceptional NFF diversity focus on the high species richness observed within local assemblages (i.e., alpha diversity) and the high species turnover (i.e., beta diversity) between habitat assemblages (Lujan et al. 2011, 2013; Fitzgerald et al. 2017; López-Delgado et al. 2019). Lowland rivers of Greater Amazonia often exhibit

Alpha diversity: species richness within a local assemblage

Beta diversity: change in species composition (spatial turnover) between habitats or across geographic or temporal gradients

remarkably high local species richness; it is common to collect 40 or more fish species along short (100 m) stretches of small rainforest streams and more than 100 species in a long day of sampling the floating macrophytes of floodplain lakes of large rivers (Albert et al. 2011c, Crampton 2011, Duarte et al. 2019). Beta diversity measures can also be high, with distinct species assemblages in large river channels, floodplain forests and oxbow lakes, and non-floodplain (*terra firme*) streams and small rivers (Albert et al. 2011b, Crampton 2011).

Beta diversity measures among habitats vary substantially across gradients of stream order (i.e., level of branching in a river network) and topographic relief. Along the longitudinal river gradient, there is higher beta diversity among low-order (i.e., small) rainforest streams than there is in high-order floodplain or river channel corridors, and there is also higher beta diversity in high-relief (i.e., rugged) uplands than low-relief (i.e., flat) lowlands. From a functional perspective, high alpha diversity implies the coexistence of many species in sympatry and syntopy, whereas high beta diversity among habitats implies species sorting based on functional attributes (López-Delgado et al. 2019). The reader should note that beta diversity as assessed among habitats is different from the similarly termed beta diversity as assessed across watershed boundaries at biogeographic scales (e.g., Dagosta & de Pinna 2017).

2.1.2. Habitat utilization.   Other hypotheses for the exceptional NFF species richness focus on the roles of functional traits and habitat utilization (Albert et al. 2011b, Lujan & Armbruster 2012, Arbour & López-Fernández 2016, Ferreira et al. 2017, Fitzgerald et al. 2017, Roxo et al. 2017, Rodrigues-Filho et al. 2018). Such traits allow taxa to specialize and subdivide aquatic habitats locally and also to occupy a wider range of habitat types throughout the vast Neotropical region.

To examine these hypotheses, we assigned all valid NFF species to one or more of eight broad macrohabitat categories assessed at >100-m scale (**Figure 2*a***). We found that a majority of NFF species (3,592 species or approximately 60% of the total) inhabit small *terra firme* rainforest streams and rivers. This result is expected from a quantitative habitat perspective, since lowland streams and rivers constitute a great majority of total habitat volume in the lowland Amazon and other Neotropical river basins, as they do in other drainage systems of the world. Smaller water-ways (with stream orders 1–3) represent approximately 88% of the total South American drainage network, with median widths of 1–10 m along river-run segments at bank-full flow (Albert et al. 2011c, figure 2.4). In lowland Amazonia, these smaller waterways run under a closed canopy and have predominantly allochthonous primary production (Castro & Polaz 2020). Larger water-ways, with stream orders 4–10, median widths of 1,000–10,000 m, and a higher proportion of autochthonous production, constitute only 12% of the drainage network as measured by river length. However, larger waterways occupy a much greater proportion of the total habitat space, with a channel footprint of approximately 18,200 km^2 or >80% of the total drainage network (Downing et al. 2012).

It is important to note that each of these broad macrohabitat categories encompasses a substantial range of environmental variables at the macro- (100–1,000 m), meso- (10–100 m), and micro- (1–10 m) scales. Some of these variables are known to affect the species and functional composition of local NFF assemblages (Rodrigues-Filho et al. 2018, Duarte et al. 2019). Certain NFF species are adapted to high mountain lakes above 4,000 m elevation, torrential mountain rivers, seasonal swamps and ephemeral pools, caves and aquifers, moist leaf litter, coastal estuaries, and mangroves. As with other freshwater fishes of the world, many NFF species exhibit specializations for life in particular portions of the river continuum, including macroscale longitudinal gradients from headwaters to mouth (from low to high stream orders); mesoscale lotic, lentic, and ephemeral or seasonal habitats; and microscale habitats like substrate composition (e.g., soft, sand, gravel/rocks, submerged wood, vegetation) and position within the river cross-profile

vertically (e.g., benthic, pelagic, surface) and laterally (e.g., channel, littoral, floodplain) (Albert et al. 2011b, Lujan et al. 2013, Rodrigues-Filho et al. 2018, Duarte et al. 2019). Fishes adapted to larger waterways (with stream orders 4–10) also represent a heterogeneous set of environmental conditions with varied functional (trophic, locomotor) traits, ranging from an extensive network of small rainforest streams flowing under a closed canopy to the seasonally variable oxbow lakes and flooded forests of broad river floodplains and deep river channels (up to 120 m depth) of the large lowland rivers.

Many NFF taxa also possess specialized phenotypes enabling them to inhabit rivers with one or more of three distinct water chemistry profiles (Val & de Almeida-Val 1995, van der Sleen & Albert 2017). A majority of NFF species (4,311 species or 71% of the NFF total) inhabit clearwater rivers draining the Brazilian and Guianas shields (e.g., Essequibo, Tapajós, Tocantins, Upper Paraná, Xingu) with slightly acidic waters (pH = 6.1–6.7) and high transparency (low sediment load; **Figure 2*b***). Another large fraction (1,480 species or 24.6%) inhabits muddy (sediment and nutrient-rich) waters draining the Andean Cordilleras (e.g., Amazon, Madeira, Magdalena, Orinoco, Paraguay) with approximately neutral pH (6.8–7.0). Fishes of muddy waters constitute the largest biomass in the lowland Neotropics and form the basis of regional aquatic food webs and inland fisheries (Goulding et al. 2019). A third major fraction (864 species or 14.3%) are blackwater specialists with adaptations to inhabit acidic waters, some with a pH as low as 3.5, in the range of the gastric juices of many vertebrate predators. Blackwater fishes include many with bright coloration, like the giant peacock bass (*Cichla temensis*) and the Neon and Cardinal tetras (*Paracheirodon* spp.).

2.1.3. Trophic specializations. A third set of hypotheses for high NFF diversity focuses on trophic or feeding specializations as contributing to the high species richness in local assemblages and regional species pools (Lujan et al. 2011, Duarte et al. 2019, Evans et al. 2019). Although NFFs consume a wide variety of food items, currently available data suggest that trophic specializations contribute only modestly to the lineage diversity of the NFF fauna as a whole. A majority of NFF species are generalist benthic invertivores (**Figure 2*c***), adventitiously consuming a variety of small-bodied aquatic arthropods, especially the larvae of Ephemeroptera (mayflies), Diptera (true flies and midges), Plecoptera (stoneflies), and Trichoptera (caddisflies). Benthic invertivores do exhibit a heterogeneous range of phenotypes and dietary preferences, with some species and clades having generalized phenotypes and eurytrophic (broadly adapted feeding) habits and many taxa having specialized (stenotrophic) phenotypes and habits. Some specialized trophic syndromes include benthic sifting in geophagine cichlids (López-Fernández et al. 2013, Arbour & López-Fernández 2016), pharyngeal crushing in heroine cichlids (Kautt et al. 2018), and substrate probing with grasp-suction feeding in apteronotid electric fishes (Evans et al. 2018). The major dietary categories in **Figure 2*c*** predict many trophic roles and phenotypes, for example those associated with algivorous, detritivorous, and piscivorous habits. Species representing these trophic categories commonly exhibit a characteristic suite of morphological and physiological traits, including specialized aspects of sensory, feeding, digestive, and locomotor biology (Lujan et al. 2011, Lujan & Armbruster 2012, Silva et al. 2016, Evans et al. 2019).

2.2. Systematics

This section summarizes the major patterns of taxonomic and biogeographic diversity observed among NFF taxa. Viewing diversity patterns through a phylogenetic or taxonomic lens highlights the roles of individual clades and traits in the process of NFF diversification (Albert et al. 2011b, Bloom et al. 2013).

2.2.1. Taxonomic diversity patterns. The >6,200 known NFF species have been assigned by taxonomists to 854 genera, 95 families, and 39 orders. As with most biotas on Earth, NFF diversity is unevenly distributed among higher taxa; most of the diversity is concentrated in a just few clades, while most NFF clades exhibit low diversity (Albert et al. 2011c). Indeed, most NFF species are members of just five major clades conventionally ranked as taxonomic orders: Siluriformes (catfishes), Characiformes (tetras, piranhas, and allies), Cyprinodontiformes (killifishes, rivulids, and allies), Cichliformes (cichlids), and Gymnotiformes (Neotropical electric fishes). Together these five clades account for 87% of all NFF species and 96% of Amazonian fish species (Reis et al. 2016, van der Sleen & Albert 2017, Dagosta & de Pinna 2019). The combined total of all the other 34 NFF orders includes only 783 species, and 24 of these orders have fewer than 10 species each, mainly clades of otherwise marine fish that entered Neotropical freshwaters during the Neogene (∼23–2.6 Ma) and Quaternary (∼2.6–0 Ma) (Bloom & Lovejoy 2017). This highly uneven distribution of diversity among clades is observed across diversity metrics, such as species richness, phenotypic disparity, and functional differences.

> **Speciation:** process by which an ancestral species gives rise to one or more daughter species, during which intraspecific variation is transformed into interspecific diversity

The uneven distribution of NFF diversity is also observed over a wide range of taxonomic scales. NFF phylogenies resemble those of other taxa in exhibiting fractal-like tree topologies in which the most species-rich orders have only a few species-rich families and many species-poor families, and the most species-rich families have only a few species-rich genera and many species-poor genera (van der Sleen & Albert 2017, figure 3). In a taxonomy based on phylogenetic systematics, such patterns reflect a highly unbalanced tree topology (Mooers & Heard 1997). Such topologies are predicted by neutral models of lineage diversification and are widely observed in the NFF fauna (Albert & Carvalho 2011, table 7.2).

One measure of the evenness (or unevenness) of species among clades is the value of the exponent (b) in a power function ($y = ax^b$) fit to the distribution of species richness per clade against rank-clade diversity. In this function, more negative values of b indicate a greater dominance of the most species-rich clades. Among 66 phylogenetically independent NFF clades, b was empirically determined to be −2.27, while a similar analysis of 88 clades of North American freshwater fishes found the value of b to be −1.35 (Albert et al. 2011a, figure 5.9b). The more negative value of b in the more species-rich fauna is consistent with neutral models of biodiversification (e.g., Worm & Tittensor 2018) in which accumulated time for diversification and low extinction rates are stronger predictors of diversity than different rates of speciation among taxa due to functional differences (see Section 2.3.3 below).

At the other end of the diversity spectrum, the long tail of species-poor clades includes some species that are ecologically abundant and have important functional roles in certain environments. For example, several large-bodied fish species are locally abundant apex predators in whitewater floodplains, including the South American lungfish *Lepidosiren paradoxa* (Lepidosireniformes), which grows to a standard length of 125 cm; the Pirarucu or Paiche *Arapaima gigas* (Osteoglossiformes), which grows to a standard length of 450 cm; and the Silver Arowana *Osteoglossum bicirrhosum* (Osteoglossiformes), which grows to a standard length of 120 cm. As top predators, these species exert disproportionate effects on the food webs of floodplain lakes. As measured by evolutionary persistence, these three taxa may also be viewed as evolutionary successes, with phylogenetic origins tens of millions of years ago during the Paleogene (∼66–23 Ma) or Upper Cretaceous (∼101–66 Ma) periods (López-Fernández & Albert 2011). The existence of such ecologically abundant and evolutionarily persistent, although species-poor, taxa highlights the multiple alternative strategies leading to long-term ecological and evolutionary success.

2.2.2. Clade-specific traits. The taxonomic perspective illuminates the potential role of certain derived traits that may affect rates of net diversification. Such organismal traits, often

referred to as key innovations, have been hypothesized to spur speciation or slow extinction in fishes (Liem 1973, Wainwright et al. 2012). Alternative mechanisms for how key innovations may affect diversification include their effects on (*a*) dispersal capacity (i.e., vagility) and gene flow, (*b*) ability to use new or newly available resources (i.e., ecological opportunity), (*c*) capacity for reproductive isolation (Seehausen & Wagner 2014), (*d*) evolvability or capacity to respond to selection (Rabosky 2014), and (*e*) lability or capacity to produce functional trait variation.

Evolutionary lability can be affected by the evolution of phenotypic modularity (i.e., developmental compartmentalization) and the evolution of ecophenotypically plastic tissues. The evolution of modularity and plasticity have been linked to increased craniofacial disparification in some NFF clades (Evans et al. 2017a,b; Burress et al. 2018a; Burns & Sidlauskas 2019). Some key innovations may also have prominent ecosystem-level consequences, including life-history traits that result in high abundances and biomass in detritivorous characins, planktivorous electric fishes, and piscivorous catfishes that support riverine food webs and regional commercial fisheries (Melo et al. 2013, Goulding et al. 2019).

Some key innovations may have a long phylogenetic fuse, contributing to diversity only after a lengthy wait time in the context of newly derived traits or environmental conditions. For example, whole genome duplication in the most recent common ancestor of teleost fishes is hypothesized to have increased the evolvability of the neuroendocrine system, thereby contributing to the phenomenal diversity of reproductive modes and life-history strategies of teleost fishes (Smith & Wootton 2016). Whole genome duplication did not immediately produce rapid diversification (Santini et al. 2009) but rather provided multiple copies of opsin and myocyte membrane protein genes, which later became used in the diversification of visual and electric organ phenotypes, respectively, in certain teleost subclades (e.g., Gallant et al. 2014, Hauser et al. 2017).

Key innovations may also increase the capacity for species to coexist in local (syntopic) assemblages. In taxa where they have been closely studied, the most prominent effects of key innovations generally turn out to be on the evolution of diet, microhabitat, and sexual communication signals, e.g., in cichlids (Tougard et al. 2017; Burress et al. 2018a,b; Kautt et al. 2018) and electric fishes (Crampton et al. 2016, Craig et al. 2017, Lehmberg et al. 2018). Certain other derived traits, like smaller adult body size and more specialized habitat tolerances, may act systematically to reduce dispersal and genetically isolate populations over time. By reducing dispersal and gene flow, these traits are expected to increase chances for both speciation and extinction, i.e., Vrba's Effect Hypothesis (see Albert et al. 2011c). Dispersal capacity in association with ecological opportunity has been correlated with net diversification rates in all of the three most species-rich NFF families: Loricariidae (Silva et al. 2016, Roxo et al. 2017), Characidae (Melo et al. 2016a, Wendt et al. 2019), and Cichlidae (Arbour & López-Fernández 2016, Tougard et al. 2017). In these families the species tend toward relatively small adult body sizes (an average standard length of 20.0 cm) and restricted geographic distributions (Albert et al. 2011a, figure 5.7; Dagosta & de Pinna 2019).

By the same token, larger adult body sizes and less specialized habitat tolerances are expected to increase dispersal and therefore reduce rates of speciation and extinction over time, thereby lowering net species turnover (Dagosta & de Pinna 2017). This effect is well illustrated by comparing the families Loricariidae (armored suckermouth catfishes) and Pimelodidae (long-whiskered catfishes). Loricariidae is known from >990 species, most of which grow to relatively small adult body sizes (an average standard length of 16.2 cm) and which possess predominantly benthic, algivorous, and philopatric life histories that increase their chances of geographic isolation. By contrast, Pimelodidae is known from just 114 species, all with much larger adult body sizes (an average standard length of 60.5 cm), most of which are migratory with broad geographic distributions across the major lowland river basins.

Dispersal:
evolutionary expansion of species geographic range

Vagility:
dispersal capacity

Ecological opportunity:
evolutionary diversification in the context of exposure to new, or newly available, resource(s)

Certainly many other factors contribute to differences in the diversity of fish groups. Many ancient NFF taxa with small adult body sizes and low vagility exhibit low diversity (e.g., *Scoloplax*, *Phreatobius*), and some ancient, large-bodied taxa (e.g., *Arapaima*, *Osteoglossum*, *Lepidosiren* mentioned in Section 2.2.1) with potentially high vagility also have low diversity (Albert et al. 2011a, figure 5.5). It seems that small (although not the smallest) modal adult body size, low vagility, ecological specializations, and ancient origins are properties of all of the species-rich clades, although none of these factors is sufficient in isolation.

Any complete discussion of NFF diversity must note that a majority of the species are members of a single clade, the Ostariophysi. This clade is a super-radiation of teleost fishes represented by 4,035 Neotropical species and almost 11,000 species globally. Ostariophysi includes approximately 68% of all freshwater fish species in the world and 67% of NFF fish species in three orders: Siluriformes (catfishes), Characiformes (tetras, piranhas, and allies) and Gymnotiformes (Neotropical electric fishes) (**Figure 2*d***). Among phylogenetically independent NFF clades, five of the eight most diverse clades are ostariophysans, i.e., Characoidea, Loricarioidea, Pimelodoidea, Doradoidea, Gymnotiformes, and Erythrinoidea (Albert et al. 2011a, table 5.1).
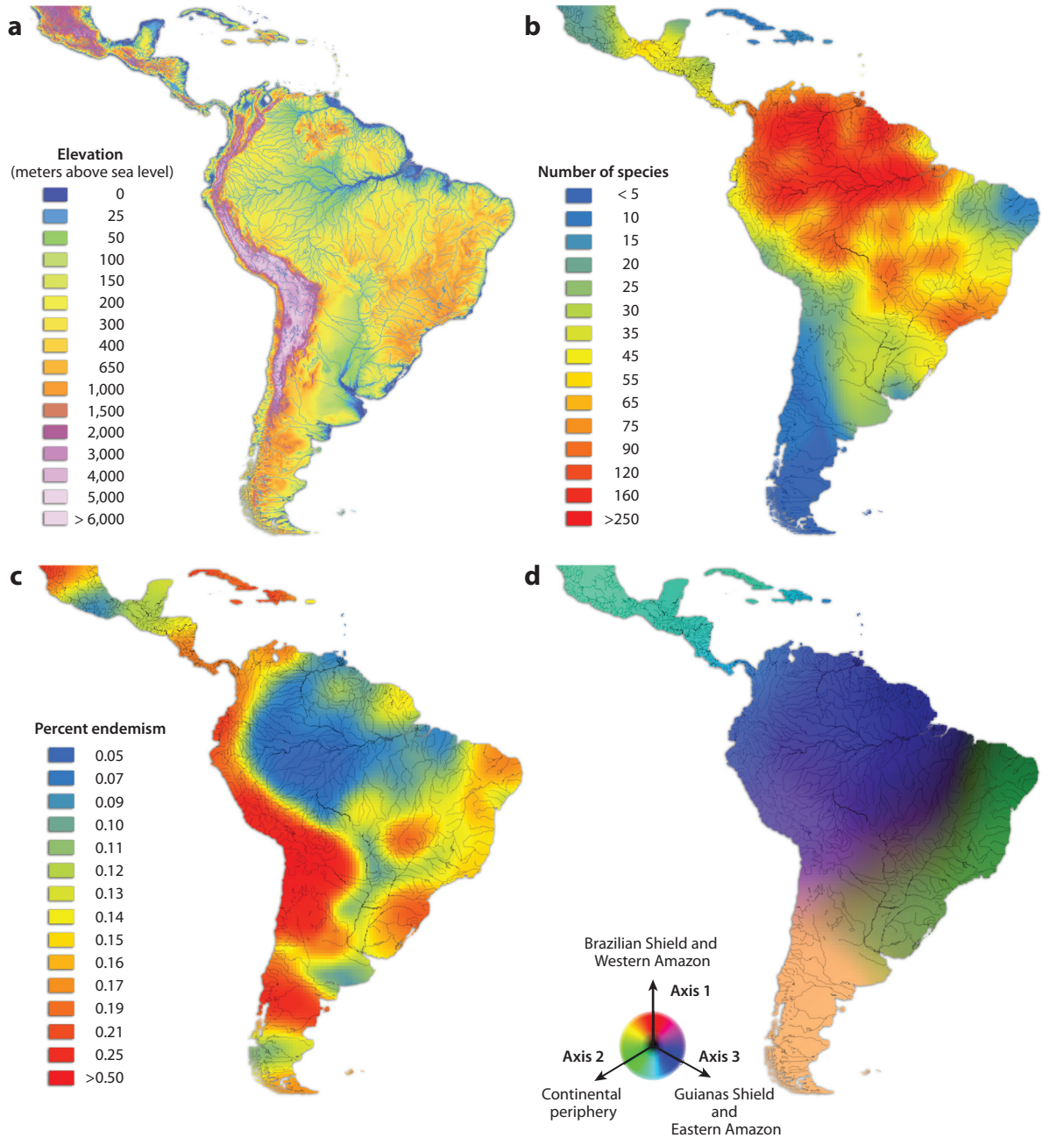
2.2.3. Major biogeographic patterns.

Many large-scale biodiversity patterns observed in NFF taxa are similar to those of other Neotropical taxa, including both terrestrial (e.g., birds, mammals, flowering plants, lepidopteran and coleopteran insects) and aquatic (e.g., decapod crustaceans, opisthobranch mollusks, odonate and ephemeropteran insects) clades. Some of the most prominent of these biodiversity patterns include (*a*) a latitudinal diversity gradient with more diversity near the equator, (*b*) a mid-domain effect with more species near the center of biogeographic regions, (*c*) Tobler's first law of geography in which near things are more related or similar than are distant things (i.e., spatial autocorrelation), and (*d*) an inverse relationship between species richness and geographic range size (Albert et al. 2011b). These near-universal patterns help reveal general mechanisms of organismal diversification, mechanisms that are not sensitive to taxon-specific dispersal modes or other life-history attributes, genetic background, or the effects of historical contingency.

2.2.3.1. *Biogeographic patterns of fishes and terrestrial taxa.*

Many NFF taxa exhibit a distinctive core-periphery pattern of contrasting biodiversity measures. This core-periphery pattern is characterized by high species richness at the continental core and high species endemism at the continental periphery (Albert et al. 2011c, 2017). Similar contrasting core-periphery patterns are widely observed in Neotropically distributed taxa, including birds, mammals, snakes, frogs, and plants (Antonelli et al. 2018b, Azevedo et al. 2020, Vasconcelos et al. 2019). The regions with the highest NFF species richness are the low-relief river basins of Greater Amazonia centered on the Amazon and Orinoco basins (**Figure 3*b***). There are also secondary NFF species-richness hotspots in the Choco (Pacific slope of Colombia), the upper Paraguay basin (Pantanal wetlands) of central Brazil and Paraguay, and the Atlantic rainforest of southeastern Brazil. By contrast, the regions with the highest species endemism are in the high-relief upland basins of the continental periphery, especially the Mexican plateau, the Greater Antilles, the Pacific slope, and the Southern Cone (**Figure 3*c***).

A core-periphery pattern is also observed in the phylogenies of many NFF taxa. In these clades most species are members of a single subclade distributed across Greater Amazonia, in which one or more early-branching subclades with low diversity are located at the geographic periphery, e.g., Atlantic coastal rivers of the Guianas, Southeastern Brazil, or the Southern Cone (Albert et al. 2017). Some examples of this core-periphery phylogenetic pattern are known in electric fishes (Hypopomidae and Gymnotidae; Tagliacollo et al. 2016) and catfishes (Doradidae, Arce

Continental core: biogeographic center of a continent characterized by relatively high species richness and relatively low species endemism

Continental periphery: biogeographic margins of a continent characterized by relatively low species richness and relatively high species endemism

et al. 2013; Trichomycteridae, Ochoa et al. 2020). This core-periphery phylogenetic pattern is predicted from a neutral model of diversification that produces higher rates of both speciation and extinction (i.e., greater temporal species turnover) toward the center of a biogeographic region and the accumulation of early-branching low-diversity clades toward the geographic periphery (Albert et al. 2017) (see Section 2.3.3 on landscape evolution).

(*Caption appears on following page*)

Figure 3 (*Figure appears on preceding page*)

Major biogeographic patterns in the Neotropical freshwater fish (NFF) fauna. Data on geographic distributions for 6,088 species collated from multiple sources; see references in Dagosta & de Pinna (2019). (*a*) Topography. Note the vertical exaggeration at lower elevations. (*b*) Species richness (SR) was calculated as counts of species per 1.5° grid cell and interpolated using nearest-neighbor interpolation (NNI) with smooth curves. Note the Greater Amazonian core with the highest SR and the continental periphery with lower SR. Note also secondary SR hotspots in the Choco (Pacific slope of Colombia), the Pantanal of central Brazil and Paraguay, and the Atlantic rainforest of southeastern Brazil. (*c*) Species endemism (SE) was calculated as the mean weighted endemism per grid cell and interpolated using NNI. Note the lowest SE values are in low-relief lowland river basins (i.e., Amazon, Orinoco, La Plata), and the highest SE values are in in high-relief upland basins. (*d*) Beta diversity was calculated as a Bray-Curtis dissimilarity matrix among grid cells converted into linear estimates of three spatial vectors by non-metric multidimensional scaling (NMDS) interpolated using NNI. Note the geographic turnover in species composition along three axes, representing the faunas of the Brazilian Shield and Western Amazon (*red*), Guianas Shield and Eastern Amazon (*blue*), and the species-poor regions on the continental periphery (*green*).

As in many biotas, the Neotropical ichthyofauna exhibits a hollow-curve (i.e., power-function) frequency distribution of species' geographic range sizes, in which a few species have exceptionally broad geographic ranges and many species have small ranges (Dagosta & de Pinna 2019). More than half of all NFF species are restricted to a single ecoregion or river basin, and 90% are known from five or fewer ecoregions (Albert et al. 2011c, figure 2.6). This pattern is especially prominent in the high-relief uplands at the continental margins (Albert et al. 2011c, 2018b). As a result, the upland fauna exhibits high beta diversity (i.e., taxonomic turnover) across watershed boundaries, among rivers draining landforms with distinct water chemistry profiles, and across the high-relief upland basins of the continental periphery (**Figure 2d**; Albert et al. 2011c, Dagosta & de Pinna 2017, Oberdorff et al. 2019).

Several nominal NFF morphospecies are widespread across most of tropical northern South America, including the characiform *Hoplias malabaricus* (Erythrinidae), the gymnotiforms *Gymnotus carapo* (Gymnotidae) and *Sternopygus macrurus* (Sternopygidae), the siluriforms *Rhamdia quelen* (Heptapteridae) and *Callichthys callichthys* (Callichthyidae), and the synbranchiform *Synbranchus marmoratus* (Synbranchidae). Some of these broadly distributed NFF morphospecies have been sampled across their geographic range for morphometric, genetic, and karyotype differences. These taxa have been shown to exhibit a range of divergence levels and patterns, which has been interpreted alternatively to represent a single widespread polytypic species (Craig et al. 2017), a paraphyletic ancestral morphospecies and one or more daughter species (Craig et al. 2019), incipient species (Ferreira et al. 2017), or multiple cryptic or pseudocryptic species (Cardoso et al. 2018a).

2.2.3.2. Biogeographic patterns of fishes.   Other major diversity patterns differ qualitatively between terrestrial and aquatic taxa. These patterns can help illuminate how phenotypes associated with feeding, habitat utilization, dispersal, and sexual signaling affect gene flow and population structure, and ultimately, the macroevolutionary processes of speciation, extinction, and dispersal (Albert et al. 2011c). Whereas many terrestrial taxa exhibit a mid-elevation peak in species richness (birds, mammals, flowering plants), species richness for freshwater fishes is greatest at low elevations (see Hoorn et al. 2018). Other prominent differences between terrestrial and aquatic taxa include the roles of large rivers as barriers or corridors for dispersal and gene flow (Musher et al. 2019), the effects of Euclidean versus dendritic geometry on landscape connectivity and population genetic isolation (Smith et al. 2010, Dias et al. 2014, Carvajal-Quintero et al. 2019), and the position of diversity peaks along elevation gradients (Antonelli et al. 2018a, Hoorn et al. 2018).

The larger NFF fauna is composed of several regionally defined faunas, each with somewhat distinct although overlapping taxonomic compositions. Three of these regional faunas have ancient origins during the Paleogene or Upper Cretaceous (Tagliacollo et al. 2015, 2017): (*a*) the Amazonian Lowlands and the Pacific slope of northern South America, including the Amazon,

Orinoco, and Essequibo basins and the Pacific basins of Colombia and Ecuador; (*b*) Amazon Craton, including upland basins on the Guiana and Brazilian Shields and their Amazonian and Atlantic coastal drainages; and (*c*) the Southern Cone, including the Pacific basins of Chile and Atlantic Basins of Argentina, the diversity of which was severely reduced by late Cenozoic climate change. The NFF fauna also includes at least two distinct Neogene faunas: (*a*) an upland fauna of the Northern Andes (Colombia to Bolivia, above 500 m) and (*b*) a mixed upland and lowland fauna of the Caribbean Plate, including nuclear and southern Middle America and the Greater Antilles. Examples of taxa exhibiting these distinct biogeographic patterns in northern South America are provided by Dagosta & de Pinna (2019).

2.3. Evolution

The NFF fauna provides a rich natural laboratory in which to study mechanisms of biodiversification and the formation of species-rich tropical biotas. Some NFF clades are becoming widely used in the fields of genetics and genomics, evolutionary morphology, animal behavior, historical biogeography, and biodiversity conservation, among others. This section provides a brief overview of current thinking on the mechanisms of biodiversification in the NFF fauna, with references to this burgeoning literature.

2.3.1.   Temporal patterns.   The main temporal patterns of diversification in NFF clades can be summarized as follows: (*a*) deep divergence times for species and higher taxa, (*b*) relatively even rates of net diversification with little evidence for rapid bursts (i.e., adaptive radiations) or busts (i.e., mass extinctions), and (*c*) low extinction rates. Recent comparative analyses of freshwater fishes have globally emphasized the role of long evolutionary histories with relatively low extinction rates as main drivers of the exceptional diversity of Neotropical fishes (Lundberg et al. 1998, Albert et al. 2011c, Miller & Román-Palacios 2019). Accumulating evidence from multilocus time-calibrated phylogenies suggests relatively even expansion of lineage diversity during the Neogene in many groups, without major bursts or busts of speciation and extinction (e.g., Tagliacollo et al. 2016, Costa et al. 2017, Roxo et al. 2017, Tougard et al. 2017, Alda et al. 2018, Bloom & Egan 2018, Bragança & Costa 2018, Cardoso et al. 2018b, Amorim & Costa 2019, Bernt et al. 2019, Burns & Sidlauskas 2019, Craig et al. 2019). Direct evidence from fossils (Lundberg 1998, Reis 1998, Malabarba et al. 2010) and time-calibrated molecular phylogenies indicate Paleogene dates for the origins of most of the higher taxa (ranked as genera and above) and a fauna entirely composed of modern genera by the Late Miocene (∼11.6–5.3 Ma; Hoorn et al. 2010, Albert et al. 2018b). Thus, with few exceptions, supraspecific NFF taxa ranked as genera or above are older than the river basins and aquatic macrohabitat structures that they inhabit today.

Evidence to date indicates ancient Mesozoic and Paleogene origins for the Amazonian aquatic fauna (Lundberg 1998, López-Fernández & Albert 2011). Although the NFF fossil record is relatively sparse, there are some spectacular examples of species exhibiting modern phenotypes by the Eocene (∼56.0–33.9 Ma). Two notable examples are the callichthyid catfish †*Corydoras revelatus* (Reis 1998) and the geophagine cichlid †*Gymnogeophagus eocenicus* (Malabarba et al. 2010), both collected from the Lower-Middle Eocene Lumbrera Formation (∼40.0–56.0 Ma) in northwestern Argentina. Both of these species are phenotypically derived members nested highly within the modern diversity of their respective families (Lundberg 1998, Hoorn et al. 2010, López-Fernández & Albert 2011). Deep divergence times among lineages are also supported by time-calibrated molecular phylogenies of many family-level taxa, which recover Early Miocene (∼23.0–16.0 Ma) or older dates for the origins of many species and genera (e.g., Bragança & Costa 2018, Bernt et al. 2019). This interpretation is reinforced by data on biodiversity patterns of fish genera across the Northern Andes, which exhibit similar species per genus ratios, indicating these taxa had already

Adaptive radiation:
evolutionary diversification where adaptation and speciation are coupled via natural selection, and where therefore most species are adaptively diverged from one another

diversified to modern levels before the uplift of the Eastern Cordillera of Colombia began in the Late Miocene (~12.0 Ma; Albert et al. 2006).

River capture:
geomorphological process that moves the location of watershed divides between adjacent river basins, simultaneously separating and merging portions of drainage networks and their resident biotas

2.3.2. Evolutionary radiations.

Phylogenetic, biogeographic, and paleontological data from the NFF fauna indicate the high biodiversity of the fauna arose over a period of tens of millions of years during which rates of speciation exceeded those of extinction (Rull 2011, Miller & Román-Palacios 2019). In other words, ecological limits do not seem to have substantively constrained the production of NFF diversity. In this regard the NFF fauna resembles other biotas that are among the world's most species rich (Linder & Verboom 2015, Worm & Tittensor 2018, Miller & Román-Palacios 2019).

Basin-wide assemblages of NFF species also resemble those of freshwater fishes worldwide in being polyphyletic and having been assembled primarily by means of dispersal (Albert & Carvalho 2011, Albert et al. 2011b, Fitzgerald et al. 2017, Tagliacollo et al. 2017). The many species that coexist locally are rarely if ever one another's closest relatives. This means that speciation is rarely the reason for high local species richness of NFF assemblages. Indeed, sympatrically distributed sister-species pairs are vanishingly rare in the fishes of lowland Amazonia (Albert et al. 2011c, table 2.4), and sympatric congeners whose geographic ranges overlap substantially are rarely sister species (Crampton et al. 2016, Amorim & Costa 2019, Thomaz et al. 2019). It seems almost a rule of Neotropical ichthyology that sympatric fish assemblages accumulated by means of dispersal and ecological coexistence and not by in situ speciation (Albert et al. 2011b, Silva et al. 2016, Lambert et al. 2019, Thomaz et al. 2019).

There are, however, several cichlid clades that pose interesting possible exceptions to this rule (Hulsey et al. 2010, Seehausen & Wagner 2014). Phenotypic plasticity and resource or habitat partitioning may have contributed to the speciation process in several riverine clades of *Crenicichla* (with a total of 95 species) in large rapids of the Amazon and Uruguay basins and in *Amphilophus* (with a total of 23 species) in volcanic lakes of Central America (Burress et al. 2016, 2018a,b; Kautt et al. 2018). Furthermore, several deep clades of Neotropical cichlids (e.g., Geophagini, Cichlasomatini, Heroini) have been suggested to represent the ghosts of adaptive radiations past (Arbour & López-Fernández 2016). However, even among cichlids, adaptive diversification may be an exception to the rule. In the second most species-rich Neotropical cichlid genus, *Apistogramma* (with 94 species), sister species have allopatric distributions, and diversity appears to have accumulated incrementally (Tougard et al. 2017).

A distinctive geographic feature of freshwater ecosystems in northern South America is the extreme dearth of ancient (non-floodplain) lakes. Lacustrine fish flocks are therefore restricted to volcanic lakes of Central America and high-elevation Altiplano lakes in the Andes. However, even the lacustrine assemblage of 23 species of *Orestias* pupfishes (Cyprinodontiformes) in Lake Titicaca is polyphyletic, including no sister-species pairs (Albert et al. 2011c and references therein).

2.3.3. Landscape evolution.

Certain landscape evolution processes can alter the hydrological connections of adjacent river basins, resulting in the concomitant separation and merging of portions of drainage networks and, as a result, modifying the genetic and demographic connections of their resident aquatic biotas (Dias et al. 2014; Albert et al. 2006; 2018a,b). Here we describe the pronounced effects of two such landscape evolution processes on diversification in NFF taxa: erosion-driven river capture and climate-driven eustatic (global) sea-level oscillations.

2.3.3.1. River capture.

River capture, sometimes referred to as stream piracy, is a geomorphological process operating perennially on all continental surfaces. River capture is fascinating from a biogeographic perspective because it moves the location of watershed divides between adjacent
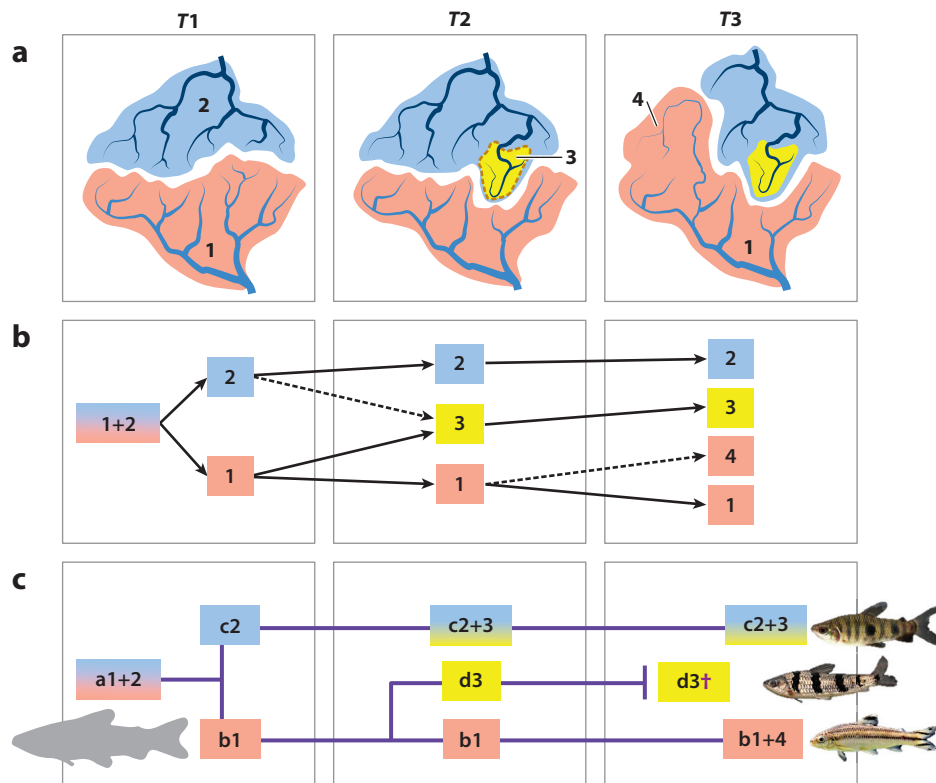
Figure 4

Effects of river capture on macroevolutionary diversification in obligate aquatic and riverine taxa. $T1$–$T3$ are time steps 1–3. (*a*) Colored regions indicate different geographic areas such as river basins, watersheds, and dispersal barriers. (*b*) Changing patterns of area connectivity through time. Solid arrows indicate area identity or vicariance events; dashed arrows indicate area identity or geodispersal events. (*c*) Hypothetical phylogeny of fishes on the evolving landscape. Species are indicated by letters and areas by numbers. The purple cross denotes extinct species. Note at $T2$ the concordant allopatric origin of species d in area 3 and the range expansion of species c into area 3, such that species c and d are sympatric but not sister species. Note also at $T3$ the range expansion of species b into area 4, the extirpation of species c from area 4, and the extinction of range-restricted species d. Figure adapted from Albert et al. (2017).

river basins, simultaneously separating and merging portions of drainage networks and their resi-dent biotas (Albert et al. 2017) (**Figure 4**). River capture therefore plays a pronounced role in the evolutionary diversification of obligate riverine and riparian taxa (Tagliacollo et al. 2015, Albert et al. 2018a, Musher et al. 2019). By separating previously connected river segments, river cap-ture subdivides species' geographic ranges, increasing genetic isolation among populations, and thereby increasing rates of both speciation and extinction (i.e., temporal species turnover). Yet by merging other portions of the drainage network, river capture also facilitates dispersal (i.e., ge-ographic range expansion) among populations, thereby decreasing genetic isolation and slowing rates of speciation and extinction.

The complex effects of river capture that promote diversification in obligate riverine and ripar-ian taxa are predicted by theory and demonstrated by computer simulations (Burridge et al. 2006, Albert & Antonelli 2017, Lyons et al. 2020). These complex effects have also been demonstrated

empirically in several NFF taxa (Albert & Carvalho 2011, Albert et al. 2011c, Carvalho & Albert 2011, Tagliacollo et al. 2015, Roxo et al. 2017). River capture is so efficacious at driving diversification in obligate riverine and riparian taxa at least in part because these groups generally exhibit higher genetic variation among populations distributed across a dendritic (versus Euclidean) landscape (Fagan 2002).

2.3.3.2. *Pleistocene climate oscillations.*   Pleistocene global climate oscillations have also had important biogeographic effects on NFF diversity and distributions, affecting shorelines, regional precipitation regimes, and vegetation cover (Hoorn et al. 2010, Svenning et al. 2015). Fluctuating sea levels directly affect the quality and quantity of habitat areas for animal and plant species on continental lowlands (López-Fernández & Albert 2011, Roxo et al. 2014). Pleistocene sea-level fluctuations resulted in the periodic formation and erosion of riverine floodplains known as the Irion cycle. During interglacial periods, high sea levels back up and slow the flow of lowland sediment-rich (i.e., white-water) rivers, allowing sediments to settle out and build up to form floodplains. When sea levels fall during glacial periods, floodplains erode as the river channel cuts down to the lower base level. Irion cycles strongly affect the number and distribution of NFF species on lowland Amazonian white-water floodplains, one of the most ecologically productive and species-dense continental ecosystems on Earth.

Pleistocene climate oscillations also strongly affect the historical connectivity of river mouths and therefore genetic connections between obligate riverine and riparian taxa (Dias et al. 2014, Albert et al. 2018a). The highest sea level in the past 10 million years was +35 m above modern (∼5.5 Ma), and the lowest level was –122 m at the Last Glacial Maximum (∼21–25 ka). These sea levels correspond to a total of 46 (maximum) and 16 (minimum) separate river mouths discharging to the sea, as compared with 35 separate river mouths on the modern landscape. Responses of sub-basins to sea-level fluctuations depend on elevation and topography, with stronger responses in Eastern Amazon lowlands and the Maranhão coastal plain; responses are also stronger when sea levels rise than when they fall. Global climate oscillations and sea-level fluctuations over the past 10 million years increased extinction rates in the Eastern Amazon lowlands, affecting the biodiversity and biogeography of fishes in northern South America.

3. MACROEVOLUTIONARY MODELS

> Dominance in biodiversity, including the extreme species richness ranked as hyperdiversity, is attained through a fortunate combination of small size, right demographic factors, preemption during colonization and subsequent incumbency, and a suite of key adaptations potent in opening new niches or excluding competitors
>
> —Wilson (2003, p. 14)

Data on the ecology, evolution, and systematics of NFFs generally support Wilson's four causes of hyperdiversity (Albert et al. 2011c). All of the most diverse NFF clades have (*a*) relatively small adult modal body sizes on the order of 10 cm, (*b*) ancient origins as ecosystem incumbents with stem-group ages in the Cretaceous and crown-group radiations in the Paleogene, (*c*) relatively limited dispersal capacities promoting population fragmentation and phylogenetic differentiation in dendritic river networks under the continual influence of river capture separating and merging tributaries, and (*d*) a suite of key adaptations involving habitat and trophic specialization and dispersal limitation that promote the coexistence of high species richness in local assemblages and demographic fragmentation across the landscape.

There are, however, two important caveats to this Wilsonian interpretation. First, not all fish clades with small adult body size, ancient origins, limited dispersal capacity, or putative key

adaptations are in fact diverse, and many clades with these attributes are actually species poor. In other words, the presence of these attributes is a necessary but not sufficient condition for high diversity. Second, it is perhaps better to view the contributions to organismal diversification as acting in a probabilistic rather than a deterministic manner. Details of the geological and climatic contexts always matter, as do the spatial and temporal coincidences in how traits are acquired, the outsized role of rare or unique genetic and environmental events, and the omnipresent contributions of accident and chance, i.e., historical contingency.

3.1. Cradles and Museums of Diversity

Biodiversity data for NFF taxa indicate different modes of diversification in continental South America and in more insular regions of the Greater Antilles and Central America (GACA). These data for South America are complex, supporting patterns that resemble both the cradle and museum models (Albert et al. 2011c, figure 2.15). Data for the GACA region are generally much simpler, more readily matching a cradle model alone. Under a macroevolutionary perspective, an evolutionary cradle is a region where rates of speciation exceed those of extinction, and an evolutionary museum is a place where lineages have resisted extinction over geological time (sensu Rangel et al. 2018). Within continental South America, peripheral geographic regions exhibit many patterns associated with evolutionary museums (e.g., the upland Brazilian and Guianas Shields, the Atlantic coasts of Brazil and Guianas, the Pacific slope of Colombia and Ecuador) or even evolutionary graves (e.g., Altiplano, the Southern Cone). All these peripheral regions have depauperate fish faunas due to high rates of extinction during the Neogene and Quaternary.

The evolutionary museum model is consistent with many NFF clades originating during the Paleogene in clearwater basins draining the upland Brazilian and Guianas Shields, while the evolutionary cradle model is consistent with diversification of these same clades during the Neogene in lowland tropical basins (Albert & Carvalho 2011, figure 7.3). This dual museum/cradle model successfully predicts the exceptionally high species richness in the Amazon-Orinoco-Guianas region that constitutes the biogeographic core of the continent and the high species endemism in river basins of the biogeographic periphery. High diversification rates in the core region were promoted by allopatric speciation in the spatial geometry of fragmented dendritic river networks and a lengthy history of river-capture events over tens of millions of years that episodically fragmented and merged adjacent river segments (Tagliacollo et al. 2015; Albert et al. 2017, 2018a,b; Machado et al. 2018; Thomaz et al. 2019).

While the fossil record of NFF taxa in the Cenozoic is relatively sparse, several localities are known from the Miocene (∼23.0–5.3 Ma) that support a museum model characterized by low extinction rates (Lundberg 1998, Albert & Carvalho 2011). Fish assemblages from the La Venta Formation in Colombia, the Urumaco Formation in Venezuela, and the Pebas formation in Peru display a fauna that is almost entirely modern at the genus level by the early or middle Miocene (Lundberg 1998, López-Fernández & Albert 2011). To emphasize this point, only one Miocene-age fossil fish taxon (†*Acregoliath rancii*) is not ascribed to an extant genus or species group, and this taxon is known from a few isolated scales. In other words, with this trivial exception, all known fish fossils from the Miocene are readily attributed to modern taxa.

In combination, these paleontological, molecular phylogenetic, and biogeographic results suggest that the phenotypes and lineages that characterize modern generic and family-level Amazonian fish clades emerged in the early Cenozoic or Late Cretaceous (Hoorn et al. 2010, Albert et al. 2018b). Thus, in terms of its taxonomic composition, lineage diversity, and phenotypic disparity (a proxy for functional diversity), Amazonian fishes and the species-rich Amazonian

aquatic ecosystem originated tens of millions of years before the geological formation of the modern Amazon and Oronoco river basins (∼10 Ma).

Continental radiation:
evolutionary diversification where adaptation and speciation are decoupled, and where adaptation may occur more slowly than speciation, resulting in functional redundancy

3.2. Continental Versus Adaptive Radiations

Biodiversity data for South American freshwater fishes are more consistent with a model of continental radiation rather than adaptive radiation (Albert et al. 2017). The adaptive radiation model posits a tight functional link between the processes of adaptation and speciation in which both processes are generated by natural selection (Stroud & Losos 2016). By contrast, the continental radiation model relaxes this link, allowing the processes governing phenotypic and lineage divergences to be more decoupled from one another (Simões et al. 2016). In a continental radiation, diversification occurs in several distinct steps dispersed across biogeographic space and through evolutionary time, including allopatric divergence of lineages due to genetic drift and/or selection, geographic range expansion, and neutral or adaptive genetic and phenotypic divergences among species in secondary sympatry (Burns & Sidlauskas 2019, Czekanski-Moir & Rundell 2019). The continental radiation model also predicts more rapid divergence of secondary sexual phenotypes in sympatry than of functional traits related to habitat utilization and trophic specialization (Arnegard et al. 2010).

The continental radiation model is supported by newly emerging phylogenomic studies using thousands of genetic loci among closely related species. These studies indicate that speciation in continentally distributed clades of South American fishes usually occurs in allopatry, often accompanied by incomplete lineage sorting or hybridization (Roxo et al. 2019, Ochoa et al. 2020). However, an intriguing possible exception to this general pattern may be Paleogene diversification of body shapes within tropical South American members of the Cichlinae (Arbour & López-Fernández 2016).

Although many studies have demonstrated the action of natural selection in driving trait evolution in NFF taxa, speciation in these taxa often involves additional factors like secondary gene flow and assortative mating (e.g., Cui et al. 2013, Reznick 2016, Richards & Martin 2017). Ecological speciation in NFF taxa is mostly restricted to peripheral areas of the GACA (e.g., Langerhans et al. 2007, de León et al. 2014, Seehausen & Wagner 2014) or temperate areas of southern South America (e.g., Burress et al. 2016). The diverse fish fauna of the GACA region with >600 species is dominated by just two clades with Miocene origins in the region, heroine cichlids and poeciliids, which together constitute >80% of the GACA fish fauna (Matamoros et al. 2015, Tagliacollo et al. 2017).

Phylogenetic, biogeographic, and ecological data from many NFF clades, including those in both South America and the GACA region, indicate that speciation often occurs in allopatry by neutral divergence due to genetic drift (e.g., Picq et al. 2016) and is often accompanied by changes in chromosomal organization (e.g., Cardoso et al. 2018a). One study (Beheregaray et al. 2015) arguing for ecological speciation in some Amazonian fishes is not well supported due to limits in the study design. That project sampled only parts of the species' natural geographic ranges and did not sample outgroup taxa to determine the polarity of the allelic differences, i.e., to establish if the populations are diverging or converging across their contact zone.

3.3. Ecological Opportunity in a Continental Radiation

While the adaptive radiation model predicts ecological opportunity and ecological release to be main drivers of phenotypic and lineage diversification, lineage accumulation is not predicted to be as limited by ecological opportunities in a continental radiation as in temperate zone or island

radiations (Derryberry et al. 2011). What then, if any, is the role of ecological opportunity in a tropical continental radiation?

As with other diverse tropical biotas, NFF diversification involved a lengthy history of organisms adapting to external environmental challenges posed by geological and climatic events and by the evolution of other taxa. Indeed, much of the phenotypic disparity observed among NFF taxa arose from the different ways that taxa have been able to respond to environmental challenges given their unique phenomic traits and genomic capacities (Burns & Sidlauskas 2019). If ecological opportunity may be reinterpreted as the integration of external environmental and internal organismal factors in the process of adaptive evolution, then this process is readily supported as an important factor in NFF diversification (Arbour & López-Fernández 2016, Roxo et al. 2017).

The evolution of snout lengths in gymnotiform electric fishes provides a clear example of how an internal ontogenetic constraint interacts with external adaptive functions to influence macroevolutionary changes. Certain gymnotiform clades have evolved foreshortened or elongate snout morphologies; each phenotype has evolved several times independently and usually in association with the evolution of trophic specializations of the types in **Figure 2c**. In these gymnotiform clades, snout evolution always arises from allometric changes in the growth of the braincase and snout regions of the skull along the brachycephalic to dolichocephalic (i.e., heterocephalic) axis of skull shape variance (Evans et al. 2017b).

In gymnotiforms, foreshortened (i.e., brachycephalic) snouts always evolve by decreasing, and elongate (i.e., dolichocephalic) snouts by increasing, the amount of developmental integration between these two skull regions. Therefore, in these fishes, adaptations to an external environmental factor (i.e., food type) are facilitated by functionally appropriate changes generated by an internal organismal factor (i.e., developmental integration) that guides macroevolutionary disparification of craniofacial morphologies. Interestingly, heterocephaly is usually the developmental line of least evolutionary resistance (*sensu* Marroig & Cheverud 2005) for craniofacial evolution in vertebrates (Evans et al. 2017a). Indeed, several gymnotiform taxa employ changes in developmental integration to produce extreme sexual dimorphism in snout length used in sexual agonistic and courtship behaviors (Evans et al. 2018).

Developmental integration: increasing the interactions among growth fields during ontogeny such that their phenotypes become more correlated or less compartmentalized

4. SUMMARY

Here we identify nine major biodiversity patterns of the NFF fauna. Some patterns resemble those of terrestrial taxa worldwide, including (*a*) a species–area (and species–water discharge) relationship among major river basins, (*b*) a hollow-curve (power-function) species-richness frequency distribution in which most species are members of only a few highly diverse clades and most clades are species poor, (*c*) exponential (log-linear) accumulation of lineages through time, (*d*) a mid-domain effect with more species near the continental core, and (*e*) a core-periphery pattern with contrasting measures of species richness and species endemism. Whereas the Amazon and Orinoco river basins have extraordinarily high fish species density and number of species with broad geographic ranges (i.e., low percent species endemism), river basins of the geographic periphery have lower species richness and higher percent species endemism. Most NFF clades exhibit their highest diversity in the Amazon and Orinoco river basins, and many early-branching and low-diversity clades are located at the geographic periphery.

Some NFF biodiversity patterns resemble those of other freshwater faunas but not terrestrial taxa worldwide, including (*f*) lowland (not mid-elevation) areas with highest species richness and (*g*) the importance of drainage network position and river network connectivity through time in lineage diversification. Yet other NFF biodiversity patterns are distinct, especially (*h*) Paleogene/ Upper Cretaceous (not Neogene) origins for most genus and family-level higher taxa and (*i*) little

biotic turnover associated with the Late Neogene rise of the Isthmus of Panama or Pleistocene global climate oscillations.

4.1. Major Biogeographic Patterns

Many patterns of biodiversity and biogeography in Amazonian fishes are universal (e.g., latitudinal species gradient, species-area relationship), typical of continental taxa (e.g., core versus periphery arrangement of species richness and endemism, respectively), or distinct for fishes (e.g., lineages generally constrained to individual basins; maximum diversity at lowest elevations). Other patterns emerge from the unique geographic history of the region (e.g., the Amazon basin as the area of highest diversity). Species richness and endemism are spatially heterogeneous, with the highest species richness being in the Greater Amazonian core and the highest species endemism in the continental periphery. Most species have small geographic ranges, with more than half restricted to a single ecoregion, and there is a high beta diversity of species turnover across the landscape. These patterns result both from dispersal limitation relative to historical events (isolation across basin boundaries) and from environmental filtering.

Most speciation in NFF fishes occurred in allopatry, with vicariances attributed to tectonic uplifts, river capture, marine transgressions, and habitat changes associated with global and regional climate change. Vicariant speciation and geodispersal across the semipermeable watersheds of adjacent tributary basins contributed to the accumulation of species-rich faunas over geological time scales. All basin-wide species assemblages are polyphyletic, even within individual genera, suggesting diversification through many rounds of divergence in allopatry, followed by dispersal and subsequent coexistence in sympatry.

4.2. Major Temporal Patterns

The great diversity of Amazonian fishes accumulated over tens of millions of years and over a continental arena. There is little evidence for adaptive radiations, and the high levels of Amazonian species richness did not arise recently, nor rapidly, nor under geographically restricted conditions. Fossil data show that many of the phenotypes and lineages of modern Amazonian fishes are ancient, some dating to the Paleogene or earlier. These radiations were characterized by low rates of extinction, due in part to the capacity of many species to coexist in lowland assemblages and to the immense area, heterogeneity, and interconnectedness of the aquatic habitats. The Neotropical region is unique among the Earth's continental ecosystems in retaining the high species richness generated during the global greenhouse of the Late Cretaceous and early Paleogene. This exceptional fauna is therefore in part relictual, having persisted through a fortuitous combination of geological, climatological, and especially biogeographic processes.

4.3. Conservation

Freshwater organisms and ecosystems throughout the world face immense challenges in the Anthropocene (Albert et al. 2020). Human activities are dramatically altering the distribution and flows of surface, subsurface, and atmospheric waters at regional scales, undermining the resilience of aquatic, riparian, and coastal ecosystems. Neotropical freshwaters are increasingly threatened by many human activities. The most immediate and widespread impacts come from habitat degradation, due to deforestation and other land-use changes from expanding agricultural frontiers, and from the construction of dams and other water diversion and impoundment structures. These structures drown rapids and alter water flow regimes, changing the hydrological connectivity among portions of river drainage networks. Additional important threats vary by region, including

land-use changes and pollution associated with urbanization and mining, overfishing, competition, predation and diseases from the introduction of invasive exotic species, and salination and coastal erosion from rising seas (Dudgeon 2019).

The conservation status of NFF species varies by region, and many rivers are threatened by anthropogenic activities (Reis et al. 2016). Yet in the early decades of the twenty-first century many Neotropical aquatic ecosystems remain relatively well preserved as compared with other regions of the world. Many waterways and aquatic ecosystems of lowland Amazonia retain their exceptionally high native biodiversity. Much of the lowland Amazon, for example, is still covered by dense tropical rainforests and other distinct natural ecosystems, which, although impacted by millennia of human activities, continue to support a large biomass of lowland tropical rainforests (Antonelli et al. 2018a). However, as with most tropical regions worldwide, Neotropical waterways are facing rapid environmental deterioration and conservation threats from a variety of human activities (Castello & Macedo 2016, Pelicice et al. 2017).

Conserving freshwater biotas is an obligation and a challenge for human societies around the world. Proactive conservation policies and enforcement measures are required to preserve the Neotropical ichthyofauna, the most species-rich vertebrate fauna on Earth. The survival of many NFF species into the near future is uncertain, especially for species inhabiting headwater streams, many of which have limited distributional ranges. The singular diversity of Neotropical freshwaters accumulated over a period of tens of millions of years, and once damaged or destroyed, this vital ecosystem will never be replaced. As with all unique forms of life on Earth, extinction is forever.

DISCLOSURE STATEMENT

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