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Evolutionary Innovations and the Diversification of Fishes

By

NICK PEOPLES
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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in

Population Biology

in the

OFFICE OF GRADUATE STUDIES

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DISSERTATION ABSTRACT

Rates of lineage diversification vary considerably across the tree of life. This variation can be caused by ecological factors such as interspecific competition, environmental factors such as habitat expansions, and by evolutionary innovations, which are organismal traits that confer new functions or major increases in performance and have the potential to alter evolutionary trajectories. Often, lineages that possess an innovation may undergo diversification at a faster rate than closely related clades that lack the same trait. Though the ability to produce novel traits can vary between clades and may drive ecological transitions, the impact of differences in the pace at which innovations evolve at macroevolutionary scales has been overlooked. Complex teeth are one innovation that contributed to the evolutionary success of major vertebrate lineages such as mammals and squamates. In my first chapter, I investigate the evolution of complex teeth across ray-finned fishes (Actinopterygii), which represent over half of vertebrate diversity, to show that evolutionary lability of tooth complexity, but not complexity itself, spurs rapid diversification. Speciation rates are 5x higher when transitions between simple and complex teeth occur rapidly. I find that African cichlids are unique across all fishes; they are dominated by lineages that transition between simple and complex teeth at unparalleled rates. This innovation interacted with the ecological versatility of complex teeth to spur rapid adaptive radiations in Lakes Malawi, Victoria, and Barombi Mbo. The marked effect on diversification stems from the tight association of tooth complexity with microhabitat and diet. These results show that phylogenetic variation in how innovations evolve can have a stronger effect on patterns of diversification than the innovation itself. Investigating the impact of innovations through this new lens will likely implicate more traits in causing heterogeneous diversification rates across the tree of life.

In addition to affecting rates and patterns of lineage diversification, innovations can also alter the trajectory and pace of phenotypic diversification. Innovations bring lineages into new ecological zones and as a result, can subject lineages to new functional demands and selective pressures. However, innovations can have varied effects on morphology, which is determined by both intrinsic properties of the innovation and extrinsic properties of the adaptive zone. In this chapter, I show that the evolution of complex teeth with multiple cusps has mixed effects on the trajectory of dental evolution in the adaptive radiation of cichlid fishes in Lake Tanganyika. Complex teeth first evolved outside Lake Tanganyika and facilitated a secondary incursion of riverine lineages into the lake through herbivorous niches. Complex dentitions are more restricted and segregate along multiple axes of dental diversity. This dental novelty generated by the evolution of complex teeth was likely spurred by adaptive evolution pulling traits towards distinct optima. By accelerating the diversification of tooth cusps, complex teeth unlocked a new axis of dental diversity, leading to diversification within but not between ecological niches. These patterns are likely driven by the functional demands of herbivory. These results show that innovations can shape the tempo and mode of morphological change through both intrinsic and extrinsic effects. Because the consequence of innovation is often varied, these results highlight the need to jointly consider these effects to better understand the macroevolutionary diversity of innovations and their outcomes.

In Chapters 1 and 2, I show that innovations can shape the trajectory of evolution through a variety of mechanisms. However, considering the effects of innovations independently ignores the functional and evolutionary interactions among multiple innovations in the same species. Two innovations that have underpinned the ecological and evolutionary success of ray-finned fishes are large teeth and highly protrusible jaws, which both independently expanded the

diversity of prey capture strategies by unlocking new prey types and feeding modes. In this chapter, I explore the functional relationship between these innovations across ray-finned fishes using high-speed videography and phylogenetic comparative methods. I find that these two innovations are functionally and evolutionarily incompatible because there is an overarching tradeoff between jaw protrusion and tooth size. Having large teeth decreases the kinematic diversity of prey capture by restricting species to overtake prey predominantly by swimming, while highly protrusible jaws are only found in species with small teeth. The space within tooth-bearing bones may impose this constraint, by limiting the maximum tooth size of species with gracile jaws adapted for high mobility and jaw protrusion. Nevertheless, some species break this constraint on tooth size through novel adaptations that accommodate exceptionally large teeth, unlocking new feeding modes which may have expanded the nature of aquatic feeding and influenced the ecosystems themselves. Although both high jaw protrusion and large teeth separately expanded prey capture strategies in fishes, they are generally not found in combination and are evolutionarily incompatible.

In my fourth chapter, I return to a central question in evolutionary biology – why, and how, do species diversification rates vary through time. Multiple lines of evidence indicate that speciation and extinction events are concentrated unevenly through space and time, shaping the global distribution of extant biodiversity. Habitats often modulate these dynamics over short timescales by determining the ecological landscape and providing substrate for diversification. In parallel, innovations can evolve and allow lineages to capitalize on ecological opportunity, which often differs between habitats. This raises questions about whether biotic and abiotic heterogeneity across habitats also influenced diversification rate over geological timescales. In this chapter, I show that the rate of species diversification varies through time according to major

differences between aquatic habitats for ray-finned fishes. Phylogeny-wide net diversification rates have accelerated 1.5-1.7x in reef-associated, freshwater benthopelagic and freshwater demersal lineages, which inhabit complex benthic habitats, while remaining constant through time in other less complex habitats such as the pelagic zone. Combined evidence across multiple diversification models implicates the rise of benthic-feeding fishes, which capitalized on functional feeding innovations and the reorganization of benthic resources following the Paleocene-Eocene Thermal Maximum, in driving this pattern of rate increase through time. Our results show that the global biodiversity of ray-finned fishes is shaped by a combination of clade-specific pulses and tree-wide rate shifts, an outcome of the dynamic interplay between the traits of species and the features of the habitats they occupy.

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To those close to me, I think it was clear that I had a (somewhat excessive) interest in fish from a young age. I knew that I wanted to study fish before I even knew that getting a PhD was an option. My parents, Chris and Linda, who always gave their unwavering encouragement and support to me to pursue those interests, even when it meant transforming the basement into an aquarium room or driving me to random parking lots to swap fish with random strangers. My brother Timothy and sister Natalie always joined my relentless pursuit of any form of fishing on family vacations. I was lucky to have my grandparents (Nana and Grandpap) close by growing up, and it was my grandfather Rudy who instilled in me my appreciation for fish, fishing, and the natural world. The first fish I caught and held was with him in the Outer Banks (I decided I would keep the fish in a cardboard box in the sun), and I like to think that's where it all started.

I was incredibly lucky to have the support of many mentors who so often went out of their way to create opportunities for me. At Duke, Stuart Pimm supported my very external and often ambiguous interests in cichlid research. Les Kaufman got me into the field for the first time as an eager, unknowing undergrad finding their own way to Kenya. John Okechi became my Kenyan father and now great collaborator and friend. My qualifying exam (Jay Stachowicz, Phil Ward, Chris Martin, Rachael Bay, and Brian Moore), and dissertation (Phil Ward, Chris Martin) committees challenged me methodologically and to see the big picture in my work. My PhD advisor, Peter Wainwright, has been the most incredible advisor I could have asked for. Peter challenged me to ask bigger questions and then trusted me to develop my own research program. More importantly, Peter has shown me how to carry a research career with integrity and rigor yet to never lose sight of the reasons for why I am in this field. Working with Peter for 5 years has been an absolute joy.

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Chapter 1

Evolutionary lability of a key innovation spurs rapid diversification

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Introduction

Variation in the processes of speciation and extinction has led to an uneven distribution of species across both geographic regions and the tree of life^{1-4,13}. Evolutionary innovations are often cited when diversity varies considerably between clades, a classic example being the pharyngeal jaw of cichlid fishes¹⁴. These traits shape phenotypic, ecological, or lineage diversification rates by facilitating access to new adaptive peaks, often by unlocking previously inaccessible resources^{5,15-17}. However, clades can differ in their capacity to generate novelty^{6-9,18}. Variation in how innovations evolve across clades has been overlooked as a feature, or as an innovation itself, that shapes macroevolutionary diversification patterns. This necessitates an altered approach to key innovation studies in which differences in evolutionary lability – the rate at which an innovation is gained and lost – could instead generate the observed differences in species diversity. This effect on diversification may be strong when the trait is tightly coupled with one or more axes of divergence and change in the state of the trait leads to ecological shifts⁵. Increased lability of ecological innovations may be a feature of many adaptive radiations because niche expansion is crucial during the speciation process¹⁹. However, identifying

phylogenetic variation in lability requires replicated innovations across broad taxonomic scales to overcome the confounding effect of macroevolutionary singularity¹⁸.

The teeth of jawed vertebrates show marked increases in complexity through the addition of tooth cusps over the last 200 million years²⁰. Teeth with multiple cusps are a key innovation in the traditional sense. Their origination left a detectable effect on the tempo of mammalian and squamate evolution by expanding the range of achievable diets and increasing energy intake efficiency^{10–12}. Complex teeth have also evolved in multiple orders of ray-finned fishes (Actinopterygii)²¹, whose over 35,000 extant species constitute half of vertebrate diversity. The success of some fish groups is frequently attributed to functional innovations, many related to the feeding apparatus, that stimulate new evolutionary trajectories^{22,23}. Complex teeth, although found in lineages that occupy a range of habitats, are often associated with herbivory in productive shallow-water environments^{24,25}. Transitions between simple and complex teeth are thus linked to change in diet and habitat, two important speciation axes in fishes²⁶. These observations suggest that high lability of tooth complexity may be important for generating exceptional species diversity in adaptive radiations, where diversification along these axes proceeds rapidly. Moreover, the replicated evolutionary origins and subsequent losses of complex teeth across fishes affords an opportunity to identify differences in lability across a broad phylogenetic scale. Here, we reconstruct the evolution of complex teeth for over 88% of extant ray-finned fishes to address how phylogenetic variation in the evolution of this vertebrate innovation has shaped the tempo of evolutionary diversification in fishes. We test explicit predictions that tooth complexity and its evolutionary lability are key innovations for African cichlids, a famous example of adaptive radiation. By interrogating the effects of each across

phylogenetic scales, we show that exceptional diversification is explained by differences in lability rather than complexity itself.

Results and Discussion

Heterogeneous evolution of complex teeth

To study the evolution of tooth complexity across ray-finned fishes, we classified 30,915 extant species as having simple (single cusp) or complex (multiple cusps) teeth in their oral jaws (Supplementary Table 11), generating one of the largest datasets of a vertebrate morphological trait. Bayesian maximum a posteriori (MAP) ancestral state reconstruction and stochastic character mapping in RevBayes²⁷ reveals multiple evolutionary origins of tooth complexity across extant actinopterygians (Fig. 1a). Complex teeth have evolved at least 86 times, appearing first in the Early Cretaceous 110 million years ago, both in single lineages (e.g. *Helotes sexlineatus*, the Eastern striped grunter) and at the base of large clades (e.g. Characiformes) (Fig. 1a and Supplementary Table 8). The proportion of lineages with complex teeth has gradually increased since the Cretaceous-Paleogene (K-Pg) mass extinction (Fig. 1a,b). Despite this, complex teeth remain rare (11.7% of extant species) across fishes. Reversions to simple teeth are substantially more frequent (1.25-50x higher transition rate) and numerous (n=110 reversions). These often occur within large multicuspid clades (Fig. 1a,c) when lineages shift into specialized predatory niches (e.g. *Hydrocynus*, the African tigerfish, within *Alestidae*). The frequent reversions parallel squamate dental evolution and contrast the more unidirectional pattern in mammals¹¹, while the numerous origins (n=86) suggest complex teeth have evolved from a unicuspid state far more times in fishes than in squamates (24 independent origins¹¹) and mammals (plesiomorphic²⁸). Lineages with complex teeth dominate several diverse, primarily freshwater groups including Characiformes (*Characidae*, *Serrasalminidae*), Cyprinodontiformes

(*Cyprinodontidae*, *Goodeidae*), *Cichlidae* and *Loricariidae* (Fig. 1a,c). They also appear in the enigmatic driftfishes (*Nomeidae*, *Ariommatidae*), lanternfishes (*Myctophidae*) and pencil catfishes (*Trichomycteridae*) albeit less frequently (Supplementary Table 11).

To understand how the evolution of tooth complexity varies across Actinopterygii, we compared the fit of several Markov models of character evolution, including some that accommodate rate variation, using marginal likelihoods and Bayes factor (BF). Our results show that the gain and loss of tooth complexity is a heterogeneous process across ray-finned fishes defined by significant variation in transition rates (Fig. 1a,c,f and Supplementary Table 1) (BF=6.74). Most lineages over time, including 96.2% (n=11,075 species) of extant actinopterygians in our analyses, are defined by low evolutionary lability (Fig. 1b). This is a process where tooth complexity is gained at an exceptionally slow rate and is lost at a rate 50x faster, resulting in a single origin and retention in most descendant lineages (Fig. 1c,f). We identified an increase in the transition rate between simple and complex teeth – high evolutionary lability – originating during the Early Cretaceous (Fig. 1a,b.) and defining just 3.8% (n=433 species) of extant lineages. These taxa evolved complex teeth at a rate three orders of magnitude greater than when lability is low with a significant reduction in the relative rate at which complexity is lost (Fig. 1f). These groups – primarily African *Cichlidae*, *Liparidae*, *Mormyridae* and *Serrasalminidae* – are notable in having a high proportion of lineages with complex teeth (Fig. 1c and Extended Data Fig. 1a,b), high speciation rates, and high rates of phenotypic evolution^{29–31}.

We then explored the effects of tooth complexity and its evolutionary lability on speciation rates given that both innovations and phenotypic evolvability may shape speciation dynamics at large macroevolutionary scales^{29,32}. We used a non-parametric test for state-

dependent speciation to test for effects of tooth complexity and lability. For lability, we assigned each species to the MAP rate regime (“low” or “high”) estimated through our ancestral state reconstruction, regardless of tooth complexity. Although speciation rates for complex-toothed lineages are twice as high as simple-toothed lineages ($\lambda_{\text{complex}} = 0.239$ (sd 0.556), $\lambda_{\text{simple}} = 0.122$ (sd 0.239)), tooth complexity alone does not have a significant effect on speciation rates (one-way phylogenetic ANOVA, $F=170.58$, Cohen’s $d=0.27$, $P=0.194$; FiSSE two-tailed $P=0.06$) (Fig. 1d). Speciation rates for lineages with complex teeth are faster by 0.117 lineages my^{-1} . The high tip-ratio bias³³, low power of FiSSE compared to formal state-dependent speciation and extinction models, and numerous simple-toothed lineages with exceptional speciation rates (Extended Data Fig. 1) likely reduces our ability to detect significant rate asymmetry. However, we find that increased evolutionary lability, regardless of tooth complexity, increases speciation rates by 5x compared to when lability is low ($\lambda_{\text{low}} = 0.116$ (sd 0.206), $\lambda_{\text{high}} = 0.595$ (sd 0.958); one-way phylogenetic ANOVA; $F=1267.05$, Cohen’s $d=0.69$, $P<0.005$; FiSSE two-tailed $P<0.005$) (Fig. 1e). Speciation rates for lineages that evolve complex teeth at higher rates with a lower relative rate of cusp loss are faster by 0.479 lineages my^{-1} . Though there are many predominantly complex families (Fig. 1c), these results show that rapid switching between simple and complex teeth, but not complex teeth alone, has accelerated the diversification of some exceptional groups of ray-finned fishes.

Complexity evolves rapidly in cichlids

Among ray-finned fishes, cichlids have attracted intense interest from evolutionary biologists due to their remarkable adaptive radiations. Many advances have been made in understanding their rapid accumulation of species diversity, implicating a combination of key innovations^{14,34} and rapid ecological diversification driven by exceptional genomic potential^{31,35–}

³⁷. Yet many proposed innovations are unable to explain varying rates of species diversification³⁸. High lability of tooth complexity is concentrated within Cichlidae (>50% of all lineages with high lability) (Extended Data Fig. 7c) but separates the continental radiations of Africa and the Neotropics (Fig. 1c). All Neotropical lineages have low lability and only one (*Herotilapia multispinosa*) has complex teeth. African cichlids are dominated by lineages that rapidly transition between simple and complex teeth with a high proportion of complex-toothed lineages, a feature unique across all ray-finned fishes (Fig. 1a,c). We further explored how complexity and increased evolutionary lability shape rates of species and ecological diversification within African cichlids.

We generated an expanded dataset of tooth complexity classifications covering 92.7% of described cichlid species and representing all genera from Africa, Madagascar, and South Asia (n=1,069 species) (Supplementary Table 12). We then fit an expanded set of Hidden Markov models over a recent complete phylogeny of Cichlidae³¹ to further study how tooth complexity evolves within African cichlids. We find tooth complexity evolves in African cichlids at unparalleled rates under a process that contrasts with all other actinopterygians. Complex teeth evolve and are lost at equal rates (BF=15.46), unlike the global process where complexity is far more likely to be lost than gained (Fig. 1a,f and Supplementary Table 2). These rates vary by ten orders of magnitude across the cichlid tree (Fig. 2a,c), and an exceptionally high rate is unique to rapidly diversifying lineages (Fig. 2a), including many haplochromines endemic to Lakes Malawi and Victoria, as well as oreochromines endemic to Lake Barombi Mbo. This significant increase in lability is recent, originating within the Haplochromini ~9 mya (Fig. 2a,b).

Lability and complexity are innovations

Innovations may accelerate lineage diversification by increasing speciation, decreasing extinction, or both⁵. To formally test if tooth complexity increases rates of lineage diversification within African cichlids, we compared the fit of state-dependent speciation and extinction (SSE) models in RevBayes²⁷. We fit these models over four subtrees to account for the extreme speciation rate variation within African cichlids³¹ and compared models within each subtree using marginal likelihoods and Bayes factors. We find that tooth complexity alone cannot explain patterns of lineage diversification; character-independent models were strongly supported across all subtrees (Supplementary Table 3), suggesting speciation and extinction rates do not depend on whether lineages have simple or complex teeth.

Change in tooth complexity is primarily associated with shifts in habitat and diet in cichlids³⁹. Therefore, we tested if increased lability of tooth complexity shapes speciation and extinction rates. We assigned each species to the MAP transition rate class estimated from our ancestral state reconstruction to represent four discrete levels of lability (“very low”, “low”, “high”, “very high”). We then compared the fit of four-state hidden state speciation and extinction models and character-independent models fit across the full tree (n=1,069 species). We find decisive support (BF=175.91) for a state-dependent model where high lability consistently increases speciation rates across different background rate regimes (Fig. 2d and Supplementary Table 4). Speciation rates are 1.3-2.5x higher when lability is increased across all four hidden states (MuHiSSE-4; Fig. 2d, Extended Data Fig.4, and Supplementary Table 6). This effect on diversification is largely consistent across different relative extinction scenarios (Extended Data Fig. 5,6), as well as under a simpler model (MuHiSSE-2; Extended Data Fig. 2,3). These hidden states primarily separate riverine and Lake Tanganyika lineages from those in

Lakes Malawi, Victoria, and Barombi Mbo which have a higher background diversification rate (Extended Data Fig. 2,4). Differences in evolutionary lability explain speciation rate variation within lake radiations, between riverine lineages, and across habitats.

While we find that tooth complexity alone does not significantly affect rates of lineage diversification within African cichlids, we tested whether complexity shapes the rate of ecological diversification, a major axis of divergence in adaptive radiations¹⁹. Using primary literature, we placed 875 species into six discrete diet categories (Supplementary Table 12). We used a reversible-jump MCMC in RevBayes to estimate dietary transition rates when lineages have simple and complex teeth. Though all diets include species with both simple and complex teeth (Fig. 3c), mean transition rates between diets are 3.28x higher when lineages have complex teeth (one-way ANOVA; $df=59$, $F=15.9$, Cohen's $d=1.03$, $P<0.001$) (Fig. 3a,b). This significant increase in the rate of ecological diversification is facilitated by elevated transitions through herbivory and omnivory (Fig. 3a). Complex teeth are thought to be an adaptation to herbivory, and indeed we find evidence for correlated evolution between these traits (Pagel's model; likelihood-ratio=136.237, $P<0.0001$) (Supplementary Table 7). These results support the hypothesis of complex teeth being a crucial tool for transitions towards this axis of ecological specialization within fishes. In addition, the diverse arrangements of multiple cusps may support a greater diversity of tooth functions⁴⁰. The versatility provided by complex teeth to fill a range of ecological niches (Fig. 3a, c) likely gave lineages with complex teeth an advantage in rapidly changing rift lake environments^{41,42}.

Evolutionary rates often show time-dependency that may signal interesting biological phenomena^{29,43}. The strong effect lability has on diversification rates uncovered in our analyses indicates a speciation mode of character change in extant fishes. We suggest this uniquely high

lability of tooth complexity and complexity itself are key innovations for African cichlids, which have interacted to spur rapid diversification along multiple ecological axes of divergence. A rapid change in tooth complexity can drive divergence along depth gradients because complex teeth are tightly associated with herbivory in littoral habitat, where algal productivity is high. The dietary versatility (Fig. 3a) of complex teeth accelerates ecological diversification while rapid reversion to simple teeth can allow further ecological specialization, such as piscivory⁴⁴. Habitat and ecology are known axes of speciation in cichlids^{26,38} and divergence in tooth complexity also characterizes sympatric ecotypes at early stages of speciation⁴⁵. Haplochromine species, which have colonized Lakes Malawi and Victoria and represent replicate examples of explosive adaptive radiation, have both increased lability of tooth complexity (Fig. 2a) and a propensity to radiate in lakes³⁸. Rapid transitions between simple and complex teeth may facilitate simultaneous divergence in diet and along habitat gradients in rift lakes. Together, these innovations allowed species in Lake Malawi, Victoria, and Barombi Mbo to take advantage of expanding ecological opportunities as the lakes filled, spurring rapid adaptive radiation. Continued increases in tooth complexity within Actinopterygii and across the vertebrate tree may be necessary to maintain diversification rates as niches continue to fill and there is an inevitable push to feed at a lower trophic level^{11,46,47}.

Flexible development underlies lability

Though rare across living fishes, producing complex teeth requires only a few changes within a highly conserved developmental program^{21,48}. Clades that rapidly lose and gain complex teeth may take advantage of this by maintaining a flexible system of tooth development, leading to rapid evolutionary transitions between simple and complex teeth. Many haplochromine cichlids in lakes Malawi and Victoria undergo ontogenetic transformations in tooth complexity

through consecutive tooth replacements, where complex teeth in juveniles can be gradually replaced with simple teeth in adult fish³⁹. This ontogenetic shift in tooth complexity suggests all haplochromine lineages have the inherent developmental capacity to produce complex teeth, irrespective of adult ecology. Differential tuning of developmental pathways over ontogeny underlie trophic innovations in haplochromine cichlids⁴⁹. Change in the timing of events during tooth development⁵⁰, therefore, could be a mechanism for uniquely rapid transitions between simple and complex teeth in this exceptional group.

Conclusions

The evolution of tooth complexity shapes rates of lineage diversification across the largest group of vertebrates, not by an effect of complexity itself but by a strong effect of increased evolutionary lability. We further show that both tooth complexity and lability are key innovations for African cichlids that interact to catalyze species and ecological diversification in rift lake radiations and explain some of the highest speciation rates seen in vertebrates. Our results offer a counterpoint to the traditional view of evolutionary innovations as traits that rarely evolve and directly increase rates of lineage diversification⁵. Differences in how innovations evolve across clades can have an even greater effect on diversification when the trait is tightly coupled to an axis of divergence, such as diet or habitat. This new concept of evolutionary innovation – increased lability of a discrete trait, or an ‘evolutionary seesaw’ – reflects clade-specific differences in evolvability that could explain macroevolutionary variation in speciation rates²⁹. Defining traits as key innovations through this novel lens is supported when the trait is linked to known speciation axes, and developmental mechanisms may underlie the differences in lability³². Identifying these differences in lability requires broad comparative studies and multiple origins of the trait, two criteria often missing from traditional key innovation studies.

Considering evolutionary innovations in this manner will likely implicate many previously overlooked traits as important drivers of the widespread variation in species diversification rates across the tree of life.

Methods

Tooth complexity classification

We generated a dataset of tooth complexity classifications for 30,915 extant species of ray-finned fishes⁵¹, which represents all families, from the existing literature (Supplementary Table 11). We considered species to have complex teeth if primary teeth with more than one cusp were present on the maxilla, premaxilla, or dentary of adult fish. Though complex teeth may vary in the total number of cusps (2-12), this binary classification reflects a major evolutionary transition with functional implications, following the traditional view of a key innovation. The data include all orders of Actinopterygii and missing data is not biased towards any particular group. We generated an expanded dataset for cichlid lineages from Africa, South Asia, and Madagascar (n=1,069) representing 92.7% of taxonomically valid species described before 2019 (Supplementary Table 12)³¹. All categorizations are based on adult fish. We quantified the proportion of lineages with complex teeth and proportion of lineages with high lability for each family that had at least one complex lineage and at least 10 species in the tree built with genetic sequence data (n=31 families). These criteria excluded Anablepidae (5 species in tree), Scoloplacidae (1 species in tree), Astroblepidae (0 species in tree), Ephippidae (8 species in tree), Scatophagidae (3 species in tree), Nomeidae (9 species in tree), and Ariommatidae (3 species in tree). We calculated these proportions for Neotropical and African cichlids, two monophyletic groups, separately to highlight the striking difference between these two continental radiations (Extended Data Fig. 1b).

Phylogenies

We used the Fish Tree of Life¹ as the backbone phylogenetic hypothesis for our analyses across ray-finned fishes. Because phylogenies built using birth-death polytomy resolvers and similar methods break down natural phylogenetic patterns and should not be used for analyses of trait evolution⁵², we pruned our data to match a time-calibrated tree built using only genetic sequence data (n=11,508 species) for all comparative analyses; we recognize the total number of transitions is likely underestimated because of this. For our analyses within cichlids, we used the time-calibrated phylogeny of McGee et al.³¹, which includes all taxonomically valid species prior to 2019 pruned to include the 1,069 species for which we had data on tooth complexity.

Character evolution models

We used continuous time Markov models implemented in RevBayes v1.2.1 to study the evolution of tooth complexity across ray-finned fishes and within cichlids. For our dataset across ray-finned fishes (n=11,508 species), we fit equal rates (ER) and unequal rates (ARD) models. Because the evolution of a trait can vary across large phylogenies^{53,54}, we fit additional ER and ARD models each with two hidden rate categories (HR2-ER, HR2-ARD). These hidden states are rate classes that allow transition rates to vary between classes but impose the constraints of the ER and ARD models within each class. We set a prior of 200 transitions across the tree. We also fit a model that allowed the process (i.e., ER or ARD) to vary across the tree, and rates to vary between the processes (H2-variable). For this, we set a prior of 200 transitions under ARD, 400 transitions under ER, and 10 transitions between these processes. We used a power posterior analysis and stepping-stone sampler to estimate marginal likelihoods for each model. The simulation used 10 stones sampling 1000 states from each step with 5000 generation burn-in. For our expanded cichlid dataset (n=1,069 species), we fit ER, ARD, HR2-ER, HR2-ARD models,

as well as ER and ARD models with four hidden rate categories (HR4-ER, HR4-ARD). We used a prior of 100 transitions between simple and complex teeth. We again used a power posterior analysis and stepping-stone sampler to estimate marginal likelihoods for each model. The simulation used 100 stones, sampling 1000 states from each steps with a burn-in of 10,000 generations. When comparing all character evolution models, we used a flat Dirichlet prior distribution for the root state frequencies. We compared model fit using Bayes factors, where $\ln[\text{BF}(M_0, M_1)] = \ln[P(X | M_0)] - \ln[P(X | M_1)]$ and $\ln[P(X | M_0)]$ is the log-transformed marginal likelihood of M_0 . To estimate ancestral states and transition rates for the best-fit model (HR2-ARD) across ray-finned fishes, we ran two independent MCMC replicates for 50,000 generations with 10% burn-in, with root state frequencies set to an equal probability of simple across either hidden rate categories²¹. We verified both chains converged on the same posterior distribution with Kolmogorov-Smirnov (KS) tests in the R package ‘convenience’⁵⁵, with a precision (α) level of 0.01, and retained a single run for analyses (Extended Data Fig. 7k-p). For cichlids (HR4-ER), we ran two independent MCMC replicates for 50,000 generations with 10% burn-in and set root state frequencies to an equal probability of simple or complex across the two lower hidden rate categories due to complete uncertainty of the root state under a flat Dirichlet distribution. We verified convergence with KS tests ($\alpha=0.01$) and retained a single run for analyses (Extended Data Fig. 8e-j). We note that the MCMC sampler in RevBayes requires fewer generations to reach convergence because each generation is more computationally intensive. We used ‘*ancestralStateTree*’ to compute the maximum a posteriori (MAP) ancestral state estimation and ‘*characterMapTree*’ to generate the MAP character history through stochastic character mapping⁵⁶. We visualized these MAP reconstructions using the R package ‘RevGadgets’ v1.2.1⁵⁷.

State-dependent diversification

We used the non-parametric test FiSSE⁵⁸ to test for state-dependent diversification across our ray-finned fish dataset (n=11,508 species) because of the computational burden of other models (i.e. HiSSE) in a Bayesian framework is prohibitive at this scale. The R functions used to run FiSSE can be found at https://github.com/macroevolution/fisse/tree/master/run_fisse. FiSSE analyses used a tolerance of 0.1 and a parsimony rate type because our ancestral state reconstruction suggests the use of an M-k rate type is not supported (Fig. 1f and Supplementary Table 1). Two-tailed *P* values are reported. We estimated speciation rates using the DR statistic⁵⁹. This metric is considered an estimate of “recent” speciation because it puts more weight on recent splitting events compared to splitting events deeper in the tree. Accordingly, the DR statistic may be robust to issues of non-identifiability⁶⁰. To supplement our FiSSE analyses, we conducted phylogenetic ANOVAs on log-transformed DR values in the R package ‘phytools’ v2.0.4⁶¹. Group sample sizes were 10,361 simple and 1,147 complex species, and 11,075 low and 433 high species for FiSSE and ANOVA tests for effects of tooth complexity and lability, respectively. We used Cohen’s *d* to estimate effect sizes, where *d* is the difference in group means divided by the pooled standard deviation. For our expanded cichlid dataset, we fit a suite of formal Bayesian state-dependent speciation and extinction (SSE) models in RevBayes v1.2.1. Character evolution models indicated the evolution of tooth complexity is a heterogeneous process across African cichlids (Fig. 2a and Supplementary Table 2), which violates an assumption of SSE models⁶². SSE models fit over the full phylogeny did not converge, likely for this reason. To accommodate this, and better account for the extreme diversification rate heterogeneity within Cichlidae³¹, we created four subtrees. Our subtrees represented Lake Tanganyika (n=200 of 240 estimated species³⁵; sampling fraction 0.83), Lake Malawi (n=374 of

399 described species³¹; sampling fraction 0.94), Lake Victoria region superflock (LVRS) (n=169 of a conservative 300 estimated species; sampling fraction 0.56) and lineages in rivers and smaller lakes (n=317 of 318 described species³¹; sampling fraction 0.99). We excluded lineages endemic to Lake Barombi Mbo from this analysis. For each subtree, we fit the same set of seven state-dependent speciation and extinction models which included BiSSE⁶², character-independent models⁶³ with two, three, and four rates (CID-2, CID-3, CID-4), and HiSSE models⁶³ with two, three, and four hidden states (HiSSE-2, HiSSE-3, HiSSE-4) in RevBayes. For all models, we used a normal distribution as the prior distribution of the log speciation and extinction rates, with mean $\ln(\#taxa/2)$ divided by the age of the tree. The prior expected number of character transitions was unique to each subtree; Lake Tanganyika (10 transitions), Lake Malawi (200 transitions), Lake Victoria (50 transitions), Rivers (20 transitions). We used power posterior analysis and stepping-stone sampling to calculate marginal likelihoods of all (n=28) models, with 50 stones sampling 1000 states from each step and 5,000 generations burn-in. We compared models within each subtree using Bayes Factors. Models did not properly mix or reach convergence over the LVRS subtree, possibly due to the young age and high number of species in Lake Victoria itself. For these reasons, we do not interpret state-dependent patterns for the LVRS subtree.

To test if differences in evolutionary lability affect rates of lineage diversification, we first assigned all species to the MAP transition rate class estimated from our ancestral state reconstruction. The level of evolutionary lability was thus a binary trait for ray-finned fishes (low, high) and a four-state trait for cichlids (very low, low, high, very high). We again used FiSSE and phylogenetic ANOVA to compare speciation rates, estimated using the DR statistic, for different levels of lability across ray-finned fishes. For cichlids, we compared four-state

HiSSE models with two (MuHiSSE-2), three (MuHiSSE-3), and four (MuHiSSE-4) hidden states as well as CID-2, CID-3, and CID-4 models fit over the full tree ($n=1,069$ tips), using power posterior analysis and stepping-stone sampling (10 stones, 1000 samples from each step, 5,000 generation burn-in) to estimate marginal likelihoods; we compared the models using Bayes factors. Bayesian hidden-state models inherently test the null hypothesis of character independent diversification; if this hypothesis is supported the posterior distribution of diversification rates within a hidden state would overlap. We interpret this, along with formal model comparison, as combined evidence to support or refute the hypothesis of character-dependent diversification. Models had a prior of 10 transitions in the level of lability. We used MCMC to sample the posterior distribution of speciation and extinction rates for the MuHiSSE-2 and MuHiSSE-4 model to account for instability in the marginal likelihood estimates of more complex models (Supplementary Table 4). We ran two independent chains of 50,000 generations with 10% (MuHiSSE-2) and 20% (MuHiSSE-4) burn-in and verified convergence of the runs with KS tests ($\alpha=0.01$) (Extended Data Fig. 9). We combined the runs and verified convergence of the combined runs using ‘*checkConvergence*’ in the R package ‘convenience’ ($\alpha=0.01$).

Distinguishing between high rates

Ancestral state reconstructions assume that diversification rates are uncorrelated to the character state; high transition rates may therefore be a product of high diversification rates. To address this, we first we fit a HiSSE model using the R package ‘castor’⁶⁴ to estimate transition rates between simple and complex teeth while accounting for differences in speciation and extinction between lineages (100 trials, 100 bootstrap samplings, sampling fraction=0.328). Convergence failed after multiple optimization attempts, likely due to the high tip bias towards species with simple teeth and rarity of lineages with high lability, which decreases the power and

reliability of SSE models^{33,62,65}. We report the estimated transition rates (Supplementary Table 10) to illustrate that qualitative patterns are similar to the HR2-ARD model. For all analyses in the main text, we use the transition rates estimated under the HR2-ARD model. Second, we conducted FiSSE tests over simulated character histories. We simulated 100 character histories of a two-state character with two hidden states (HR2-ARD) across the Actinopterygian tree (n=11,508 species) using a fixed transition matrix using the ‘*rTraitDisc*’ function in the R package ‘ape’⁶⁶. We populated the matrix with the mean rates estimated from the observed data under the HR2-ARD model, rescaled the matrix by a factor of 100, and set equilibrium frequencies equal across all states. For each simulation, we pulled out tip states and conducted FiSSE tests. We find that under the simulated data, tip speciation rates are similar between low and high lability (Extended Data Fig. 7d,e). Median lambda values for low and high lability are similar ($\lambda_{\text{low}} = 0.134$, $\lambda_{\text{high}} = 0.120$). The distribution of two-tailed p-values for FiSSE tests under the simulated data falls outside the significance level of 0.05 (96/100 tests) (Extended Data Fig. 7f).

Extinction rate assumptions

To test if our results were robust to assumptions on the extinction rate, we followed the methods of Zenil-Ferguson et al.⁶⁷ to place lower bounds on the prior distribution of extinction rates for both the MuHiSSE-2 and MuHiSSE-4 models. Extinction rates (μ) were defined as a linear function, where $\mu = A * \lambda + \delta$ where $A = 0.7, 0.8, 0.9, 1, 1.1$ and δ is a random variable that allows extinction to be greater than $A*\lambda$. A represents a lower bound on the extinction fraction (μ / λ), so $A = 0.7$ defines that extinction rates must be at least 70% of speciation rates. For each A , we estimated speciation and extinction rates under the MuHiSSE-2 and MuHiSSE-4 model using MCMC; each chain was run for 100,000 generations. The posterior distributions of

speciation and net diversification rates for all values of A (0.7, 0.8, 0.9, 1.0, 1.1) and when μ and λ are freely estimated are shown in Extended Data Fig. 3 (MuHiSSE-2) and Extended Data Fig. 6,7 (MuHiSSE-4). We find that when $A=0.7, 0.8, 0.9$, the effect of lability on net diversification rates is consistent with the patterns under the free MuHiSSE-2 and MuHiSSE-4 model (Extended Data Fig. 3a,5), but the magnitude of the estimated rates is reduced. When $A=1.0$, we find that the posterior distribution of rates largely overlap within all hidden rate categories. When $A=1.1$, we find the reverse effect, where increased lability appears to decrease net diversification rates. This reversed effect is likely due to the constraint of extinction being at minimum 110% of speciation rates; if a trait markedly increases speciation rates, then the corresponding extinction rates are forced to be high, resulting in a large effect on net diversification. Indeed, we find that when net diversification is negatively affected by increased lability for $A=1.1$, speciation rates show a strong positive effect of lability (Extended Data Fig. 3b,6). These patterns are consistent with the results of Zenil-Ferguson et al.⁶⁷, indicating that high lability has a greater absolute effect on net diversification than low lability.

Transition rate prior sensitivity

The inferred number of character transitions, character histories, and transition rates, may be influenced by the choice of the transition rate prior. To test for prior sensitivity, we estimated the parameters of the HR2-ARD model under five priors: 50, 150, 200, 250, and 400 transitions. Across priors, the relative difference between transition rates in both low and high lability categories is consistent (Supplementary Table 8) and the estimated number of transitions and transition rates are consistent across all priors when lability is low (Supplementary Table 9). We find that increasing the prior number of transitions above 200 results in inflated transition rates when lability is high, driven by rapid consecutive state changes along the same branch (Extended

Data Fig. 7a). Increasing the prior decreases the number of lineages with high lability and further isolates African cichlids as the dominant lineages with high lability (Extended Data Fig. 7b,c). We replicated our FiSSE analyses to test for an effect of lability from the ancestral state reconstructions across all five priors. We find that the effect of lability on tip speciation rates is insensitive to the choice of prior on the transition rate; lineages with high lability have significantly higher tip speciation rates across all five priors (50: $\lambda_{\text{low}} = 0.114$, $\lambda_{\text{high}} = 0.28$, two-tailed $p < 0.05$; 150: $\lambda_{\text{low}} = 0.116$, $\lambda_{\text{high}} = 0.507$, two-tailed $p < 0.01$; 250: $\lambda_{\text{low}} = 0.117$, $\lambda_{\text{high}} = 0.744$, two-tailed $p < 0.005$; 400: $\lambda_{\text{low}} = 0.117$, $\lambda_{\text{high}} = 0.818$, two-tailed $p < 0.005$). We also tested the sensitivity of transition rates within African cichlids to the prior. We estimated parameters of the HR4-ER model under three priors; 50, 100, and 150 transitions. We find that the transition rate estimates are stable under different priors (Extended Data Fig. 8a-d).

Cichlid diet data

We categorized 875 species of cichlids into six diet categories based on the dominant adult prey type of each species. Categorizations were based on primary literature (Supplementary Table 12) and FishBase⁶⁸. We classified species into one of six groups: piscivore, zooplanktivore, zoobenthivore (insects/molluscs), herbivore, omnivore, and carnivore specialist (lepidophagy and paedophagy). For all diet analyses, we used a tree pruned to include only the 875 species for which we had data on both diet and tooth complexity.

Diet evolution in cichlids

To test if tooth complexity accelerated ecological diversification, we first expanded our dietary dataset to include an associated binary state for tooth complexity (simple or complex) resulting in 12 combined diet-tooth states. All dietary classifications included both simple and

complex lineages (Fig. 3c). We used a reversible-jump MCMC in RevBayes v1.2.1 to estimate transition rates between dietary categories within simple and complex lineages. We included a “null” model where the transition rate between any state was assumed to be 0 (as some transitions likely never occur, i.e. simple piscivore to simple herbivore), and an unequal rates model that assumes the transition rate between any state is greater than 0, with a prior of 300 transitions. We set a prior probability of 0.25 that any transition rate is equal to 0. The MCMC was run for 10,000 generations, sampling every generation with 10% burn-in. We verified convergence of the transition rate estimates using ‘*checkConvergence*’ in the ‘convenience’ R package ($\alpha=0.01$; effective sample size (ESS) > 625 for all transition rate parameters). Mean rates were calculated from the posterior distribution for further analyses. We directly estimated the posterior probability that any rate was equal to 0; rates were fixed to be 0 if the posterior probability was estimated to be >0.75. We log+0.01 transformed mean rates to fulfil assumptions of normality (Shapiro-Wilk test; $W=0.96$, $p>0.05$) and used an ANOVA test to compare rates when lineages have complex teeth ($n=30$) to when lineages have simple teeth ($n=30$). We estimated the effect size with Cohen’s d . To test for correlated evolution between complex teeth and herbivory, we first converted our dietary classifications into a binary trait of herbivores and non-herbivores. We then used the ‘*fitPagel*’ function in the R package phytools v2.0-4⁶¹ to fit independent and dependent trait evolution models⁶⁹ and compared the fits with a likelihood-ratio test. Our results are robust to an alternate binarization of herbivory (herbivores vs. herbivores and omnivores) (Supplementary Table 7).

Figures

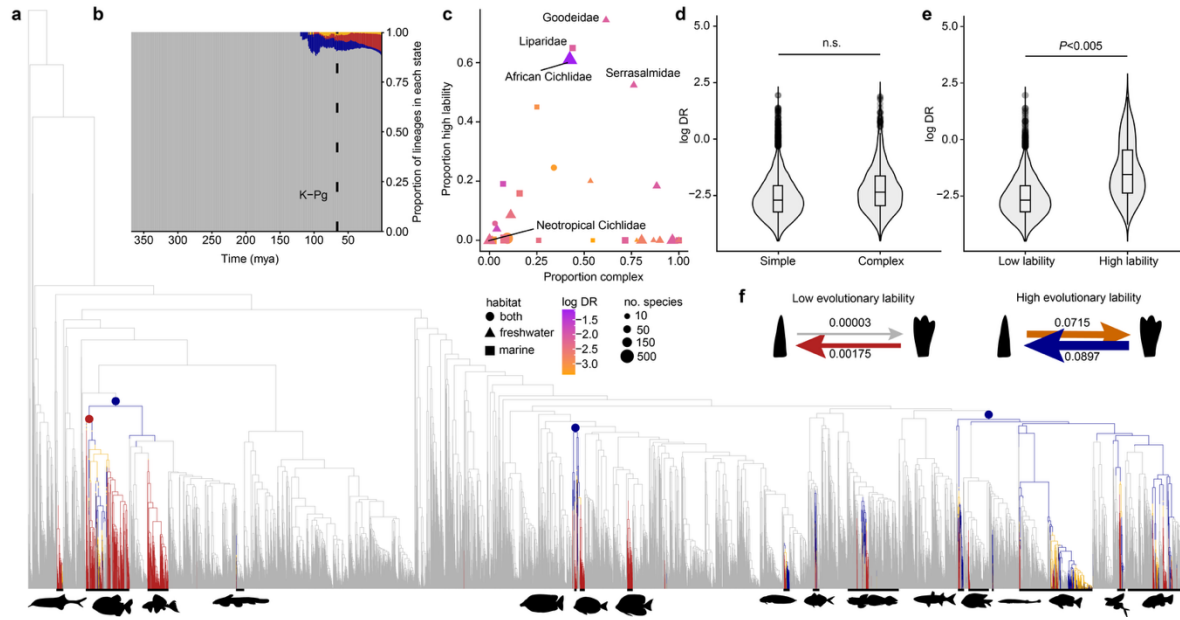


Figure 1 | The evolutionary dynamics of tooth complexity across ray-finned fishes. A, Maximum a posteriori (MAP) ancestral state reconstruction of complex teeth across ray-finned fishes (n=11,508 species) using stochastic character mapping under a hidden-rates model with two rate categories. Branches are colored by tooth complexity and lability (simple low lability – grey; simple high lability – dark blue; complex low lability – red; complex high lability – gold). Major rate increases ~100 mya are marked with blue points, and the earliest transition to complex teeth is red. Outlines represent highlighted clades (from left to right) *Mormyridae*, Characiformes, *Loricariidae*, *Mochokidae*, *Siganidae*, *Acanthuridae*, *Pomacanthidae*, *Liparidae*, *Nomeidae* and *Stromateidae*, *Gobiidae*, *Mugilidae*, *Pomacentridae*, *Gobiesocidae*, *Cichlidae*, Beloniformes, and Cyprinodontiformes. **B,** Proportion of branches in each state of tooth complexity (simple, complex) and lability (low, high) in million-year intervals. The x-axis represents time before present in million-years, progressing from left (past) to right (present). The dashed line marks the Cretaceous-Paleogene (K-Pg) boundary ~66 mya. **C,** The proportion

of lineages with complex teeth (x-axis) and high lability (y-axis) across families that have at least one lineage with complex teeth (n=31 families). Point color indicates the family mean speciation rate, shape represents habitat (freshwater, marine, both) and size indicates the number of species in the family. Distribution of log-transformed tip speciation rates between **d**, lineages with simple (n=10,361 species) and complex (n=1,147 species) teeth ($P=0.0602$) and **e**, lineages with low (n=11,075 species) and high (n=433 species) lability ($P=0.0025$). Boxplots show the median (middle horizontal line), interquartile range (box), min-max values (vertical lines) and outliers (points). Two-tailed significance was assessed using non-parametric FiSSE tests. **F**, Transition rates, reported as the number of transitions per my, between simple and complex teeth when lability is low compared to when lability is high. Arrows indicate transition direction; colors follow those of **a**.

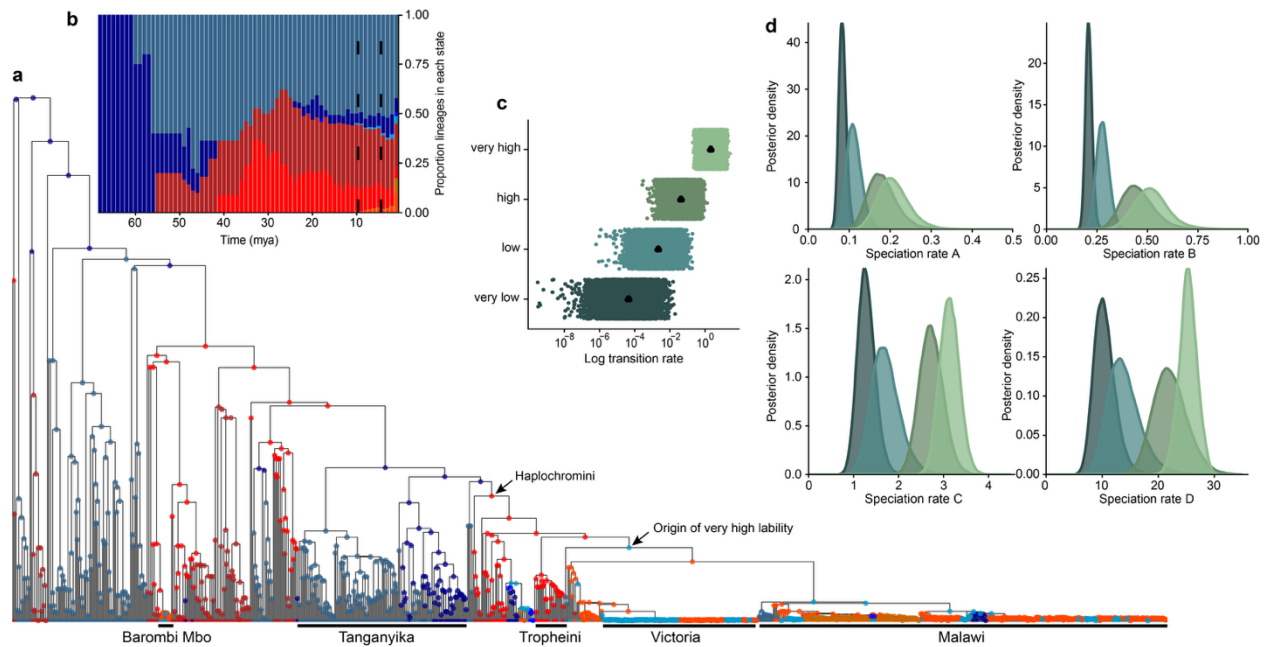


Figure 2 | Rapid evolution of tooth complexity accelerates species diversification in African

cichlids. A, MAP ancestral state reconstruction across cichlids from Africa, Madagascar, and South Asia (n=1,069 species) under an equal-rates model with four hidden rate categories.

Lineages with simple teeth are colored variations of blue, and lineages with complex teeth are colored variations of red; brighter colors represent higher rates (i.e. different levels of lability).

B, Proportion of branches in each state of tooth complexity and lability in million-year intervals.

The x-axis represents time before the present in million-years, progressing from left (past) to right (present). The dashed lines marks the age of Lake Tanganyika (9.7 mya) and Lake Malawi (~3.2 mya). All lineages endemic to Lake Victoria are found in the bar closest to the present.

C, Posterior distribution of log-transformed transition rates between simple and complex teeth. The mean of each distribution is indicated with black triangles. Note the magnitude of variation

between levels of evolutionary lability. **D,** Posterior distribution of net diversification rates

(speciation minus extinction) estimated under a MuHiSSE-4 model. Note the different scales of

baseline speciation between the four hidden states. Colors follow the varying levels of lability in

c.

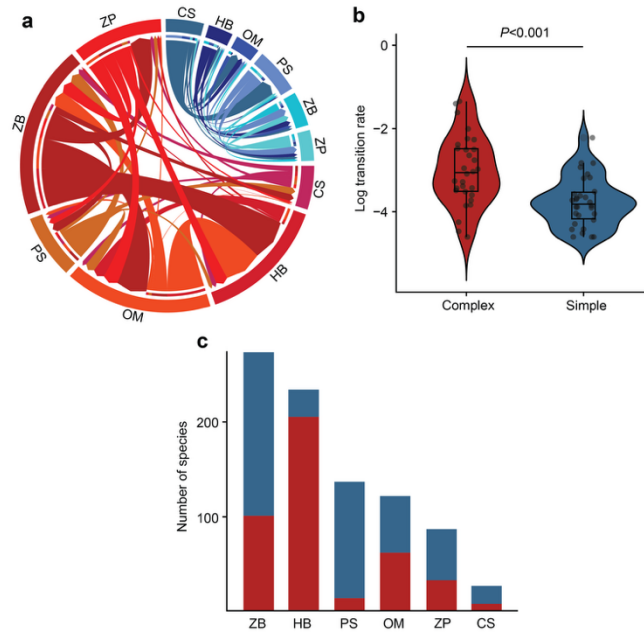
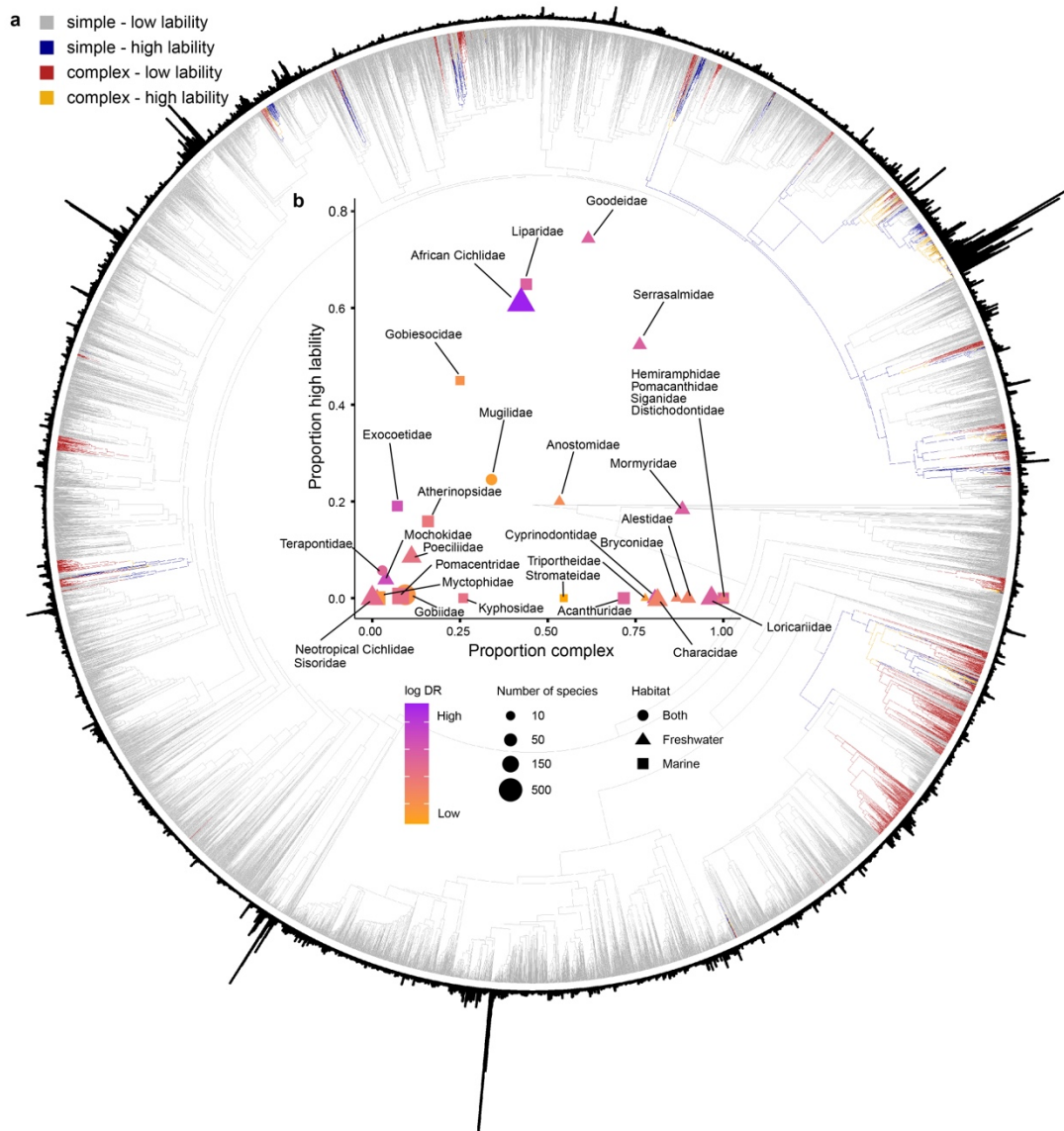
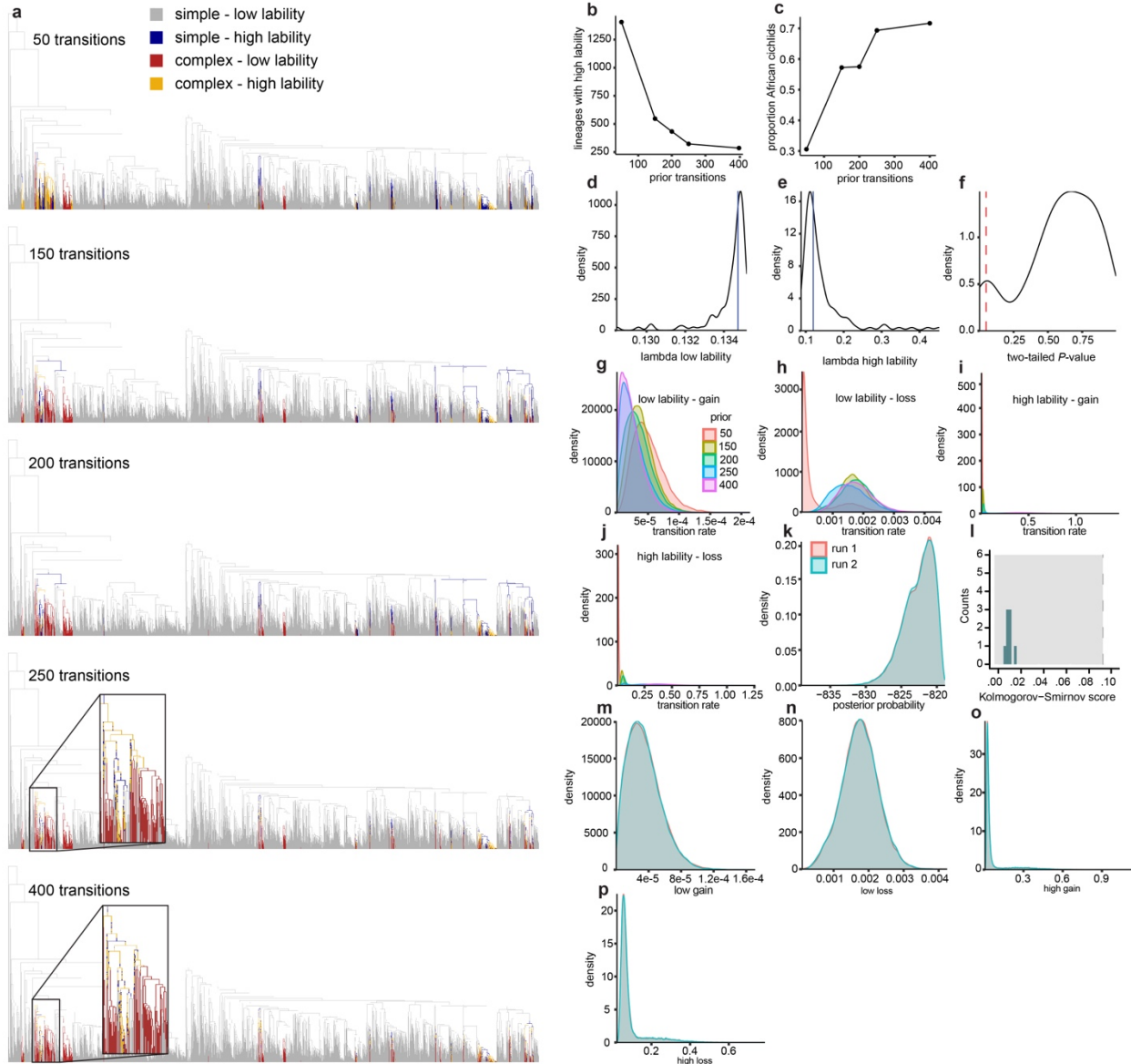


Figure 3 | State-dependent diet diversification in cichlids. **A**, Directionality and magnitude of transitions between diet categories within simple (blue hues) and complex (red hues) lineages. The maximum width of inner connections are scaled by the mean estimated transition rate, and the width of outer bars scaled by the total number of transitions to and from each category. Arrows indicate directionality. Labels correspond to diet (OM=omnivore, PS=piscivore, ZB=zoobenthivore, ZP=zooplanktivore, HB=herbivore, CS=carnivore specialist). **B**, Violin plot depicting a significant increase in mean log+0.01 transformed diet transition rates when lineages have complex teeth ($P=1.89E-4$). Each point corresponds to the mean rate of the posterior distribution estimated for each transition ($n=30$ simple, $n=30$ complex). Boxplots show the median (middle horizontal line), interquartile range (box) and min-max values (vertical lines). Statistical significance was calculated using one-way analysis of variance (ANOVA). **C**, Number of simple (blue) and complex (red) lineages within each discrete diet category.



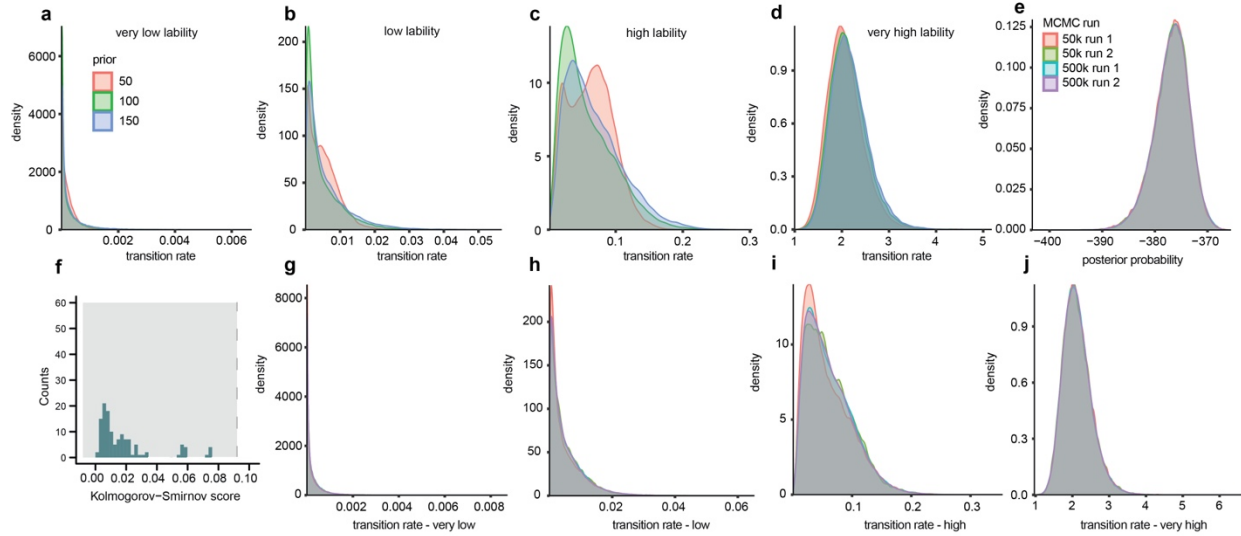
Extended data figure 1 | The phylogenetic distribution of complex teeth and high liability across ray-finned fishes. A, Maximum a posteriori stochastic character map of tooth complexity and its evolutionary liability for 11,508 species of ray-finned fishes under a HR2-ARD model. Lineages with simple teeth are light grey (low liability) and dark blue (high liability); lineages with complex teeth are red (low liability) and gold (high liability). DR statistic values, a measure of species' tip speciation rate, are plotted at the tips; longer bars indicate higher speciation rates. **B,** The proportion of lineages with complex teeth and high liability across families of ray-finned

fishes that have at least one complex lineage (n=31 families). The color of the points corresponds to family mean speciation rate, estimated using the DR statistic. The shape of the points represent habitat (freshwater, marine, both) and the size of the points are scaled by the total number of species. African and Neotropical cichlids are indicated separately to highlight the difference between these groups.

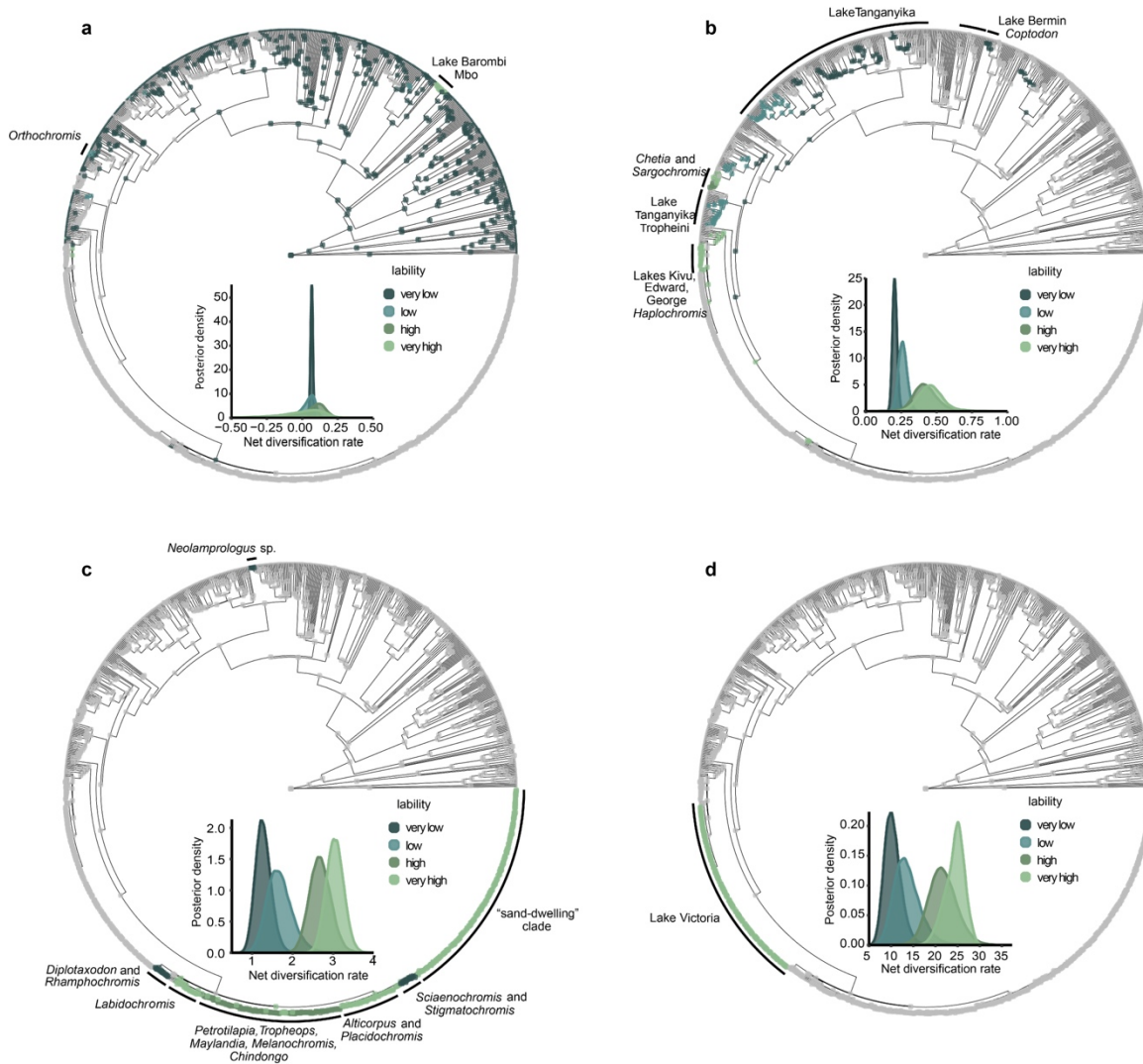


Extended data figure 2 | The evolution of tooth complexity and effect of lability are robust to assumptions on the prior transition rate in ray-finned fishes. A, Maximum a posteriori ancestral state reconstruction of tooth complexity and its evolutionary lability across ray-finned fishes (n=11,508 species) under the HR2-ARD model, under five different priors (50, 150, 200, 250, 400) for the total number of transitions. The insets for 250 and 400 highlight that rapid consecutive state changes on long branches inflate the transition rates in the “high lability” state under these priors. **B,** The total number of lineages with high lability and **c,** the proportion of

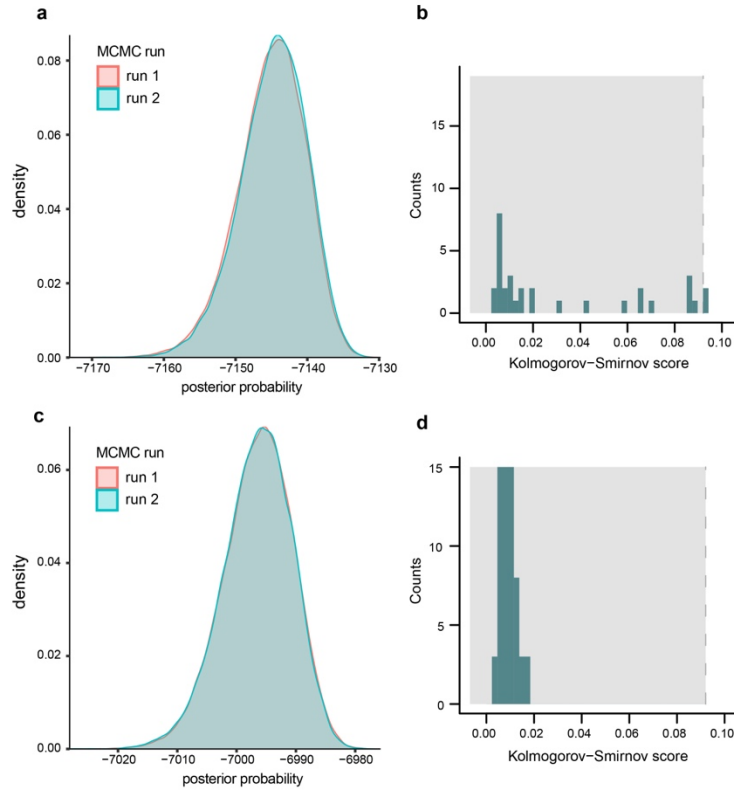
lineages with high lability that are African cichlids under five different prior number of transitions. **D-f**, Simulated data shows no effect of lability on speciation rates. Distribution of FiSSE tip speciation rates for **d**, low lability and **e**, high lability over 100 ancestral state reconstructions simulated under the observed HR2-ARD model. Median rates are indicated with blue lines. **F**, Distribution of FiSSE two-tailed p-values for 100 simulated character histories; the red line marks the significance level of 0.05. **g-j**, Transition rates are robust to assumptions on the prior number of transitions. Posterior distribution of estimated rates for **g**, low lability gain, **h**, low lability loss, **I**, high lability gain and **j**, high lability loss under the HR2-ARD model for five priors (50, 150, 200, 250, 400) on the number of transitions. **K-p**, Convergence of the HR2-ARD model. **K**, Posterior probability distribution of two replicate MCMC runs of the HR2-ARD model. **L**, Histogram of Kolmogorov-Smirnov (KS) scores for model parameters. The grey dotted line marks the threshold for the KS test of 0.0921 ($\alpha=0.01$, ESS>625), indicating that the model parameters were drawn from the same distribution for both replicate runs. Posterior distribution of transition rates across replicate MCMC runs for **m**, low lability gain, **n**, low lability loss, **o**, high lability gain, and **p**, high lability loss.



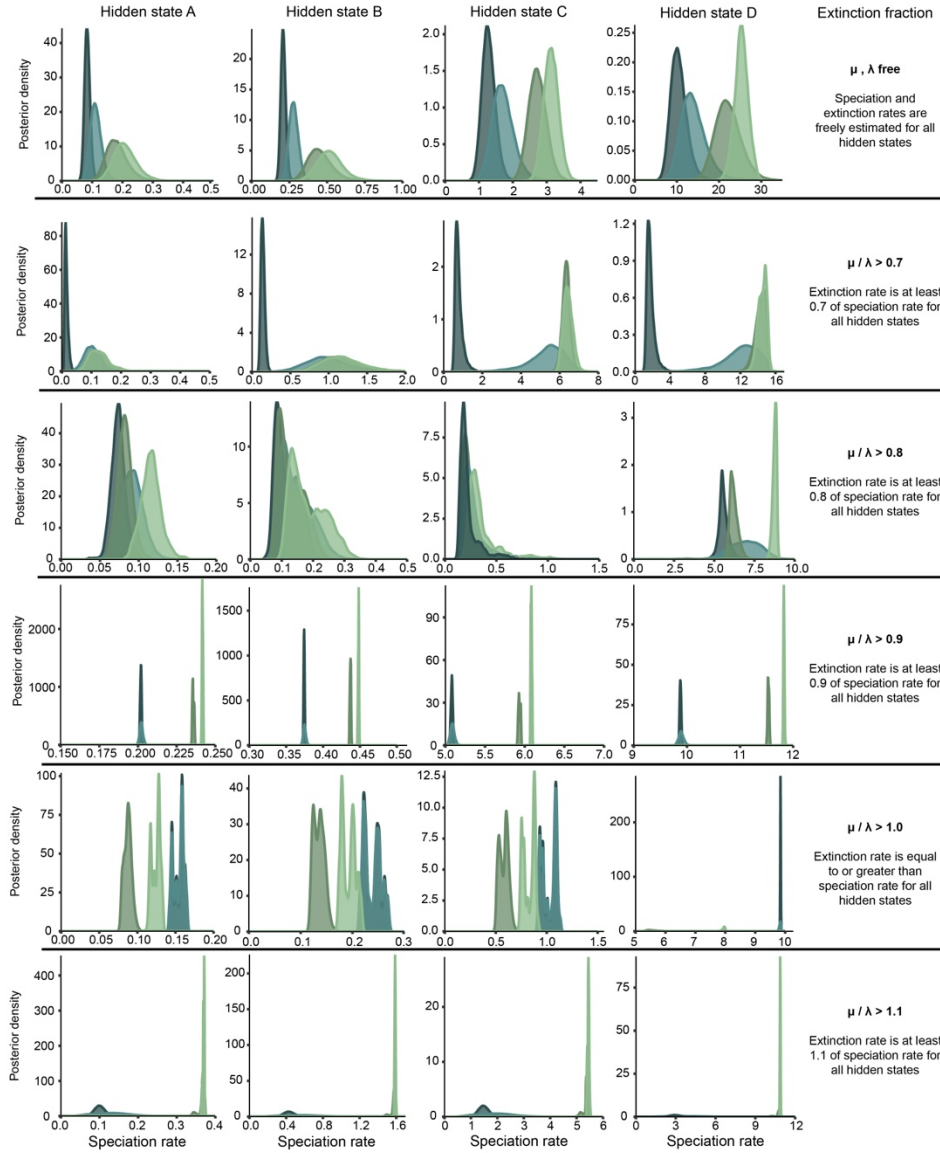
Extended data figure 3 | Convergence and prior sensitivity analyses for the HR4-ARD model in African cichlids. Posterior distribution of estimated transition rates under the HR4-ER model for three different priors (50, 100, 150) on the total number of transitions; **a**, very low liability, **b**, low liability, **c**, high liability, and **d**, very high liability. **E**, Posterior probability distribution of four replicate MCMC runs of the HR4-ER model; two chains ran for 50,000 generations and two chains ran for 500,000 generations. **F**, Histogram of Kolmogorov-Smirnov (KS) scores for model parameters. The grey dotted line marks the threshold for the KS test of 0.0921 ($\alpha=0.01$, ESS>625), indicating that the model parameters were drawn from the same distribution for all replicate runs. Running the chain longer resulted in the same posterior probability distribution and transition rate estimates despite ESS scores <625 for some parameters for 50,000 generations. Posterior distribution of transition rates across replicate MCMC runs for **g**, very low liability **h**, low liability **i**, high liability and **j**, very high liability.



Extended data figure 4 | Evolutionary lability of tooth complexity increases net diversification rates across four background rate regimes in African cichlids. Maximum a posteriori (MAP) ancestral state reconstruction of evolutionary lability for African cichlids ($n=1,069$ species) under the MuHiSSE-4 model. Lineages in **a**, hidden state A, **b**, hidden state B, **c**, hidden state C, and **d**, hidden state D are highlighted separately. Selected clades are labeled for each hidden state. The posterior distribution of net diversification rates are reported for each level of lability, within each hidden state.

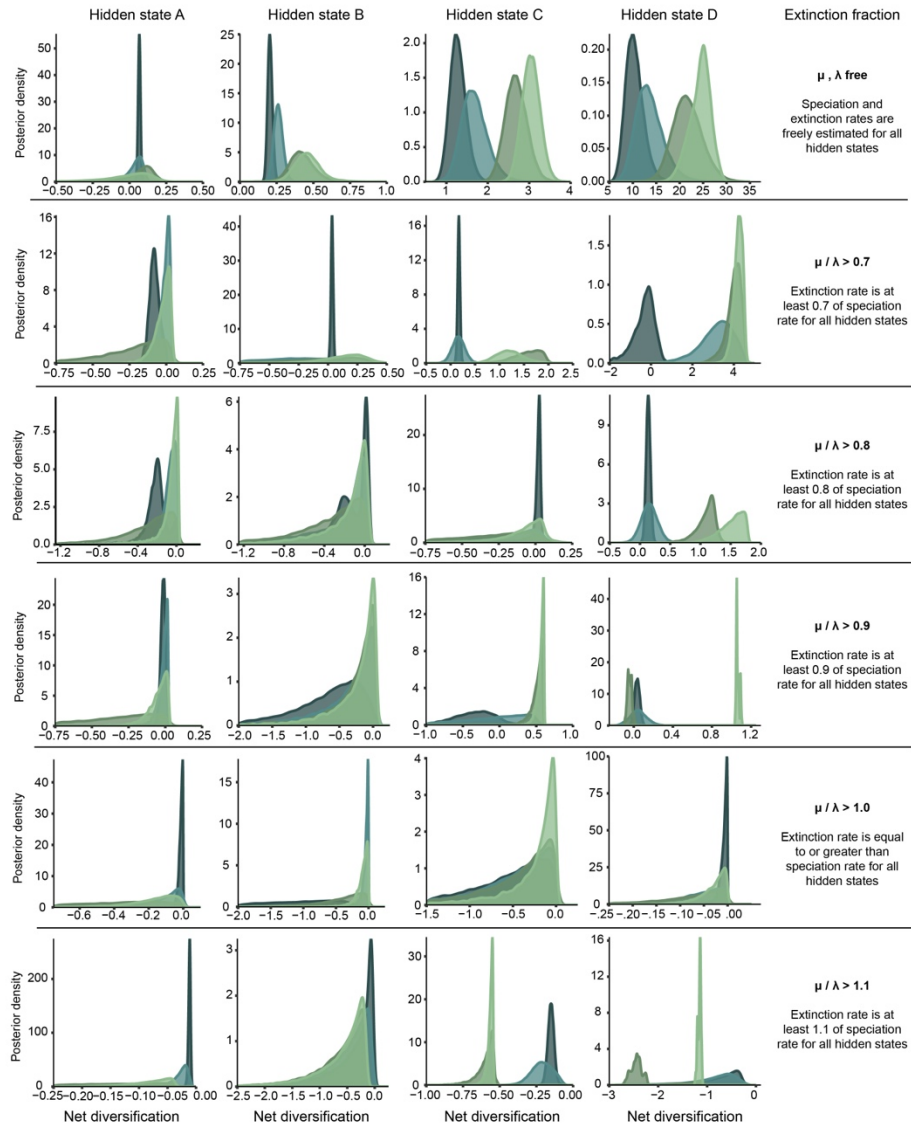


Extended data figure 5 | Convergence of the MuHiSSE-2 and MuHiSSE-4 models, fit across African cichlids. **A**, Posterior probability distribution of two replicate MCMC runs of the MuHiSSE-2 model. **B**, Histogram of Kolmogorov-Smirnov (KS) scores for MuHiSSE-2 model parameters. The grey dotted line marks the threshold for the KS test of 0.0921 ($\alpha=0.01$, ESS>625), indicating that the model parameters were drawn from the same distribution for both replicate runs. Two parameters fall just outside this threshold. **C**, Posterior probability distribution of two replicate MCMC runs of the MuHiSSE-4 model. **D**, Histogram of Kolmogorov-Smirnov (KS) scores for MuHiSSE-4 model parameters. The grey dotted line marks the threshold for the KS test of 0.0921 ($\alpha=0.01$, ESS>625), indicating that the model parameters were drawn from the same distribution for both replicate runs.

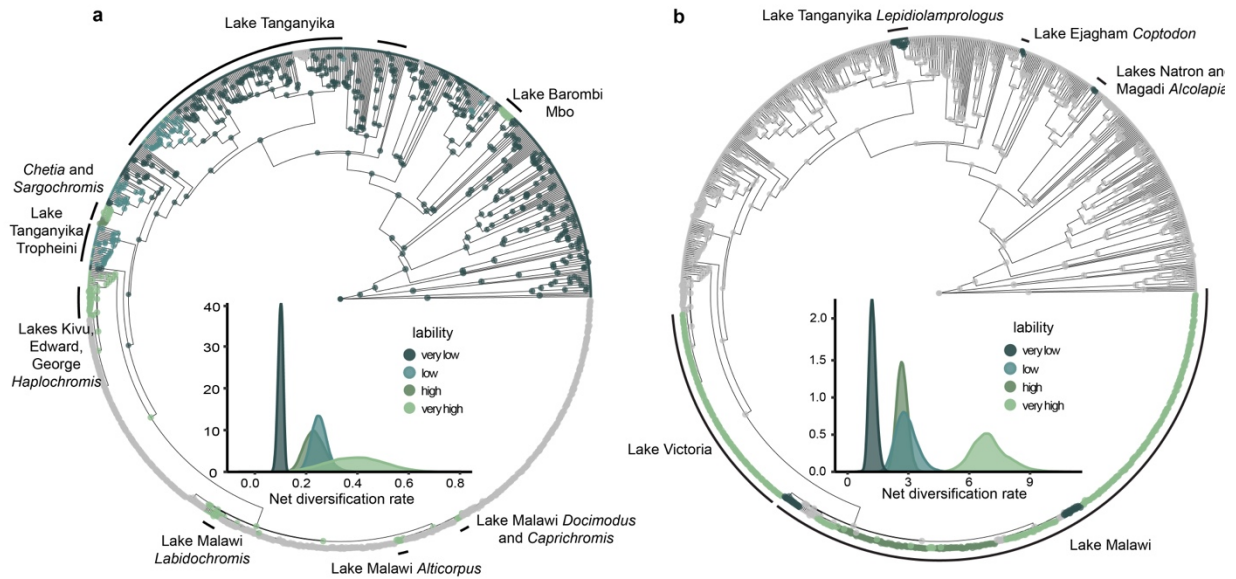


Extended data figure 6 | Net diversification rates for African cichlids under varying levels of lability and six relative extinction scenarios, estimated under a MuHiSSE-4 model.

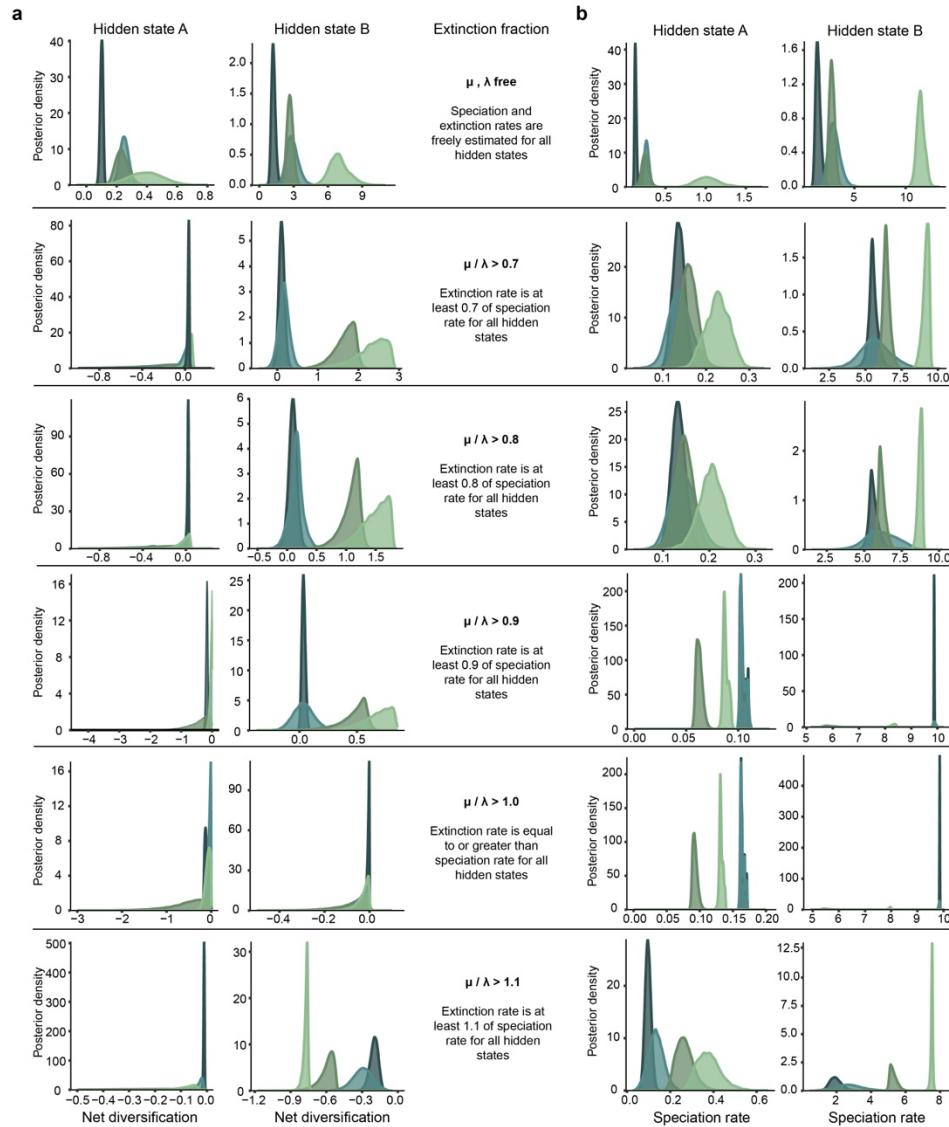
Posterior distributions of net diversification rates, measured as speciation (λ) minus extinction (μ), for four levels of lability across four hidden states (A-D). Rates were estimated under six different relative extinction scenarios by setting lower bounds on the extinction rate (μ).



Extended data figure 7 | Speciation rates for African cichlids under varying levels of lability and six relative extinction scenarios, estimated under a MuHiSSE-4 model. Posterior distributions of speciation rates for four levels of lability across four hidden states (A-D). Rates were estimated under six different relative extinction scenarios by setting lower bounds on the extinction rate (μ).



Extended data figure 8 | Net diversification and speciation rates for African cichlids under varying levels of lability and six relative extinction scenarios, estimated under a MuHiSSE-2 model. A, Posterior distributions of net diversification rates (speciation – extinction) for two levels of lability across two hidden states (A, B). **b,** Posterior distributions of corresponding speciation rates (λ). Rates were estimated under six different relative extinction scenarios by setting lower bounds on the extinction rate (μ).



Extended data figure 9 | Evolutionary lability of tooth complexity increases net diversification rates across two background rate regimes in African cichlids. Maximum a posteriori (MAP) ancestral state reconstruction of evolutionary lability for African cichlids (n=1,069 species) under the MuHiSSE-2 model. Lineages in **a**, hidden state A and **b**, hidden state B are highlighted separately. Selected clades are labeled for each hidden state. The posterior distribution of net diversification rates are reported for each level of lability, within each hidden state.

Main Text References

1. Rabosky, D. L. *et al.* An inverse latitudinal gradient in speciation rate for marine fishes. *Nature* **559**, 392–395 (2018).
2. Weir, J. T. & Schluter, D. The Latitudinal Gradient in Recent Speciation and Extinction Rates of Birds and Mammals. *Science* **315**, 1574–1576 (2007).
3. Schluter, D. & Pennell, M. W. Speciation gradients and the distribution of biodiversity. *Nature* **546**, 48–55 (2017).
4. Roelants, K. *et al.* Global patterns of diversification in the history of modern amphibians. *Proc. Natl. Acad. Sci. U.S.A.* **104**, 887–892 (2007).
5. Miller, A. H., Stroud, J. T. & Losos, J. B. The ecology and evolution of key innovations. *Trends in Ecology & Evolution* **38**, 122–131 (2023).
6. Lovette, I. J., Bermingham, E. & Ricklefs, R. E. Clade-specific morphological diversification and adaptive radiation in Hawaiian songbirds. *Proc. R. Soc. Lond. B* **269**, 37–42 (2002).
7. Martin, C. H. & Wainwright, P. C. Trophic novelty is linked to exceptional rates of morphological diversification in two adaptive radiations of *Cyprinodon* pupfish. *Evolution* **65**, 2197–2212 (2011).
8. De Alencar, L. R. V., Martins, M., Burin, G. & Quental, T. B. Arboreality constrains morphological evolution but not species diversification in vipers. *Proc. R. Soc. B.* **284**, 20171775 (2017).
9. Burns, M. D. & Bloom, D. D. Migratory lineages rapidly evolve larger body sizes than non-migratory relatives in ray-finned fishes. *Proc. R. Soc. B.* **287**, 20192615 (2020).
10. Hunter, J. P. & Jernvall, J. The hypocone as a key innovation in mammalian evolution. *Proc. Natl. Acad. Sci. U.S.A.* **92**, 10718–10722 (1995).
11. Lafuma, F., Corfe, I. J., Clavel, J. & Di-Poi, N. Multiple evolutionary origins and losses of tooth complexity in squamates. *Nat Commun* **12**, 6001 (2021).
12. Luo, Z.-X. Transformation and diversification in early mammal evolution. *Nature* **450**, 1011–1019 (2007).
13. Ricklefs, R. E. Global variation in the diversification rate of passerine birds. *Ecology* **87**, 2468–2478 (2006).
14. Liem, K. F. Evolutionary Strategies and Morphological Innovations: Cichlid Pharyngeal Jaws. *Systematic Zoology* **22**, 425–441 (1973).
15. Hunter, J. P. Key innovations and the ecology of macroevolution. *Trends in Ecology & Evolution* **13**, 31–36 (1998).
16. Rabosky, D. L. Phylogenetic tests for evolutionary innovation: the problematic link between key innovations and exceptional diversification. *Phil. Trans. R. Soc. B* **372**, 20160417 (2017).
17. Miller, Alden H. Some ecological and morphologic considerations in the evolution of higher taxonomic categories. In *Ornithologie als Biologische Wissenschaft* 84–88 (1949).
18. Title, P. O. *et al.* The macroevolutionary singularity of snakes. *Science* **383**, 918–923 (2024).
19. Schluter, D. *The Ecology of Adaptive Radiation*. (Oxford University Press, Oxford, 2000).
20. Carroll, S. B. Chance and necessity: the evolution of morphological complexity and diversity. *Nature* **409**, 1102–1109 (2001).
21. Jackman, W. R. *et al.* Manipulation of Fgf and Bmp signaling in teleost fishes suggests potential pathways for the evolutionary origin of multicuspid teeth. *Evolution and Development* **15**, 107–118 (2013).
22. Wainwright, P. C. & Longo, S. J. Functional Innovations and the Conquest of the Oceans by Acanthomorph Fishes. *Current Biology* **27**, R550–R557 (2017).

23. Wainwright, P. C., McGee, M. D., Longo, S. J. & Patricia Hernandez, L. Origins, Innovations, and Diversification of Suction Feeding in Vertebrates. *Integrative and Comparative Biology* **55**, 134–145 (2015).
24. Tebbett, S. B., Goatley, C. H. R. & Bellwood, D. R. Clarifying functional roles: algal removal by the surgeonfishes *Ctenochaetus striatus* and *Acanthurus nigrofuscus*. *Coral Reefs* **36**, 803–813 (2017).
25. Berkovitz, B. & Shellis, P. *The Teeth of Non-Mammalian Vertebrates*. (Academic Press, London, 2017).
26. Seehausen, O. & Wagner, C. E. Speciation in Freshwater Fishes. *Annu. Rev. Ecol. Evol. Syst.* **45**, 621–651 (2014).
27. Höhna, S. *et al.* RevBayes: Bayesian Phylogenetic Inference Using Graphical Models and an Interactive Model-Specification Language. *Syst Biol* **65**, 726–736 (2016).
28. Yamanaka, A. Evolution and development of the mammalian multicuspid teeth. *Journal of Oral Biosciences* **64**, 165–175 (2022).
29. Rabosky, D. L. *et al.* Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. *Nat Commun* **4**, 1958 (2013).
30. Melo, B. F. *et al.* Accelerated Diversification Explains the Exceptional Species Richness of Tropical Characoid Fishes. *Systematic Biology* **71**, 78–92 (2021).
31. McGee, M. D. *et al.* The ecological and genomic basis of explosive adaptive radiation. *Nature* **586**, 75–79 (2020).
32. Pigliucci, M. Is evolvability evolvable? *Nat Rev Genet* **9**, 75–82 (2008).
33. Davis, M. P., Midford, P. E. & Maddison, W. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. *BMC Evol Biol* **13**, 38 (2013).
34. Salzburger, W., Mack, T., Verheyen, E. & Meyer, A. Out of Tanganyika: Genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evol Biol* **5**, 17 (2005).
35. Ronco, F. *et al.* Drivers and dynamics of a massive adaptive radiation in cichlid fishes. *Nature* **589**, 76–81 (2021).
36. Meier, J. I. *et al.* Cycles of fusion and fission enabled rapid parallel adaptive radiations in African cichlids. *Science* **381**, eade2833 (2023).
37. Brawand, D. *et al.* The genomic substrate for adaptive radiation in African cichlid fish. *Nature* **513**, 375–381 (2014).
38. Seehausen, O. African cichlid fish: a model system in adaptive radiation research. *Proc. R. Soc. B.* **273**, 1987–1998 (2006).
39. Greenwood, P. H. *The Haplochromine Fishes of East African Lakes: Taxonomy, Biology and Evolution*. (Kraus International Publications, Munich, 1981).
40. Crofts, S. B., Smith, S. M. & Anderson, P. S. L. Beyond Description: The Many Facets of Dental Biomechanics. *Integrative and Comparative Biology* **60**, 594–607 (2020).
41. Ivory, S. J. *et al.* Environmental change explains cichlid adaptive radiation at Lake Malawi over the past 1.2 million years. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11895–11900 (2016).
42. Lyons, R. P. *et al.* Continuous 1.3-million-year record of East African hydroclimate, and implications for patterns of evolution and biodiversity. *Proc. Natl. Acad. Sci. U.S.A.* **112**, 15568–15573 (2015).
43. Henao Diaz, L. F., Harmon, L. J., Sugawara, M. T. C., Miller, E. T. & Pennell, M. W. Macroevolutionary diversification rates show time dependency. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 7403–7408 (2019).
44. Mihalitsis, M. & Bellwood, D. R. Functional groups in piscivorous fishes. *Ecology and Evolution* **11**, 12765–12778 (2021).

45. Magalhaes, I. S., Lundsgaard-Hansen, B., Mwaiko, S. & Seehausen, O. Evolutionary divergence in replicate pairs of ecotypes of Lake Victoria cichlid fish. *Evolutionary Ecology Research* **14**, 381–401 (2012).
46. Corn, K. A. *et al.* The rise of biting during the Cenozoic fueled reef fish body shape diversification. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2119828119 (2022).
47. Price, S. A., Hopkins, S. S. B., Smith, K. K. & Roth, V. L. Tempo of trophic evolution and its impact on mammalian diversification. *Proc. Natl. Acad. Sci. U.S.A.* **109**, 7008–7012 (2012).
48. Albertson, R. C. Genetic Basis of Adaptive Shape Differences in the Cichlid Head. *Journal of Heredity* **94**, 291–301 (2003).
49. Duenser, A. *et al.* Developmental tinkering of gene regulation facilitated super rapid adaptive radiation. Preprint at <https://doi.org/10.1101/2024.01.24.577063> (2024).
50. Yamanaka, A. *et al.* Developmental process of the modern house shrew's molars: implications for the evolution of the tribosphenic molar in Mesozoic mammals. *Evolution* qpad228 (2023) doi:10.1093/evolut/qpad228.
51. Peoples, N., Burns, M. D., Mihalitsis, M. & Wainwright, P. C. Evolutionary lability of a key innovation spurs rapid diversification. Figshare <https://doi.org/10.6084/m9.figshare.25661859> (2024).
52. Rabosky, D. L. No substitute for real data: A cautionary note on the use of phylogenies from birth-death polytomy resolvers for downstream comparative analyses: BRIEF COMMUNICATIONS. *Evolution* **69**, 3207–3216 (2015).
53. Beaulieu, J. M., O'Meara, B. C. & Donoghue, M. J. Identifying Hidden Rate Changes in the Evolution of a Binary Morphological Character: The Evolution of Plant Habit in Campanulid Angiosperms. *Systematic Biology* **62**, 725–737 (2013).
54. Boyko, J. D. & Beaulieu, J. M. Generalized hidden Markov models for phylogenetic comparative datasets. *Methods Ecol Evol* **12**, 468–478 (2021).
55. Fabreti, L., Höhna, S. & Magee, A. convenience: Convergence assessment for phylogenetic inference. (2024).
56. Bollback, J. P. SIMMAP: Stochastic character mapping of discrete traits on phylogenies. *BMC Bioinformatics* **7**, (2006).
57. Tribble, C. M. *et al.* RevGadgets: An R package for visualizing Bayesian phylogenetic analyses from RevBayes. *Methods Ecol Evol* **13**, 314–323 (2022).
58. Rabosky, D. L. & Goldberg, E. E. FiSSE: A simple nonparametric test for the effects of a binary character on lineage diversification rates. *Evolution* **71**, 1432–1442 (2017).
59. Jetz, W., Thomas, G. H., Joy, J. B., Hartmann, K. & Mooers, A. O. The global diversity of birds in space and time. *Nature* **491**, 444–448 (2012).
60. Louca, S. & Pennell, M. W. Extant timetrees are consistent with a myriad of diversification histories. *Nature* **580**, 502–505 (2020).
61. Revell, L. J. phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol* **3**, 217–223 (2012).
62. Maddison, W. P., Midford, P. E. & Otto, S. P. Estimating a Binary Character's Effect on Speciation and Extinction. *Systematic Biology* **56**, 701–710 (2007).
63. Beaulieu, J. M. & O'Meara, B. C. Detecting Hidden Diversification Shifts in Models of Trait-Dependent Speciation and Extinction. *Syst Biol* **65**, 583–601 (2016).
64. Louca, S. & Pennell, M. W. A General and Efficient Algorithm for the Likelihood of Diversification and Discrete-Trait Evolutionary Models. *Systematic Biology* **69**, 545–556 (2020).
65. Helmstetter, A. J. *et al.* Trait-dependent diversification in angiosperms: Patterns, models, and data. *Ecology Letters* **26**, 640–657 (2023).

66. Paradis, E. & Schliep, K. ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. *Bioinformatics* **35**, 526–528 (2019).
67. Zenil-Ferguson, R., McEntee, J. P., Burleigh, J. G. & Duckworth, R. A. Linking Ecological Specialization to Its Macroevolutionary Consequences: An Example with Passerine Nest Type. *Systematic Biology* **72**, 294–306 (2023).
68. Froese, R. & Pauly, D. FishBase. www.fishbase.org.
69. Pagel, M. Detecting correlated evolution on phylogenies: a general method for the comparative analysis of discrete characters. *Proc. R. Soc. Lond. B* **255**, 37–45 (1994).

Supplementary Discussion

Classification of simple and complex teeth

In this study, we classified tooth complexity as a binary trait. Our binary classification represents a major evolutionary transition, from between simple and complex in any form, that follows the traditional definition of a key innovation and has functional implications. We classified a species as complex if teeth with more than one cusp were present on any dentigerous bones in the oral jaws. Lineages with complex teeth thus include species with teeth that have two (e.g., Loricariidae, Mormyridae) to as many as twelve cusps (e.g., Acanthuridae, Characidae). We recognize that our phylogenetic comparative analyses based on this binary classification ignore any transitions in cusp number within complex species. In other studies of vertebrates, transition rates of the number of cusps were found to be higher within complex lineages; the transition rate from two to three cusps is higher than the rate from one to two cusps in squamates¹. We expect a similar pattern to be true in fishes. Some groups (e.g., Characiformes) have considerable variation in the number of cusps within complex lineages while others (e.g., African cichlids) have only two or three. However, we expect that the effect of a transition within complex lineages (i.e., from three to four cusps) on macroevolutionary processes is considerably weaker than a transition from simple to complex teeth in any form.

The development of complex teeth

In vertebrates, the process of tooth development and replacement is a conserved process that has given rise to a diversity of dentitions through modification to the same underlying machinery^{2,3}. Formation of complex teeth from a simple, conical ancestral form may require minimal modification to existing tooth development mechanisms⁴. Early experimental studies in mammals implicate extracellular signaling ligands, primarily fibroblast growth factors (FGFs)

and bone morphogenetic proteins (BMPs), in controlling cusp number and morphology^{5,6}. The antagonistic actions of these signaling molecules control outcomes at multiple stages of tooth morphogenesis, including tooth formation and position and the formation of the primary enamel knot (EK). BMPs and FGFs secreted from the primary EK can inhibit or promote the development of additional Eks (and as a result, tooth cusps) because the number and spacing of Eks determines the number of cusps⁷⁻⁹. Experimental studies in fishes, including African cichlids, show that manipulating any single pathway containing candidate activators or inhibitors of cusp development produce changes in the number of tooth cusps^{4,10}. These signaling pathways include BMP, FGF, Hedgehog (Hh), Notch, and Wnt/ β -catenin. This supports the idea that change in tooth complexity in cichlids has a simple genetic basis that can respond rapidly to selective pressure^{11,12}, as activation or inhibition of any single pathway is enough to produce extreme changes in tooth complexity.

The observed ontogenetic change in tooth complexity, from complex to simple, in many African cichlids implies that every individual has the developmental capacity to produce both simple and complex teeth. We suggest this ontogenetic change may play an important role in causing the elevated macroevolutionary transition rates in this clade. The redundancy of development pathways that affect tooth complexity in cichlids¹⁰ provides multiple avenues for significant morphological change to occur. Fine-tuning of gene regulation through ontogeny facilitates trophic innovations in African cichlids¹³. This ontogenetic change might allow tooth complexity to respond rapidly to selection by maintaining a level of phenotypic plasticity. Indeed, cichlids often show plastic responses in trophic morphology¹⁴⁻¹⁶. Moreover, we see population-level variation in tooth complexity in putative species complexes at early stages of divergence, particularly in Lake Victoria, that may be linked to diet and microhabitat (N.P.

unpublished data,¹⁷). Genetic assimilation may result in the canalization of simple or complex teeth during or following the speciation process¹⁸. We suggest this scenario is consistent with the association between elevated macroevolutionary transition rates and ontogenetic change, and the resulting effect of this lability on speciation rates in this study.

Supplementary Tables

Supplementary Table 1 | Actinopterygian families that contain lineages with complex oral jaw teeth. Within each family, genera that contain at least one species with complex teeth are listed. Orders follow Near and Thacker (2024)¹⁹.

Order	Family	Genera with complex species	Reference
Perciformes	Liparidae	<i>Liparis</i> , <i>Polypera</i> , <i>Crystallichthys</i> , <i>Elassodiscus</i> , <i>Rhinoliparis</i> , <i>Careproctus</i> , <i>Temnocora</i> , <i>Crystallias</i> , <i>Paraliparis</i> , <i>Allocareproctus</i>	20–28
Atheriniiformes	Anablepidae	<i>Jenynsia</i>	29
Atheriniiformes	Poeciliidae	<i>Poeciliopsis</i> , <i>Limia</i> , <i>Poecilia</i>	30–32
Atheriniiformes	Goodeidae	<i>Allodontichthys</i> , <i>Ameca</i> , <i>Ataeniobius</i> , <i>Chapalichthys</i> , <i>Characodon</i> , <i>Goodea</i> , <i>Ilyodon</i> , <i>Skiffia</i> , <i>Xenophorous</i> , <i>Xenotaenia</i> , <i>Xenotoca</i>	33–38
Atheriniiformes	Cyprinodontidae	<i>Aphanius</i> , <i>Cyprinodon</i> , <i>Jordanella</i> , <i>Cualac</i> , <i>Floridichthys</i> , <i>Megupsilon</i>	29,39
Siluriformes	Amphiliidae	<i>Tetracamphilius</i>	40
Siluriformes	Trichomycteridae	<i>Trichogenes</i>	41
Siluriformes	Scoloplacidae	<i>Scoloplax</i>	42
Siluriformes	Astroblepidae	<i>Astroblepus</i>	43
Siluriformes	Sisoridae	<i>Glaridoglanis</i>	44
Siluriformes	Heptateridae	<i>Phenacorhamdia</i>	45
Siluriformes	Mochokidae	<i>Atopochilus</i> , <i>Atopodontus</i> , <i>Euchilichthys</i>	46–48
Siluriformes	Loricariidae	<i>Brochiloricaria</i> , <i>Dasylicaria</i> , <i>Farlowella</i> , <i>Furcodontichthys</i> , <i>Harttia</i> , <i>Harttiella</i> , <i>Hemiodontichthys</i> , <i>Ixinandria</i> , <i>Lamontichthys</i> , <i>Limatulichthys</i> , <i>Loricaria</i> , <i>Loricariichthys</i> , <i>Metaloricaria</i> , <i>Paraloricaria</i> , <i>Pseudoloricaria</i> , <i>Pterosturisoma</i> , <i>Ricola</i> , <i>Rineloricaria</i> , <i>Spatuloricaria</i> , <i>Sturisoma</i> , <i>Sturisomatichthys</i> , <i>Delturus</i> , <i>Hemipsilichthys</i> , <i>Lithogenes</i> , <i>Microplecostomus</i> , <i>Hirtella</i> , <i>Isbrueckerichthys</i> , <i>Neoplecostomus</i> , <i>Pareiorhaphis</i> , <i>Pareiorhina</i> , <i>Acestridium</i> , <i>Hypoptopoma</i> , <i>Otocinclus</i> , <i>Corumbataia</i> , <i>Curculionichthys</i> , <i>Epactionotus</i> ,	49–78

		<i>Hisonotus, Microlepidogaster, Otothyris, Otothyropsis, Parotocinclus, Gymnotocinclus, Plesioptopoma, Rhinolekos, Acanthicus, Ancistrus, Andeancistrus, Araichthys, Baryancistrus, Chaetostoma, Cordylancistrus, Corymbophanes, Dekeyseria, Dolichancistrus, Exastilithoxus, Hemiancistrus, Hopliancistrus, Hypancistrus, Lasiancistrus, Leporacanthicus, Lithoxus, Megalancistrus, Micracanthicus, Neblinichthys, Panaqolus, Panaque, Parancistrus, Paulasquama, Pseudacanthicus, Pseudancistrus, Pseudolithoxus, Pseudoqolus, Scobinancistrus, Soromonichthys, Spectracanthicus, Transancistrus, Yaluwak, Aphanotorulus, Hypostomus, Isorineloricaria, Pterygoplichthys, Pogonopoma, Pseudorinelepis, Rhinelepis, Ancistomus, Cryptancistrus, Guyanancistrus, Leptoancistrus, Peckoltia, Peckoltichthys, Nannoplecostomus</i>	
Osteoglossiformes	Mormyridae	<i>Boulengeromyrus, Brienomyrus, Brevimyrus, Campylomormyrus, Gnathonemus, Hippopotamyrus, Hyperopisus, Isichthys, Ivindomyrus, Marcusenius, Mormyrops, Mormyrus, Paramormyrops, Pollimyrus, Stomatorhinus, Cryptomyrus, Petrocephalus</i>	46,79–87
Myctophiformes	Myctophidae	<i>Diogenichthys</i>	88
Blenniiformes	Mugilidae	<i>Agonostomus, Chaenomugil, Chelon, Joturus, Mugil, Neomyxus</i>	89–95
Atheriniformes	Atherinopsidae	<i>Atherinops, Colpichthys</i>	96
Atheriniformes	Exocoetidae	<i>Cypselurus</i>	97
Atheriniformes	Hemirhamphidae	<i>Arrhampus, Chriodorus, Euleptorhamphus, Hemiramphus, Hyporhamphus, Oxyporhamphus</i>	98–102
Centrarchiformes	Girellidae	<i>Girella</i>	103
Acanthuriformes	Pomacanthidae	<i>Apolemichthys, Centropyge, Chaetodontoplus, Genicanthus, Holacanthus, Paracentropyge, Pomacanthus, Pygoplites</i>	104, observation of specimens

Centrarchiformes	Terapontidae	<i>Helotes</i>	105
Acanthuriformes	Ephippidae	<i>Platax, Proteracanthus, Rhinoprenes, Tripterodon, Zabidius</i>	106
Acanthuriformes	Scatophagidae	<i>Scatophagus, Selenotoca</i>	107
Cichliformes	Pomacentridae	<i>Abudefduf</i>	observation of specimens
Acanthuriformes	Siganidae	<i>Siganus</i>	observation of specimens
Acanthuriformes	Acanthuridae	<i>Acanthurus, Ctenochaetus, Prionurus, Paracanthurus, Zebrasoma</i>	observation of specimens
Scombriformes	Nomeidae	<i>Psenes</i>	108
Scombriformes	Ariommatidae	<i>Ariomma</i>	109
Scombriformes	Stromateidae	<i>Pampus, Peprilus</i>	110,111
Gobiesociformes	Gobiesocidae	<i>Diademichthys, Acyrtops, Acyrtus, Derilissus, Gobiesox, Tomicodon,</i>	112–133
Gobiiformes	Oxudercidae	<i>Sicyopterus, Sicydium, Cotylopus, Lentipes, Akihito, Parasicydium, Stiphodon, Awaous, Tridentiger, Evorthodous, Apocryptodon, Apocryptes, Boleophthalmus</i>	134–166
Cithariniformes	Distichodontidae	<i>Belonophago, Congocharax, Distichodus, Dundocharax, Eugnathichthys, Hemigrammocharax, Ichthyborus, Mesoborus, Microstomatichthyborus, Monostichodus, Nannaethiops, Nannocharax, Neolebias, Paradistichodus, Paraphago, Phago, Xenocharax</i>	167
Cithariniformes	Citharinidae	<i>Citharinidium, Citharinops, Citharinus</i>	167
Characiformes	Alestiidae	<i>Alestes, Alestopetersius, Arnoldichthys, Bathyaethiops, Brachypetersius, Brycinus, Bryconaethiops, Hemigrammopetersius, Ladigesia, Micralestes, Nannopetersius, Petersius, Phenacogrammus, Rhabdalestes, Tricuspidaletes</i>	168
Characiformes	Acestrorhynchidae	<i>Acestrorhynchus, Gilbertolus, Gnathocharax, Heterocharax, Hoplocharax, Roestes</i>	observation of specimens
Characiformes	Anostomidae	<i>Abramites, Anostomoides, Anostomus, Gnathodolus, Hypomasticus, Laemolyta, Leporellus, Leporinus, Megaleporinus, Petulanos, Pseudanos, Rhytiodus, Sartor, Schizodon</i>	169–174

Characiformes	Bryconidae	<i>Brycon, Chilobrycon, Henochilus</i>	175–177
Characiformes	Chalceidae	<i>Chalceus</i>	178
Characiformes	Characidae	<i>Acrobrycon, Amazonspither, Argopleura, Astyanax, Bryconamericus, Carlastyanax, Ceratobranchia, Cheirodon, Chrysobrycon, Compsura, Coptobrycon, Corynopoma, Creagrutus, Ctenobrycon, Deuterodon, Diapoma, Eretmobrycon, Erythrocharax, Gephyrocharax, Glandulocauda, Gymnocorymbus, Hasemania, Hemibrycon, Hemigrammus, Heterocheirodon, Hollandichthys, Hyphessobrycon, Inpaichthys, Jupiaba, Knodus, Kolpotocheirodon, Lepidocharax, Leptagoniates, Lophiobrycon, Markiana, Microschemobrycon, Mimagoniates, Moenkhausia, Myxiops, Nematobrycon, Odontostilbe, Oligosarcus, Petitella, Phenacogaster, Piabina, Planaltina, Poptella, Prionobrama, Pristella, Probolodus, Prodontocharax, Protocheirodon, Pseudochalceus, Rhinobrycon, Serrapinnus, Stethaprion, Stygichthys, Tetragonopterus, Thayeria</i>	179–227
Characiformes	Chilodontidae	<i>Caenotropus</i>	172,228
Characiformes	Crenuchidae	<i>Ammocryptocharax, Crenuchus, Poecilocharax</i>	179,229–231
Characiformes	Gasteropelecidae	<i>Carnegiella, Gasteropelecus, Thoracocharax</i>	232
Characiformes	Hemiodontidae	<i>Argonectes, Bivibranchia, Hemiodus</i>	233
Characiformes	Iguanodectidae	<i>Iguanodectes</i>	204
Characiformes	Lebiasinidae	<i>Lebiasina, Nannostomus</i>	234,235
Characiformes	Parodontidae	<i>Apareiodon, Parodon, Saccodon</i>	236–238
Characiformes	Serrasalminidae	<i>Colossoma, Mylesinus, Myleus, Ossubtus, Pristobrycon, Pygocentrus, Pygopristis, Serrasalmus, Tometes, Utiaitichthys</i>	239–249
Characiformes	Triportheidae	<i>Clupeacharax, Triportheus</i>	250,251
Blenniiformes	Cichlidae	See Supplementary Table 12	

Supplementary Table 2 | Estimated number of transitions between each state under the MAP ancestral state reconstruction of tooth complexity for 11,508 species of ray-finned fishes. Low and high refer to the two hidden states in the model, reflecting differences in lability. The number of transitions are reported for five different prior transitions (50 / 150 / 200 / 250 / 400). The results under the prior of 200 (in bold) are reported in the main text.

From	To	Transition type	Number of transitions
simple-low	complex-low	gain complex teeth	6 / 6 / 8 / 7 / 8
simple-low	simple-high	increase lability	31 / 36 / 16 / 48 / 24
simple-low	complex-high	gain complex teeth, increase lability	1 / 0 / 1 / 3 / 9
complex-low	simple-low	lose complex teeth	0 / 24 / 27 / 26 / 27
complex-low	simple-high	lose complex teeth, increase lability	1 / 3 / 0 / 1 / 3
complex-low	complex-high	increase lability	3 / 10 / 11 / 22 / 28
simple-high	simple-low	decrease lability	17 / 119 / 113 / 101 / 60
simple-high	complex-low	gain complex teeth, decrease lability	2 / 5 / 8 / 12 / 10
simple-high	complex-high	gain complex teeth	58 / 65 / 78 / 242 / 362
complex-high	simple-low	lose complex teeth, decrease lability	1 / 0 / 0 / 5 / 36
complex-high	complex-low	decrease lability	3 / 56 / 62 / 71 / 83
complex-high	simple-high	lose complex teeth	64 / 60 / 83 / 248 / 374

Supplementary Table 3 | Comparison of discrete character evolution models fit over the ray-finned fish phylogeny (n=11,508 species). Bayes factors (BF) are reported in comparison to the best-fit model, HR2-ARD (in bold). A second set marginal likelihood estimation under the same power posterior analysis and stepping sampler is shown in parentheses to demonstrate that the marginal likelihood estimates are stable using 10 stones.

Model	Marginal likelihood	ln BF	BF
ER	-1116.615 (-1116.688)	273.5266	6.1815E+118
ARD	-949.2979 (-950.1362)	106.2095	1.33721E+46
HR-variable	-873.8059 (-872.8403)	30.7175	2.18998E+13
HR2-ER	-844.9976 (-846.8524)	1.9092	6.747688488
HR2-ARD	-843.0884 (-843.0934)	-	-

Supplementary Table 4 | Mean estimated transition rates between simple and complex teeth across ray-finned fishes under 5 priors. The relative difference in rates reported as the ratio of the rate of loss to the rate of gain.

	Low gain	low loss	high gain	high loss	ratio-low	ratio-high
50	0.000050788	0.0004784	0.0095337	0.013	9.42	1.36
150	0.00003964	0.001698	0.0243	0.0495	42.84	2.04
200	0.000034685	0.0017515	0.0715	0.0897	50.5	1.25
250	0.00002683	0.001542	0.2606	0.2309	57.47	0.89
400	0.000023939	0.001759	0.4535	0.4062	73.48	0.9

Supplementary Table 5 | Transition rates between simple and complex teeth across ray-finned fishes under a HiSSE model fit using the ‘castor’ R package under a Bayesian HR2-ARD model in RevBayes.

Transition type	rate (HiSSE with ‘castor’)	rate (HR2-ARD)
gain complex teeth – low lability	0.1657	0.00003
lose complex teeth – low lability	72.1088	0.00175
gain complex teeth – high lability	136.0097	0.0715
lose complex teeth – high lability	107.7286	0.0897

Supplementary Table 6 | Tooth complexity and diet classifications for Cichlidae, covering all genera from Africa, Madagascar, and South Asia. If genera contain species with both simple and complex teeth, both are listed. For genera that contain species of multiple different diet classifications, all diets within that genera are listed.

Genus	Tooth complexity	Diet	Tooth complexity references	Diet references
<i>Abactochromis</i>	Complex	Zoobenthivore	252	253
<i>Alcolapia</i>	Complex	Herbivore	254	255,256
<i>Asprotilapia</i>	Complex	Herbivore	257,258	259
<i>Cheilochromis</i>	Complex	Zoobenthivore	260	255
<i>Chetia</i>	Complex	Piscivore, Zoobenthivore	261,262	255,262,263
<i>Chindongo</i>	Complex	Herbivore, Omnivore, Zooplanktivore	253,264	253,255,265,266
<i>Coelotilapia</i>	Complex	Herbivore	267	255
<i>Congolapia</i>	Complex		268	
<i>Coptodon</i>	Complex	Herbivore, Omnivore, Zooplanktivore	269,270	255,269,270

<i>Ctenochromis</i>	Complex	Omnivore	258,271	272
<i>Cunningtonia</i>	Complex	Zooplanktivore	257,258	255
<i>Cyathochromis</i>	Complex	Herbivore	273	255,266
<i>Cyclopharynx</i>	Complex	Herbivore	274	255
<i>Danakilia</i>	Complex	Herbivore	254	255
<i>Docimodus</i>	Complex	Carnivore specialist	260,275	255,266
<i>Eclectochromis</i>	Complex	Zoobenthivore	276	255
<i>Etia</i>	Complex	Zoobenthivore	277	278
<i>Etroplus</i>	Complex	Herbivore	279	255
<i>Fossorochromis</i>	Complex	Zoobenthivore	276	255
<i>Genyochromis</i>	Complex	Carnivore specialist	273	255
<i>Gobiocichla</i>	Complex	Herbivore	257	255
<i>Heterotilapia</i>	Complex	Omnivore	280	278
<i>Interochromis</i>	Complex	Herbivore	258	255
<i>Iodotropheus</i>	Complex	Omnivore	253	253
<i>Iranocichla</i>	Complex	Herbivore	281,282	255
<i>Katria</i>	Complex		279	
<i>Konia</i>	Complex	Zoobenthivore	283	255
<i>Labeotropheus</i>	Complex	Herbivore	253	253,255
<i>Lichnochromis</i>	Complex	Piscivore	260	273
<i>Limnotilapia</i>	Complex	Omnivore	258	255,284
<i>Lithochromis</i>	Complex	Herbivore	285	285
<i>Maylandia</i>	Complex	Herbivore, Zooplanktivore, Zoobenthivore	264,286	253,255,265,266
<i>Mbipia</i>	Complex	Herbivore, Omnivore	285	255
<i>Mchenga</i>	Complex	Zooplanktivore	276,287	255,288
<i>Melanochromis</i>	Complex	Herbivore, Omnivore, Piscivore, Carnivore specialist	253	253,255
<i>Naevochromis</i>	Complex	Carnivore specialist	260	255
<i>Neochromis</i>	Complex	Herbivore, Omnivore	285	255,285
<i>Orthochromis</i>	Complex	Herbivore, Omnivore	289–291	255

<i>Oxylapia</i>	Complex		279	
<i>Pelmatolapia</i>	Complex	Herbivore	292	255
<i>Petrochromis</i>	Complex	Herbivore	293	294
<i>Petrotilapia</i>	Complex	Herbivore, Omnivore	273,295	253,255,295
<i>Pharyngochromis</i>	Complex		296	
<i>Pseudetroplus</i>	Complex	Omnivore	279	255
<i>Pseudocrenilabrus</i>	Complex	Omnivore, Zoobenthivore	291,297	255
<i>Pseudosimochromis</i>	Complex	Herbivore	294	259,294
<i>Pseudotropheus</i>	Complex	Herbivore, Omnivore, Zooplanktivore, Zoobenthivore	276	253,255
<i>Ptychochromis</i>	Complex	Omnivore, Zoobenthivore	279	255
<i>Ptychochromoides</i>	Complex	Omnivore	279	255
<i>Sarotherodon</i>	Complex	Herbivore, Omnivore, Zoobenthivore	254	255
<i>Schwetzochromis</i>	Complex	Herbivore	274	255
<i>Simochromis</i>	Complex	Herbivore	258	259
<i>Taeniolethrinops</i>	Complex	Zoobenthivore	260	255
<i>Thoracochromis</i>	Complex		291	
<i>Tilapia</i>	Complex	Herbivore	257,270	255,298,299
<i>Tropheops</i>	Complex	Herbivore	264	253,264
<i>Tropheus</i>	Complex	Herbivore	258	255
<i>Baileychromis</i>	Simple	Piscivore	300	300
<i>Bathybates</i>	Simple	Piscivore	258	255
<i>Benitochromis</i>	Simple	Omnivore	301	278,302
<i>Boulengerochromis</i>	Simple	Piscivore	303	255
<i>Buccochromis</i>	Simple	Piscivore	260,276	255
<i>Callochromis</i>	Simple	Zoobenthivore	258	255
<i>Caprichromis</i>	Simple	Carnivore specialist	260	255,304
<i>Cardiopharynx</i>	Simple	Zooplanktivore	258	255
<i>Chalinochromis</i>	Simple	Zoobenthivore	305,306	255,284

<i>Champsochromis</i>	Simple	Piscivore	276	255
<i>Chilochromis</i>	Simple	Herbivore	307	278
<i>Chilotilapia</i>	Simple	Zoobenthivore	276	255
<i>Chromidotilapia</i>	Simple	Omnivore.	279,308	255
		Zoobenthivore		
<i>Congochromis</i>	Simple		309	
<i>Corematodus</i>	Simple	Carnivore specialist	276	255,260
<i>Cyathopharynx</i>	Simple	Herbivore	258	255
<i>Cynotilapia</i>	Simple	Zooplanktivore	276	255,266,276
<i>Cyphotilapia</i>	Simple	Piscivore	258	255,284
<i>Cyprichromis</i>	Simple	Zooplanktivore	258	255,284
<i>Cyrtocara</i>	Simple	Zoobenthivore	260	253,310
<i>Dimidiochromis</i>	Simple	Piscivore	260,276	255,266
<i>Diplotaxodon</i>	Simple	Piscivore, Zooplanktivore	260	255,311,312
<i>Divandu</i>	Simple	Omnivore	313	255
<i>Ectodus</i>	Simple	Herbivore	258	284
<i>Enigmatochromis</i>	Simple		314	
<i>Eretmodus</i>	Simple	Herbivore	258	255
<i>Exochochromis</i>	Simple	Piscivore	260	260
<i>Gephyrochromis</i>	Simple	Herbivore	276	315
<i>Gnathochromis</i>	Simple	Zoobenthivore, Zooplanktivore	258	316
<i>Grammatotria</i>	Simple	Zoobenthivore	258	255
<i>Greenwoodochromis</i>	Simple	Piscivore	258	284
<i>Haplotaxodon</i>	Simple	Piscivore	258	317
<i>Hemibates</i>	Simple	Piscivore	258	255
<i>Hemichromis</i>	Simple	Zoobenthivore, Piscivore	318	255
<i>Hemitaeniochromis</i>	Simple	Carnivore specialist, Piscivore	260,276	255,260
<i>Hemitilapia</i>	Simple	Herbivore	276	255
<i>Heterochromis</i>	Simple	Zoobenthivore	257	255

<i>Julidochromis</i>	Simple	Zoobenthivore	305	255
<i>Lamprologus</i>	Simple	Zoobenthivore, Piscivore, Zooplanktivore	257	255,284,316
<i>Lepidiolamprologus</i>	Simple	Piscivore	319, observation of specimens	255
<i>Lestradea</i>	Simple	Herbivore	258	255,284
<i>Limbochromis</i>	Simple	Herbivore	301	255,278
<i>Limnochromis</i>	Simple	Zoobenthivore	258	316
<i>Lobochilotes</i>	Simple	Zoobenthivore	258	255
<i>Myaka</i>	Simple	Omnivore	283	255,283
<i>Nanochromis</i>	Simple	Omnivore, Zoobenthivore	301	255
<i>Neolamprologus</i>	Simple	Zooplanktivore, Zoobenthivore, Piscivore	305	255,316,320
<i>Pallidochromis</i>	Simple	Piscivore	321	311
<i>Paracyprichromis</i>	Simple	Zooplanktivore	258	255
<i>Parananochromis</i>	Simple	Omnivore	322,323	278
<i>Paratilapia</i>	Simple	Piscivore	279	324
<i>Paretroplus</i>	Simple	Zoobenthivore	279	279,325–327
<i>Pelmatochromis</i>	Simple	Omnivore	257	255,278
<i>Pelvicachromis</i>	Simple	Herbivore, Omnivore	301	278,328,329
<i>Perissodus</i>	Simple	Carnivore specialist	258	317
<i>Plecodus</i>	Simple	Carnivore specialist	258	317
<i>Pterochromis</i>	Simple		301	
<i>Pundamilia</i>	Simple	Zooplanktivore, Omnivore, Zoobenthivore	285	255
<i>Pungu</i>	Simple	Zoobenthivore	283	255,283
<i>Reganochromis</i>	Simple	Zoobenthivore	observation of specimen	255,284
<i>Rhamphochromis</i>	Simple	Piscivore	260,276	255,311
<i>Sargochromis</i>	Simple	Zoobenthivore	262	255,330
<i>Sciaenochromis</i>	Simple	Piscivore	260	255,266
<i>Serranochromis</i>	Simple	Piscivore	262	255,263
<i>Spathodus</i>	Simple	Omnivore	258	255,284
<i>Stigmatochromis</i>	Simple	Piscivore, Zoobenthivore	260,331	255

<i>Stomatepia</i>	Simple	Piscivore, Zoobenthivore	283	283,332
<i>Taeniochromis</i>	Simple	Piscivore	276	255
<i>Tangachromis</i>	Simple	Zooplanktivore	258	255,284
<i>Tanganicodus</i>	Simple	Herbivore	258	259
<i>Teleogramma</i>	Simple	Zoobenthivore	257,333	46,278,333
<i>Telmatochromis</i>	Simple	Herbivore, Zoobenthivore	334,335	336
<i>Thysochromis</i>	Simple	Zoobenthivore	337	278,337
<i>Trematocara</i>	Simple	Zooplanktivore, Zoobenthivore	258	338
<i>Trematochromis</i>	Simple	Piscivore	258	284
<i>Triglachromis</i>	Simple	Zoobenthivore	258	255
<i>Tristamella</i>	Simple	Omnivore, Herbivore	339	255
<i>Tylochromis</i>	Simple	Zoobenthivore, Omnivore	257	255
<i>Tyrannochromis</i>	Simple	Piscivore	260,276	255
<i>Variabilichromis</i>	Simple	Omnivore	258	255
<i>Wallaceochromis</i>	Simple	Omnivore	340	340
<i>Xenochromis</i>	Simple	Zooplanktivore	258	317
<i>Xenotilapia</i>	Simple	Zoobenthivore	258,341	255
<i>Aulonocara</i>	Simple, Complex	Zoobenthivore	260,342,343	255,260
<i>Copadichromis</i>	Simple, Complex	Zooplanktivore	260,276,287,344,345	255,344
<i>Ctenopharynx</i>	Simple, Complex	Zooplanktivore, Zoobenthivore	260,311	255
<i>Haplochromis</i> *	Simple, Complex	Herbivore, Omnivore, Zooplanktivore, Zoobenthivore, Piscivore, Carnivore Specialist	257,274,285,297,307,346–352	255,297,348
<i>Labidochromis</i>	Simple, Complex	Zoobenthivore, Herbivore, Omnivore	353	253,255,353
<i>Lethrinops</i>	Simple, Complex	Zoobenthivore, Herbivore	260,276,354–356	255,355,356
<i>Mylochromis</i>	Simple, Complex	Zooplanktivore, Zoobenthivore	260,276,357	255
<i>Nimbochromis</i>	Simple, Complex	Piscivore	276,311	255

<i>Nyassochromis</i>	Simple, Complex	Zooplanktivore, Herbivore, Omnivore	260	255,344
<i>Ophthalmotilapia</i>	Simple, Complex	Herbivore	358	259
<i>Oreochromis</i>	Simple, Complex	Zooplanktivore, Omnivore, Herbivore, Zoobenthivore	254,257	255
<i>Otopharynx</i>	Simple, Complex	Piscivore, Zoobenthivore, Herbivore, Zooplanktivore, Omnivore, Carnivore specialist	260,276,331,359,360	255
<i>Placidochromis</i>	Simple, Complex	Omnivore, Zoobenthivore, Herbivore	260,276,311	255
<i>Protomelas</i>	Simple, Complex	Zoobenthivore, Carnivore specialist, Herbivore	260,276,344	255,260
<i>Steatocranus</i>	Simple, Complex	Herbivore, Zoobenthivore	46	255
<i>Tramitichromis</i>	Simple, Complex	Zoobenthivore	276	255,266
<i>Trematocranus</i>	Simple, Complex	Zooplanktivore, Zoobenthivore	260,361	255

Supplementary Table 7 | Comparison of discrete character evolution models fit over the African cichlid phylogeny (n=1,069 species). Bayes factors (BF) are reported in comparison to the best-fit model, HR4-ER (in bold).

Model	Marginal likelihood	ln BF	BF
ER	-662.044	245.9517	6.5383E+106
ARD	-648.3601	232.2678	7.458E+100
HR2-ER	-418.8311	2.7388	15.46841186
HR2-ARD	-424.2223	8.13	3394.799565
HR4-ER	-416.0923	-	-
HR4-ARD	-419.5322	3.4399	31.18383963

Supplementary Table 8 | Marginal likelihoods of Bayesian state-dependent speciation and extinction (SSE) models, each fit over a subtree of African cichlids. The CID-4 model for the Lake Victoria subtree did not mix properly during the power-posterior analysis. Best-fit models for each subtree are bold.

Model	Rivers/non-rift lakes	Lake Tanganyika	Lake Malawi	Lake Victoria
CID-2	-4724.319	-2559.727	-1576.46	-396.74
CID-3	-2368.919	-1341.914	-1563.757	-469.631
CID-4	-2317.977	-1279.22	-1561.664	*
BiSSE	-2391.691	-1293.066	-1771.429	-511.508
HiSSE-2	-2350.964	-1288.786	-1578.434	-361.792
HiSSE-3	-2370.752	-1286.176	-1568.255	-388.507
HiSSE-4	-2370.671	-1282.403	-1566.829	-343.619

Supplementary Table 9 | Marginal likelihoods and Bayes factor (BF) for SSE models using lability as a four-state discrete trait (very low, low, high, very high). Both models were fit over the full African cichlid phylogeny (n=1,069). BF's are reported in comparison to the best-fit model, MuHiSSE-4 (in bold). A second set marginal likelihood estimation under the same power posterior analysis and stepping sampler is shown in parentheses.

Model	Marginal likelihood	ln BF	BF
CID-2	-7359.293 (-7359.423)	201.765	4.22E+87
CID-3	-7255.554 (-7216.647)	98.026	3.73E+42
CID-4	-7162.698 (-12177.58)	5.17	175.91
MuHiSSE-2	-7234.33 (-7235.751)	76.802	2.26E+33
MuHiSSE-3	-7181.471 (-7176.844)	23.943	2.5E+10
MuHiSSE-4	-7157.528 (-7156.99)	-	-

Supplementary Table 10 | Mean speciation, extinction and net diversification (speciation minus extinction) rates estimated for each level of lability (very low, low, high, very high) across four hidden states (A, B, C, D) under the MuHiSSE-4 model for African cichlids. For each speciation and extinction rate mean, the standard deviation is reported in parentheses. Mean rates were taken from the posterior distribution estimated using MCMC.

State	Speciation	Extinction	Net diversification
very low – A	0.0839 (.0091)	0.0173 (.0104)	0.0666
low – A	0.1121 (.0184)	0.0745 (.0589)	0.0376
high – A	0.1816 (.0354)	0.117 (.1254)	0.0646
very high – A	0.2085 (.038)	0.2349 (.1999)	-0.0264
very low – B	0.2095 (.0164)	0.0046 (.0042)	0.2049
low – B	0.2786 (.031)	0.0172 (.0143)	0.2614
high – B	0.4534 (.0813)	0.0323 (.0457)	0.4211
very high – B	0.5205 (.0865)	0.0593 (.0577)	0.4612
very low – C	1.2859 (.195)	0.0081 (.0093)	1.2778
low – C	1.7134 (.3169)	0.0346 (.0461)	1.6788
high – C	2.7284 (.2624)	0.0461 (.0519)	2.6823
very high – C	3.1323 (.2173)	0.0874 (.0795)	3.0449
very low – D	10.4397 (1.885)	0.0785 (.1119)	10.3612
low – D	13.9104 (2.9158)	0.3329 (.5182)	13.5775
high – D	22.1519 (3.0042)	0.5052 (.8237)	21.6467
very high – D	25.3064 (1.5964)	0.9448 (.9306)	24.3616

Supplementary Table 11 | Mean speciation, extinction and net diversification (speciation minus extinction) rates estimated for each level of lability (very low, low, high, very high) across two hidden states (A and B) under the MuHiSSE-2 model for African cichlids. For each speciation and extinction rate mean, the standard deviation is reported in parentheses. Mean rates were taken from the posterior distribution estimated using MCMC.

State	Speciation	Extinction	Net diversification
very low – A	0.1288 (.0116)	0.0287 (.0174)	0.1001
low – A	0.2676 (.0298)	0.0179 (.0173)	0.2497
high – A	0.2513 (.0425)	0.0168 (.0172)	0.2345
very high – A	1.0263 (.1443)	0.6345 (.1988)	0.3918
very low – B	1.4683 (.2514)	0.2308 (.1887)	1.2375
low – B	3.0516 (.5585)	0.144 (.1627)	2.9076
high – B	2.8098 (.2732)	0.122 (.1178)	2.6878
very high – B	11.4576 (.3818)	4.4766 (.5204)	6.981

Supplementary Table 12 | Fit of independent and dependent models of character evolution using Pagel’s (1994) method across African cichlids (n=1,069 species). Models were fit using two binarizations of herbivory; one that included only strict herbivores, and an alternate that included omnivores and herbivores. Best-fit models are in bold.

Classification	Model	log-likelihood	AIC
herbivores only	Independent	-936.8434	1941.687
	Dependent	-900.7249	1809.45
herbivores and omnivores	Independent	-1038.9674	2081.935
	Dependent	-936.7457	1881.491

Supplementary References

1. Lafuma, F., Corfe, I. J., Clavel, J. & Di-Poi, N. Multiple evolutionary origins and losses of tooth complexity in squamates. *Nat Commun* **12**, 6001 (2021).
2. Tucker, A. S. & Fraser, G. J. Evolution and developmental diversity of tooth regeneration. *Seminars in Cell & Developmental Biology* **25–26**, 71–80 (2014).
3. Wise, S. B. & Stock, D. W. Conservation and divergence of Bmp2a, Bmp2b, and Bmp4 expression patterns within and between dentitions of teleost fishes. *Evolution and Development* **8**, 511–523 (2006).
4. Jackman, W. R. *et al.* Manipulation of Fgf and Bmp signaling in teleost fishes suggests potential pathways for the evolutionary origin of multicuspid teeth. *Evolution and Development* **15**, 107–118 (2013).
5. Jernvall, J., Keränen, S. V. E. & Thesleff, I. Evolutionary modification of development in mammalian teeth: Quantifying gene expression patterns and topography. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 14444–14448 (2000).
6. Jernvall, J. & Thesleff, I. Reiterative signaling and patterning during mammalian tooth morphogenesis. *Mechanisms of Development* **92**, 19–29 (2000).
7. Jernvall, J. & Jung, H. Genotype, phenotype, and developmental biology of molar tooth characters. *Am. J. Phys. Anthropol.* **113**, 171–190 (2000).
8. Salazar-Ciudad, I. & Jernvall, J. A gene network model accounting for development and evolution of mammalian teeth. *Proc. Natl. Acad. Sci. U.S.A.* **99**, 8116–8120 (2002).
9. Jernvall, J. Linking development with generation of novelty in mammalian teeth. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 2641–2645 (2000).
10. Fraser, G. J., Bloomquist, R. F. & Streelman, J. T. Common developmental pathways link tooth shape to regeneration. *Developmental Biology* **377**, 399–414 (2013).
11. Streelman, J. T. & Albertson, R. C. Evolution of novelty in the cichlid dentition. *J Exp Zool Pt B* **306B**, 216–226 (2006).
12. Streelman, J. T., Webb, J. F., Albertson, R. C. & Kocher, T. D. The cusp of evolution and development: a model of cichlid tooth shape diversity. *Evolution and Development* **5**, 600–608 (2003).
13. Duenser, A. *et al.* Developmental tinkering of gene regulation facilitated super rapid adaptive radiation. Preprint at <https://doi.org/10.1101/2024.01.24.577063> (2024).
14. Muschick, M., Barluenga, M., Salzburger, W. & Meyer, A. Adaptive phenotypic plasticity in the Midas cichlid fish pharyngeal jaw and its relevance in adaptive radiation. *BMC Evol Biol* **11**, 116 (2011).
15. Meyer, A. Phenotypic plasticity and heterochrony in *Cichlasoma managuense* (Pisces, Cichlidae) and their implications for speciation in cichlid fishes. *Evolution* **41**, 1357–1369 (1987).
16. Gunter, H. M. *et al.* Shaping development through mechanical strain: the transcriptional basis of diet-induced phenotypic plasticity in a cichlid fish. *Molecular Ecology* **22**, 4516–4531 (2013).
17. Magalhaes, I. S., Lundsgaard-Hansen, B., Mwaiko, S. & Seehausen, O. Evolutionary divergence in replicate pairs of ecotypes of Lake Victoria cichlid fish. *Evolutionary Ecology Research* **14**, 381–401 (2012).
18. Ehrenreich, I. M. & Pfennig, D. W. Genetic assimilation: a review of its potential proximate causes and evolutionary consequences. *Ann Bot* **117**, 769–779 (2016).
19. Near, T. J. & Thacker, C. E. Phylogenetic Classification of Living and Fossil Ray-finned Fishes (Actinopterygii). *Bulletin of the Peabody Museum of Natural History* **65**, 3–302 (2024).

20. Burke, Victor. Revision of the fishes of the family Liparidae. *Bulletin of the United States National Museum* **150**, 1–204 (1930).
21. Chernova, N. V. Review of Careproctus (Liparidae) of the North Atlantic and Adjacent Arctic, Including the Generic Type *C. reinhardtii*, with Rehabilitation of *C. gelatinosus* (Pallas) from Kamchatka. *Journal of Ichthyology* **45**, S1–S22 (2005).
22. Chernova, N. V. New species of the genus Careproctus (Liparidae) from the Kara Sea and identification key for congeners of the North Atlantic and Arctic. *J. Ichthyol.* **54**, 757–780 (2014).
23. Knudsen, S. W., Møller, P. R. & Gravlund, P. Phylogeny of the snailfishes (Teleostei: Liparidae) based on molecular and morphological data. *Molecular Phylogenetics and Evolution* **44**, 649–666 (2007).
24. Chernova, N. V. & Stein, D. L. Ten New Species of Psednos (Pisces, Scorpaeniformes: Liparidae) from the Pacific and North Atlantic Oceans. *Copeia* **2002**, 755–778 (2002).
25. Stein, D. L. A Review of the Snailfishes (Liparidae, Scorpaeniformes) of New Zealand, Including Descriptions of a New Genus and Sixteen New Species. *Zootaxa* **3588**, 1 (2012).
26. Balushkin, A. V. Volodichthys gen. nov. new species of the primitive snailfish (Liparidae: Scorpaeniformes) of the southern 60epidiolam. Description of new species *V. Solovjevae* sp. Nov. (Cooperation Sea, the Antarctic). *J. Ichthyol.* **52**, 1–10 (2012).
27. Balushkin, A. V. & Voskoboinikova, O. S. Revision of the genus Genioliparis Andriashev et Neelov (Liparidae, Scorpaeniformes) with description of a new species *G. kafanovi* sp. N. from the Ross Sea (Antractica). *J. Ichthyol.* **48**, 201–208 (2008).
28. Orr, J. W. & Busby, M. S. Revision of the snailfish genus Allocareproctus Pitruk & Fedorov (Teleostei: Liparidae), with descriptions of four new species from the Aleutian Islands. *Zootaxa* **1173**, 1–37 (2006).
29. Parenti, L. R. A Phylogenetic and Biogeographic Analysis of Cyprinodontiform Fishes (Teleostei, Atherinomorpha). *Bulletin of the American Museum of Natural History* **168**, 335–557 (1981).
30. Miller, Robert R. Four New Species of Viviparous Fishes, Genus Poeciliopsis, from Northwestern Mexico. *Occ. Papers* 1–11 (1960).
31. Rodriguez, C. M. Phylogenetic Analysis of the Tribe Poeciliini (Cyprinodontiformes: Poeciliidae). *Copeia* **1997**, 663 (1997).
32. Lucinda, P. H. F. & Reis, R. E. Systematics of the subfamily Poeciliinae Bonaparte (Cyprinodontiformes: Poeciliidae), with an emphasis on the tribe Cnesterodontini Hubbs. *Neotrop. Ichthyol.* **3**, 1–60 (2005).
33. Rauchenberger, M. A New Species of Allodontichthys (Cyprinodontiformes: Goodeidae), with Comparative Morphometrics for the Genus. *Copeia* **1988**, 433–441 (1988).
34. Smith, M. L. & Miller, R. R. Allotoca maculata, a New Species of Goodeid Fish from Western México, with Comments on Allotoca dugesi. *Copeia* **1980**, 408–417 (1980).
35. Miller, R. R. & Fitzsimons, J. M. Ameca splendens, a New Genus and Species of Goodeid Fish from Western México, with Remarks on the Classification of the Goodeidae. *Copeia* **1971**, 1–13 (1971).
36. Hubbs, C. L. & Turner, C. L. Studies of the Fishes of the Order Cyprinodontes. XVI. A Revision of the Goodeidae. *The Miscellaneous Publications of the Museum of Zoology, University of Michigan* 1–80 (1939).
37. Fitzsimons, J. M. A Revision of Two Genera of Goodeid Fishes (Cyprinodontiformes, Osteichthyes) from the Mexican Plateau. *Copeia* **1972**, 728–756 (1972).
38. Turner, C. L. A Contribution to the Taxonomy and Zoogeography of the Goodeid Fishes. *Science* 1–13 (1946).

39. Parenti, Lynne R. A Taxonomic Revision of the Andean Killifish Genus *Orestias* (Cyprinodontiformes, Cyprinodontidae). *Bulletin of the American Museum of Natural History* **178**, 107–214 (1984).
40. Roberts, T. R. Systematics and Osteology of Leptoglaninae a New Subfamily of the African Catfish Family Amphiliidae, with Descriptions of Three New Genera and Six New Species. *Proceedings of the California Academy of Sciences* **54**, 81–132 (2003).
41. De Pinna, M., Reis, V. & Britski, H. A New Species of Trichogenes (Siluriformes, Trichomycteridae), with a Discussion on the Homologies of the Anterior Orbital Bones in Trichomycterids and Other Loricarioids. *American Museum Novitates* **2020**, 1–27 (2020).
42. Rocha, M., Lazzarotto, H. & Py-Daniel, L. R. A New Species of Scoloplax with a Remarkable New Tooth Morphology within Loricarioidea (Siluriformes: Scoloplacidae). *Copeia* **2012**, 670–677 (2012).
43. Schaefer, Scott A. Anatomy and Relationships of the Scoloplacid Catfishes. *Proceedings of the Academy of Natural Sciences of Philadelphia* **142**, 167–210 (1990).
44. Hora, S. L. & Silas, E. G. Evolution and Distribution of Glyptosternoid Fishes of the Family Sisoridae (Order: Siluroidea). *Proceedings of the National Institute of Sciences of India* **18**, 309–322 (1952).
45. DoNascimento, C. & Milani, N. The Venezuelan species of Phenacorhamdia (Siluriformes: Heptapteridae), with the description of two new species and a remarkable new tooth morphology for siluriforms. *Proceedings of the Academy of Natural Sciences of Philadelphia* **157**, 163–180 (2008).
46. Roberts, T. R. & Stewart, Donald J. An Ecological and Systematic Survey of Fishes in the Rapids of the Lower Zaire or Congo River. *Bulletin of the Museum of Comparative Zoology* **147**, 239–315 (1976).
47. Friel, J. P. & Vigliotta, T. R. *Atopodontus adriaensi*, a new genus and species of African suckermouth catfish from the Ogooué and Nyanga River systems of Gabon (Siluriformes: Mochokidae). *Proceedings of the Academy of Natural Sciences of Philadelphia* **157**, 13–23 (2008).
48. Vigliotta, T. R. A phylogenetic study of the African catfish family Mochokidae (Osteichthyes, Ostariophysi, Siluriformes), with a key to genera. *Proceedings of the Academy of Natural Sciences of Philadelphia* **157**, 73–136 (2008).
49. Covain, R. & Fisch-Muller, S. The genera of the Neotropical armored catfish subfamily Loricariinae (Siluriformes: Loricariidae): a practical key and synopsis. *Zootaxa* **1462**, 1–40 (2007).
50. Boeseman, M. The ‘comb-toothed’ Loricariinae of Surinam, with reflections on the phylogenetic tendencies within the family Loricariidae (Siluriformes, Siluroidei). *Zoologische Verhandelingen* **116**, 3–56 (1971).
51. Paixão, A. D. C. & Toledo-Piza, M. Systematics of Lamontichthys Miranda-Ribeiro (Siluriformes: Loricariidae), with the description of two new species. *Neotrop. Ichthyol.* **7**, 519–568 (2009).
52. Isbrücker, I. J. H. & Nijssen, H. *Rhadinoloricaria* gen. nov. and *Planiloricaria*, two genera of South American Mailed Catfishes (Pisces, Siluriformes, Loricariidae). *Beaufortia* **22**, 67–80 (1974).
53. Londoño-Burbano, A. & Reis, R. E. A Taxonomic Revision of Sturisomatichthys Isbrücker and Nijssen, 1979 (Loricariidae: Loricariinae), with Descriptions of Three New Species. *Copeia* **107**, 764 (2019).
54. Reis, R. E., Pereira, E. H. L. & Armbruster, J. W. Delturinae, a new loricariid catfish subfamily (Teleostei, Siluriformes), with revisions of Delturus and Hemipsilichthys. *Zoological Journal of the Linnean Society* **147**, 277–299 (2006).
55. Schaefer, S. A. & Provenzano, F. The Lithogeninae (Siluriformes, Loricariidae): Anatomy, Interrelationships, and Description of a New Species. *Am Museum Novitates* **3637**, 1 (2008).

56. Silva, G. S. C., Roxo, F. F. & Oyakawa, O. T. Description of a new species of Pareiorhina (Siluriformes: Loricariidae) from the rio São Francisco basin, Brazil. *Zootaxa* **4107**, 381 (2016).
57. Pereira, E. H. L., Zanata, A., Cetra, M. & Reis, R. E. A Remarkable Sexually Dimorphic New Genus and Species of Neoplecostomine Catfish (Siluriformes, Loricariidae) from a Coastal Drainage of Eastern Brazil. *Copeia* **2014**, 673–681 (2014).
58. Jerep, F. C., Shibatta, O. A., Pereira, E. H. L. & Oyakawa, O. T. Two new species of Isbrueckerichthys Derijst, 1996 (Siluriformes: Loricariidae) from the rio Paranapanema basin, Brazil. *Zootaxa* **1372**, 53 (2006).
59. Langeani, Francisco. Revisão do gênero Neoplecostomus Eigenmann & Eigenmann, 1888, com a descrição de quatro novas espécies do sudeste brasileiro (Ostariophysi, Siluriformes, Loricariidae). *Comunicações do Museu de Ciências, PUCRS, série Zoologia* **3**, 3–31 (1990).
60. Pereira, E. H. L. Resurrection of Pareiorhaphis Miranda Ribeiro, 1918 (Teleostei: Siluriformes: Loricariidae), and description of a new species from the rio Iguaçu basin, Brazil. *Neotrop. Ichthyol.* **3**, 271–276 (2005).
61. Reis, R. E. & Lehmann, P. Two New Species of Acestridium Haseman, 1911 (Loricariidae: Hypoptopomatinae) from the Rio Madeira Basin, Brazil. *Copeia* **2009**, 446–452 (2009).
62. Schaefer, S. A. The Neotropical Cascudinhos: Systematics and Biogeography of the Otocinclus Catfishes (Siluriformes: Loricariidae). *Proceedings of the Academy of Natural Sciences of Philadelphia* **148**, 1–120 (1997).
63. Silva, G. S. C., Roxo, F. F., Souza, C. S. & Oliveira, C. Two new species of Corumbataia (Hypoptopomatinae: Loricariidae) from Rio Corrente, upper Rio Paraná basin, Brazil. *Zootaxa* **4483**, 317–330 (2018).
64. Roxo, F., Da Costa E Silva, G., Orrego, L. E. & Oliveira, C. Description of a new genus and three new species of Otothyriinae (Siluriformes, Loricariidae). *ZK* **534**, 103–134 (2015).
65. Azpelicueta, Maria de las Mercedes & Casciotta, Jorge. A new species of Otothyriini (Siluriformes: Loricariidae: Hypoptopomatinae) from the Rio Uruguay basin, Argentina. *Verhandlungen der Gesellschaft fur Ichthyologie Band* **4**, 81–90 (2004).
66. Ribeiro, A. C., Carvalho, M. & Melo, A. L. A. Description and relationships of Otothyropsis marapoama, a new genus and species of hypoptopomatine catfish (Siluriformes: Loricariidae) from rio Tietê basin, southeastern Brazil. *Neotrop. Ichthyol.* **3**, 489–498 (2005).
67. Carvalho, T. P., Lehmann A., P. & Reis, R. E. Gymnotocinclus anosteos, a new uniquely-plated genus and species of loricariid catfish (Teleostei: Siluriformes) from the upper rio Tocantins basin, central Brazil. *Neotrop. Ichthyol.* **6**, 329–338 (2008).
68. Reis, R. E., Pereira, E. H. L. & Lehmann A., P. A New Genus and Species of Hypoptopomatine Catfish (Siluriformes: Loricariidae) from the Upper Rio São Francisco Basin, Brazil. *Copeia* **2012**, 6–11 (2012).
69. Martins, F. D. O. & Langeani, F. Rhinolekos, a new genus with three new species of Hypoptopomatinae (Siluriformes: Loricariidae) from upper rio Paraná. *Neotrop. Ichthyol.* **9**, 65–78 (2011).
70. Chamon, C. C. Redescription of Acanthicus hystrix Agassiz, 1829 (Siluriformes: Loricariidae), with comments on the systematics and distribution of the genus. *Zootaxa* **4088**, 395 (2016).
71. De Souza, L. S., Taphorn, D. C. & Armbruster, J. W. Review of Ancistrus (Siluriformes: Loricariidae) from the northwestern Guiana Shield, Orinoco Andes, and adjacent basins with description of six new species. *Zootaxa* **4552**, (2019).
72. Lujan, N. K., Meza-Vargas, V. & Barriga-Salazar, R. Two New Chaetostoma Group (Loricariidae: Hypostominae) Sister Genera from Opposite Sides of the Andes Mountains in Ecuador, with the Description of One New Species. *Copeia* **103**, 651–663 (2015).

73. Zawadzki, C. H., Bifi, A. C. & Mariotto, S. *Araichthys loro*, a new genus and species of suckermouth armored catfish from the upper rio Tapajos basin, Brazil (Siluriformes: Loricariidae). *Ichthyological Explorations of Freshwater* **27**, 361–372 (2016).
74. Salcedo, N. J. New Species of *Chaetostoma* (Siluriformes: Loricariidae) from Central Peru. *Copeia* **2006**, 60–67 (2006).
75. Armbruster, J. W., Sabaj, M. H., Hardman, M., Page, L. M. & Knouft, J. H. Catfish Genus *Corymbophanes* (Loricariidae: Hypostominae) with Description of One New Species: *Corymbophanes kaiei*. *Copeia* **2000**, 997–1006 (2000).
76. Cardoso, A. R. & Silva, J. F. P. D. Two new species of the genus *Hemiancistrus* Bleeker (Teleostei: Siluriformes: Loricariidae) from the upper rio Uruguai basin. *Neotrop. Ichthyol.* **2**, 1–8 (2004).
77. Lujan, N. K. & Armbruster, J. W. Two New Genera and Species of Ancistrini (Siluriformes: Loricariidae) from the Western Guiana Shield. *Copeia* **2011**, 216–225 (2011).
78. Armbruster, J. W. The species of the *Hypostomus cochliodon* group (Siluriformes: Loricariidae). *Zootaxa* **249**, 1 (2003).
79. Taverne, Louis & Gery, J. Un nouveau genre de Mormyridae (Poissons Osteoglossomorphes) : *Boulengeromyrus knoepffleri* gen. sp. Nov. *Rev. Zool. Bot. Afr.* **1**, 98–105 (1968).
80. Hopkins, Carl D., Lavoue, Sebastien, & Sullivan, John P. 12. Mormyridae. In *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa* vol. 2 1456 (Museum national d'Histoire naturelle, Paris, 2007).
81. Meunier, F. J., Germain, D. & Otero, O. A histological study of the lingual molariform teeth in *Hyperopisus bebe* (Mormyridae; Osteoglossomorpha). *Cybium* **42**, 87–90 (2018).
82. Gill, Theodore. Description of a New Generic Type of Mormyroids and Note on the Arrangement of the Genus. *Proceedings of the Academy of Natural Sciences of Philadelphia* **14**, 443–445 (1862).
83. Bigorne, Remy. 15. Mormyridae. In *The Fresh and Brackish Water Fishes of West Africa* vol. 1 155–222 (Musée Royal de l'Afrique Centrale, Tervuren, 2003).
84. Kramer, B., Van Der Bank, H. & Wink, M. Marked differentiation in a new species of dwarf stonebasher, *Pollimyrus cuandoensis* sp. Nov. (Mormyridae: Teleostei), from a contact zone with two sibling species of the Okavango and Zambezi rivers. *Journal of Natural History* **48**, 429–463 (2014).
85. Sullivan, J., Lavoue, S. & Hopkins, C. D. *Cryptomyrus*: a new genus of Mormyridae (Teleostei, Osteoglossomorpha) with two new species from Gabon, West-Central Africa. *ZK* **561**, 117–150 (2016).
86. Lavoué, S., Sullivan, J. P. & Arnegard, M. E. African weakly electric fishes of the genus *Petrocephalus* (Osteoglossomorpha: Mormyridae) of Odzala National Park, Republic of the Congo (Lékoli River, Congo River basin) with description of five new species. *Zootaxa* **2600**, 1–52 (2010).
87. Kramer, B., Bills, R., Skelton, P. & Wink, M. A critical revision of the 63epidiola snoutfish, genus *Petrocephalus* Marcusen, 1854 (Actinopterygii: Teleostei: Mormyridae), from southern and eastern Africa, with the recognition of *Petrocephalus tanensis*, and the description of five new species. *Journal of Natural History* **46**, 2179–2258 (2012).
88. Nafpaktitis, Basil G. et al. Order Iniomi (Myctophiformes). In *Fishes of the Western North Atlantic* vol. 7 299 (Sears Foundation for Marine Research, New Haven, 1977).
89. Schultz, Leonard P. A Revision of the Genera of Mulletts, Fishes of the Family Mugilidae, with Descriptions of Three New Genera. *Proceedings of the United States National Museum* **96**, 377–395 (1946).

90. Kubicek, K. M., Pinion, A. K. & Conway, K. W. New records of the Mountain Mullet, *Dajaus monticola* (Bancroft, 1834), and an overview of historical records in Texas. *CheckList* **15**, 471–478 (2019).
91. Lee, Chung-Lyul & Joo, Dong-Soo. Synopsis of Family Mugilidae (Perciformes) from Korea. *Bulletin of the Korean Fisheries Society* **27**, 814–824 (1994).
92. Gonzalez-Castro, Mariano & Ghasemzadeh, Javad. Morphology and Morphometry Based Taxonomy of Mugilidae. In *Biology, Ecology and Culture of Grey Mulletts (Mugilidae)* 539 (CRC Press, Boca Raton, 2016).
93. Cardona, Luis. Food and Feeding of Mugilidae. In *Biology, Ecology and Culture of Grey Mulletts (Mugilidae)* 539 (CRC Press, Boca Raton, 2016).
94. Ebeling, A. W. The Dentition of Eastern Pacific Mulletts, with Special Reference to Adaptation and Taxonomy. *Copeia* **1957**, 173–185 (1957).
95. Collette, B. B. & Su, Jinxiang. The Halfbeaks (Pisces, Beloniformes, Hemiramphidae) of the Far East. *Proceedings of the Academy of Natural Sciences of Philadelphia* **138**, 250–302 (1986).
96. Dyer, B. S. Phylogenetic Revision of the Atherinopsinae (Teleostei, Atherinopsidae), with Comments on the Systematics of the South American Freshwater Fish Genus *Basilichthys* Girard. *Miscellaneous Publications of the Museum of Zoology, University of Michigan* 1–64.
97. Parin, N. V. & Bogorodskiy, S. V. Distribution and morphology of flying fish *Cypselurus hexazona* placed into a separate subgenus *Zonocypselurus* subgen. Nov. *J. Ichthyol.* **51**, 658–661 (2011).
98. Tibbetts, I. R. & Carseldine, L. Anatomy of a hemiramphid pharyngeal mill with reference to *arrhamphus sclerolepis krefftii* (steindachner) (64epidiola: hemiramphidae). *Journal of Morphology* **255**, 228–243 (2003).
99. Goode, G. Brown & Bean, Tarleton H. Descriptions of twenty-five new species of fish from the southern United States, and three new genera, *Letharcus*, *loglossus*, and *Chriodorus*. *Proceedings of the United States National Museum* **5**, 412–437 (1882).
100. Collete, Bruce B., Bemis, Katherine E., Parin, Nicolay V., & Shakhovskoy, Ilia B. Order Beloniformes. In *Fishes of the Western North Atlantic* vol. 10 239 (Peabody Museum of Natural History, New Haven, 2018).
101. Wibowo, K., Wahyudewantoro, G. & Fahmi, F. First record of the Philippine snubnose halfbeak, *Melapedalion breve* (Actinopterygii: Beloniformes: Hemiramphidae), from Bangka Belitung Islands, Indonesia. *AieP* **52**, 107–110 (2022).
102. Smith, J. L. B. The South African Species of the Genus *Hemirhamphus* Cuv. *Transactions of the Royal Society of South Africa* **21**, 129–150 (1933).
103. Yagishita, N. & Nakabo, T. Revision of the genus *Girella* (Girellidae) from East Asia. *Ichthyological Research* **47**, 119–135 (2000).
104. Randall, J. E. A Revision of the Indo-Pacific Angelfish Genus *Genicanthus*, with Descriptions of Three New Species. *Bulletin of Marine Science* **25**, 393–421 (1975).
105. Vari, R. P. The Terapon Perches (Percoidei, Teraponidae). A Cladistic Analysis and Taxonomic Revision. *Bulletin of the American Museum of Natural History* **159**, 175–340 (1978).
106. Cavalluzzi, M. R. Osteology, Phylogeny, And Biogeography Of The Marine Fish Family Ehippidae (Perciformes, Acanthuroidei), With Comments On Sister Group Relationships [Doctoral dissertation, College of William and Mary and Virginia Institute of Marine Science]. 1–213 (2000) doi:10.25773/V5-PM7S-3Q12.
107. Parenti, Paolo. Family Scatophagidae Bleeker 1876 – scats. *California Academy of Sciences Annotated Checklist of Fishes* 1–5 (2004).
108. Fujita, K. Notes on the nomeid fish *Psenes sio* from the eastern Pacific. *Jap. Jour. Ich.* **38**, 323–327 (1991).

109. Urano, Takashi & Mochizuki, Kenji. A Record of an Ariommid Fish, *Ariomma indica*, from Japan. *Japanese Journal of Ichthyology* **31**, 205–208 (1984).
110. Al-Qattan, E, Dadzie, S, & Abou-Seedo, F. Feeding structures of the silver pomfret *Pampus argenteus* (Euphrasen) as observed by scanning electron microscopy. *Kuwait Journal of Science & Engineering* **27**, 329–341 (2000).
111. Haedrich, Richard L. Stromateidae. In *The Living Marine Resources of the Western Central Atlantic* vol. 3 758 (FAO, Rome, 2002).
112. Briggs, John C. A Monograph of the Clingfishes (Order Xenopterygii). *Stanford Ichthyological Bulletin* **6**, 1–224 (1955).
113. Smith, J. L. B. The Clingfishes of the Western Indian Ocean and the Red Sea. *Ichthyological Bulletin* 581–597 (1964).
114. Hutchins, B. & Morrison, S. Five new fish species of the genus *Alabes* (Gobiesocidae: Cheilobranchinae). *Rec. Aust. Mus.* **56**, 147–158 (2004).
115. Conway, K. W., Moore, G. I. & Summers, A. P. A new genus and two new species of miniature clingfishes from temperate southern Australia (Teleostei, Gobiesocidae). *ZK* **864**, 35–65 (2019).
116. Hutchins, J. B. Redescription of the Clingfish *Cochleocephalus spatula* (Gobiesocidae) from Western Australia and South Australia, with the Description of a New Species from Victoria and Tasmania. *Records of the Western Australia Museum* **11**, 33–47 (1983).
117. Conway, K. W., Moore, G. I. & Summers, A. P. A New Genus and Species of Clingfish (Teleostei: Gobiesocidae) from Western Australia. *Copeia* **105**, 128–140 (2017).
118. Craig, M. T. & Randall, J. E. *Briggsia hastingsi*, a new genus and species of clingfish from Oman. *Zootaxa* **2271**, 64–68 (2009).
119. Scott, E. O. G. Observations on Some Tasmanian Fishes Part III. *Papers and Proceedings of the Royal Society of Tasmania* 113–128 (1935).
120. Jordan, David Starr & Fowler, Henry W. A review of the cling-fishes (Gobiesocidae) of the waters of Japan. *Proceedings of the United States National Museum* **25**, 413–416 (1902).
121. Briggs, J. C. A New Genus and Species of Clingfish from the Western Pacific. *Copeia* **1976**, 339 (1976).
122. Fujiwara, K., Conway, K. W. & Motomura, H. Description of a New Genus and Two New Species of Indo-Pacific Clingfishes (Gobiesocidae: Diademichthyinae) with Redescription and Reassignment of Two Species Previously Assigned to *Lepadichthys* Waite, 1904. *Ichthyology & Herpetology* **109**, (2021).
123. Conway, K. W., Stewart, A. L. & Summers, A. P. A new genus and species of clingfish from the Rangitāhua Kermadec Islands of New Zealand (Teleostei, Gobiesocidae). *ZK* **786**, 75–104 (2018).
124. Briggs, J. C. The Clingfishes (Gobiesocidae) of Panama. *Copeia* **1969**, 774 (1969).
125. Fujiwara, K., Conway, K. W., Summers, A. P. & Motomura, H. Rediagnosis of the monotypic genus *Lepadicyathus* Prokofiev 2005 (Gobiesocidae: Diademichthyinae) and redescription of *Lepadicyathus minor* (Briggs 1955), new combination. *Ichthyol Res* **69**, 489–504 (2022).
126. Shiogaki, Masaru & Dotsu, Yoshie. Two New Genera and Two New Species of Clingfishes from Japan, with Comments on Head Sensory Canals of the Gobiesocidae. *Japanese Journal of Ichthyology* **30**, 111–121 (1983).
127. Fricke, R. *Unguitrema nigrum*, a new genus and species of clingfish. *Journal of the Ocean Science Foundation* **13**, 35–42 (2014).
128. Conway, K. W., Baldwin, C. & White, M. D. Cryptic Diversity and Venom Glands in Western Atlantic Clingfishes of the Genus *Acyrtus* (Teleostei: Gobiesocidae). *PloS ONE* **9**, e97664 (2014).
129. Sparks, J. S. & Gruber, D. F. A New Mesophotic Clingfish (Teleostei: Gobiesocidae) from the Bahamas. *Copeia* **2012**, 251–256 (2012).

130. Fricke, R., Wirtz, P. & Brito, A. A new species of the clingfish genus *Apletodon* (Teleostei: Gobiiesocidae) from the Cape Verde Islands, Eastern Central Atlantic. *Ichthyol Res* **57**, 91–97 (2010).
131. Wagner, M., Kovačić, M. & Koblmüller, S. Unravelling the taxonomy of an interstitial fish radiation: Three new species of *Gouania* (Teleostei: Gobiiesocidae) from the Mediterranean Sea and redescrptions of *G. WILLDENOWI* and *G. pigra*. *Journal of Fish Biology* **98**, 64–88 (2021).
132. Fricke, R., Chen, J.-N. & Chen, W.-J. New case of lateral asymmetry in fishes: A new subfamily, genus and species of deep water clingfishes from Papua New Guinea, western Pacific Ocean. *Comptes Rendus Biologies* **340**, 47–62 (2017).
133. Bohlke, J. E. & Robins, C. R. A new genus and species of deep-dwelling clingfish from the Lesser Antilles. *Notulae Naturae (Philadelphia)* 1–12 (1970).
134. Akihito, Prince & Meguro, Katsusuke. On the Differences between the Genera *Sicydium* and *Sicyopterus* (Gobiidae). *Japanese Journal of Ichthyology* **26**, 192–202 (1979).
135. Keith, P., Hoareau, T. & Bosc, P. The genus *Cotylopus* (Teleostei: Gobiioidei) endemic to the rivers of islands of the Indian Ocean with description of a new species from Mayotte (Comoros). *Journal of Natural History* **39**, 1395–1406 (2005).
136. Watson, R. E., Keith, P. & Marquet, G. *Lentipes kaaea*, a new species of freshwater goby (Teleostei: Gobiioidei: Sicydiinae) from New Caledonia. *Bull. Fr. Pêche Piscic.* 173–185 (2002) doi:10.1051/kmae:2002010.
137. Keith, P., Marquet, G. & Watson, R. E. *Akihito 66epidi*, a new species of freshwater goby from the South Pacific (Gobiioidei: Sicydiinae). *Cybium* **31**, 471–476 (2007).
138. Pezold, F., Iwamoto, T. & Harrison, I. J. The California Academy of Sciences Gulf of Guinea Expedition (2001) V. Multivariate Analysis of Sicydiines of São Tomé & Príncipe with Redescription of *Sicydium brevifile* and *S. bustamantei* (Teleostei: Gobiidae) and a Key to West African Sicydiines. *Proceedings of the California Academy of Sciences* **57**, 965–980 (2006).
139. Watson, Ronald E. Two new subgenera of *Sicyopus*, with a 66epidiolampro of *Sicyopus zosterophorum* (Teleostei: Gobiioidei: Sicydiinae). *Aqua, Journal of Ichthyology and Aquatic Biology* **3**, 257–274 (1999).
140. Keith, P. & Marquet, G. *Stiphodon rubromaculatus*, a new species of freshwater goby from Futuna Island (Gobiioidei: Sicydiinae). *Cybium* **31**, 45–49 (2007).
141. Watson, Ronald E. *Awaous (Awaous) acritosus*, a new species of freshwater goby from souther New Guinea and northeastern Australia (Teleostei: Gobiidae). *Ichthyol. Explor. Freshwat.* **5**, 351–364 (1994).
142. Cui, R., Pan, Y., Yang, X. & Wang, Y. A new barbeled goby from south China (Teleostei: Gobiidae). *Zootaxa* **3670**, 177–192 (2013).
143. Shibukawa, K. & Taki, Y. A new gobiid fish, *Acanthogobius insularis*, from the Ryukyu Islands, Japan. *Ichthyological Research* **43**, 79–85 (1996).
144. Shibukawa, K. & Iwata, A. Review of the East Asian Gobiid Genus *Chaeturichthys* (Teleostei: Perciformes: Gobiioidei), with Description of a New Species. *Bull. Nat. Mus. Nat. Sci. Ser. A. Supp.* **7**, 31–51 (2013).
145. Kim, D. S., Kweon, S.-M. & Choi, Y. First Record of Gobiid Fish, *Astrabe fasciata* (Perciformes: Gobiidae) from Dokdo, Korea. *Korean Journal of Ichthyology* **27**, 317–320 (2015).
146. Berti, R. & Ercolini, A. *Caecogobius cryptophthalmus* n. gen. n. sp. (Gobiidae Gobiinae), the first stygobitic fish from Philippines. *Tropical Zoology* **4**, 129–138 (1991).
147. Stevenson, D. E. Systematics and Distribution of Fishes of the Asian Goby Genera *Chaenogobius* and *Gymnogobius* (Osteichthyes: Perciformes: Gobiidae), with the Description of a New Species. *Species Diversity* **7**, 251–312 (2002).

148. Larson, Helen K. A review of the Australian endemic Gobiid fish genus *Chamydogobius*, with description of five new species. *The Beagle, Records of the Museums and Art Galleries of the Northern Territory* **12**, 19–51 (1995).
149. Shiogaki, M. A new gobiid fish of the genus *Clariger* from Mutsu Bay, northern Japan. *Jap. Jour. Ich.* **35**, 127–132 (1988).
150. Larson, H. K. Review of the Gobiid fish genera *Eugnathogobius* and *Pseudogobiopsis* (Gobioidei: Gobiidae: Gobionellinae), with descriptions of three new species. *The Raffles Bulletin of Zoology* **57**, 127–181 (2009).
151. Angmalisang, D. E., Maruyama, K., Hihara, A. & Kohno, H. Occurrence Patterns and Ontogenetic Intervals of *Eutaeniichthys gilli* (Gobiidae) in Obitsu-gawa River Estuary, Tokyo Bay, Central Japan. *La mer* **58**, 83–99 (2020).
152. Randall, J. E. & Greenfield, D. W. Redescription of *Gnatholepis cauerensis* (Bleeker, 1853), with discussion of the validity of the species. *Zool. Med. Leiden* 303–308 (2007).
153. Murdy, E. O. A review of the gobioid fish genus *Gobioides*. *Ichthyological Research* **45**, 121–133 (1998).
154. Ginsburg, Isaac. Ten new American gobioid fishes in the United States National Museum, including additions to a revision of *Gobionellus*. *Journal of the Washington Academy of Sciences* **43**, 18–26.
155. McDowall, R. M. & David, B. O. *Gobiopterus* in New Zealand (Teleostei: Gobiidae), with observations on sexual dimorphism. *New Zealand Journal of Marine and Freshwater Research* **42**, 325–331 (2008).
156. Larson, H. K. A review of the mangrove goby genus *Hemigobius* (Gobioidei, Gobiidae, Gobionellinae). *The Beagle, Records of the Museums and Art Galleries of the Northern Territory* **15**, 23–42 (1999).
157. Hubbs, Carl L. Notes on the Gobioid fishes of California, with descriptions of two new genera. *Occasional Papers of the Museum of Zoology, University of Michigan* **169**, 1–6 (1926).
158. Te Winkel, Lois E. A study of *Mistichthys luzonensis* with special reference to conditions correlated with reduced size. *Journal of Morphology* **58**, 463–535 (1935).
159. Larson, H. K., Geiger, M. F., Hadiaty, R. K. & Herder, F. *Mugilogobius hitam*, a new species of freshwater goby (Teleostei: Gobioidei: Gobiidae) from Lake Towuti, central Sulawesi, Indonesia. *The Raffles Bulletin of Zoology* **62**, 718–725 (2014).
160. Iwata, A., Hosoya, S. & Larson, H. K. *Paedogobius kimurai*, a new genus and species of goby (Teleostei: Gobiidae: Gobiidae) from the west Pacific. *Rec. Aust. Mus.* **53**, 103–112 (2001).
161. Smith, J. L. B. Gobioid fishes of the families Gobiidae, Periophthalmidae, Trypauchenidae, Taenioididae, and Kraemeriidae of the Western Indian Ocean. *Ichthyological Bulletin* **13**, 185–226 (1959).
162. MacGinitie, G. E. The Natural History of the Blind Goby, *Typhlogobius californiensis* Steindac. *The American Midland Naturalist* **21**, 489–505 (1939).
163. Murdy, E. O. A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Rec. Aust. Mus., Suppl.* **11**, 1–93 (1989).
164. Murdy, Edward. Systematics of Oxudercinae. In *The Biology of Gobies* 685 (CRC Press, Boca Raton, 2011).
165. Keith, Philippe & Lord, Clara. Systematics of Sicydiinae. In *The Biology of Gobies* 685 (CRC Press, Boca Raton, 2011).
166. Shibukawa, K. & Murdy, E. O. A Redescription of the Eel Goby *Trypauchenopsis* (Gobiidae: Amblyopinae) with Comments on Relationships. *Copeia* **2012**, 527–534 (2012).

167. Vari, Richard P. Anatomy, relationships and classification of the families Citharinidae and Distichodontidae (Pisces, Characoidea). *Bulletin of the British Museum (Natural History) Zoology* **36**, 261–344 (1979).
168. Zanata, A. M. & Vari, R. P. The family Alestidae (Ostariophysi, Characiformes): a phylogenetic analysis of a trans-Atlantic clade. *Zoological Journal of the Linnean Society* **145**, 1–144 (2005).
169. Ramirez, J. L., Birindelli, J. L. O. & Galetti, P. M. A new genus of Anostomidae (Ostariophysi: Characiformes): Diversity, phylogeny and biogeography based on cytogenetic, molecular and morphological data. *Molecular Phylogenetics and Evolution* **107**, 308–323 (2017).
170. Assega, F. M. & Birindelli, J. L. O. Taxonomic revision of the genus Anostomoides (Characiformes: Anostomidae). *Zootaxa* **4646**, 124–144 (2019).
171. Myers, G. S. & De Carvalho, A. A Remarkable New Genus of Anostomin Characid Fishes from the Upper Rio Xingu in Central Brazil. *Copeia* **1959**, 148 (1959).
172. Sidlauskas, B. L. & Vari, R. P. Phylogenetic relationships within the South American fish family Anostomidae (Teleostei, Ostariophysi, Characiformes). *Zoological Journal of the Linnean Society* **154**, 70–210 (2008).
173. Garavello, J. C. & Santos, G. M. Two new species of Leporinus Agassiz, 1829 from Araguaia-Tocantins system, Amazon basin, Brazil (Ostariophysi, Anostomidae). *Braz. J. Biol.* **69**, 109–116 (2009).
174. Machado-Evangelista, M., Esguícero, A. L. H., Arcifa, M. S. & Pereira, T. N. A. Diet and ecomorphology of Leporinus reticulatus (Characiformes: Anostomidae) from the upper Rio Jurueña, MT, Brazil: ontogenetic shifts related to the feeding ecology. *Acta Amaz.* **45**, 383–392 (2015).
175. Lima, F. C. T. A revision of the cis-andean species of the genus Brycon Müller & Troschel (Characiformes: Characidae). *Zootaxa* **4222**, (2017).
176. Howes, G. Review of the genus Brycon (Teleostei: Characoidei). *Bulletin of the British Museum of Natural History (Zoology)* **43**, 1–47 (1982).
177. Castro, R. M. C., Vari, R. P., Vieira, F. & Oliveira, C. Phylogenetic Analysis and Redescription of the Genus Henochilus (Characiformes: Characidae). *Copeia* **2004**, 496–506 (2004).
178. Zanata, A. M. & Toledo-Piza, M. Taxonomic revision of the South American fish genus Chalceus Cuvier (Teleostei: Ostariophysi: Characiformes) with the description of three new species. *Zoological Journal of the Linnean Society* **140**, 103–135 (2004).
179. Menezes, N. A. Description of five new species of Acestrocephalus Eigenmann and redescription of A. sardina and A. boehlkei (Characiformes: Characidae). *Neotrop. Ichthyol.* **4**, 385–400 (2006).
180. Arcila, D., Vari, R. P. & Menezes, N. A. Revision of the Neotropical Genus Acrobrycon (Ostariophysi: Characiformes: Characidae) with Description of Two New Species. *Copeia* **2013**, 604–611 (2013).
181. Bührnheim, C. M., Carvalho, T. P., Malabarba, L. R. & Weitzman, S. H. A new genus and species of characid fish from the Amazon basin: the recognition of a relictual lineage of characid fishes (Ostariophysi: Cheirodontinae: Cheirodontini). *Neotrop. Ichthyol.* **6**, 663–678 (2008).
182. De Brito, P. S., Guimarães, E. C., Katz, A. M., Piorski, N. M. & Ottoni, F. P. Taxonomic status of Aphyocharax avary Fowler, 1913, Aphyocharax pusillus Günther, 1868 and Chirodon 68epidiol Günther, 1869 (Characiformes, Characidae). *Zoosystematics and Evolution* **94**, 393–399 (2018).
183. Eigenmann, C. H. The American Characidae. *Memoirs of the Museum of Comparative Zoology, Harvard College* **43**, 209–310 (1921).
184. Eigenmann, C. H. On further collections of fishes from Paraguay. *Annals of the Carnegie Museum* **4**, 110–157 (1907).

185. Cucalón, R. V. *et al.* Phylogeography of the Chocó Endemic Rainbow Characin (Teleostei: Rhoadsia). *Ichthyology & Herpetology* **110**, 138–155 (2022).
186. Mirande, J. M., Jerep, F. C. & Vanegas-Ríos, J. A. Phylogenetic relationships of the enigmatic *Carlastyanax aurocaudatus* (Eigenmann) with remarks on the phylogeny of the Stevardiinae (Teleostei: Characidae). *Neotrop. Ichthyol.* **11**, 747–766 (2013).
187. Chernoff, B. & Machado-Allison, A. Characid Fishes of the Genus *Ceratobranchia*, with Descriptions of New Species from Venezuela and Peru. *Proceedings of the Academy of Natural Sciences of Philadelphia* **142**, 261–290 (1990).
188. Soto, M. Á. *Et al.* Evolution of trans-Andean endemic fishes of the genus *Cheirodon* (Teleostei: Characidae) are associated with chromosomal rearrangements. *Rev. Chil. De Hist. Nat.* **91**, 1–8 (2018).
189. Weitzman, S. H. & Thomerson, J. E. A New Species of Glandulocaudine Characid Fish, *Hysteronotus Myersi*, from Peru. *Proceedings of the California Academy of Sciences* **38**, 139–156 (1970).
190. Fink, W. & Weitzman, S. H. *The So-Called Cheirodontin Fishes of Central America with Descriptions of Two New Species (Pisces: Characidae)*. (Smithsonian Institution Press, Washington, D.C., 1974).
191. Langeani, F. & Serra, J. P. *Coptobrycon bilineatus* (Ellis, 1911) (Characiformes: Characidae): redescription and comments on its phylogenetic relationships. *Neotrop. Ichthyol.* **8**, 727–736 (2010).
192. Vari, R. P. & Harold, A. S. *Phylogenetic Study of the Neotropical Fish Genera Creagrutus Günther and Piabina Reinhardt (Teleostei: Ostariophysi: Characiformes), with a Revision of the Cis-Andean Species*. (Smithsonian Institution Press, Washington, D.C., 2001).
193. Benine, R. C., Lopes, G. A. M. & Ron, E. A new species of *Ctenobrycon* Eigenmann, 1908 (Characiformes: Characidae) from the río Orinoco basin, Venezuela. *Zootaxa* **2715**, 59–67 (2010).
194. Menezes, N. A. A new species of *Cynopotamus Valenciennes*, 1849 (Characiformes, Characidae) with a key to the species of the genus. *Zootaxa* **1635**, 55–61 (2007).
195. Da Silva Gonçalves, C. & Cestari, C. Grazing of the lambari fish *Deuterodon iguape* is associated with mouth morphology. *Zoologischer Anzeiger* **274**, 127–130 (2018).
196. Menezes, N. A. & Weitzman, S. H. A systematic review of *diapoma* (69epidiola: characiformes: 69epidiolam: stevardiinae: diapomini) with descriptions of two new species from southern Brazil. *Pap. Avulsos Zool.* **51**, 59–82 (2011).
197. Fink, W. A New Genus and Species of Characid Fish from the Bayano River Basin, Panama (Pisces: Cypriniformes). *Proceedings of the Biological Society of Washington* **88**, 331–344 (1976).
198. Netto-Ferreira, A. L., Birindelli, J. L. O., De Sousa, L. M., Mariguela, T. C. & Oliveira, C. A New Miniature Characid (Ostariophysi: Characiformes: Characidae), with Phylogenetic Position Inferred from Morphological and Molecular Data. *PLoS ONE* **8**, e52098 (2013).
199. Giovannetti, V., Toledo-Piza, M. & Menezes, N. A. Taxonomic revision of *Galeocharax* (Characiformes: Characidae: Characinae). *Neotrop. Ichthyol.* **15**, e160040 (2017).
200. Menezes, N. A. & Weitzman, S. H. Systematics of the neotropical fish subfamily Glandulocaudinae (Teleostei: Characiformes: Characidae). *Neotrop. Ichthyol.* **7**, 295–370 (2009).
201. Benine, R. C., Melo, B. F., Castro, R. M. C. & Oliveira, C. Taxonomic revision and molecular phylogeny of *Gymnocorymbus* Eigenmann, 1908 (Teleostei, Characiformes, Characidae). *Zootaxa* **3956**, 1–28 (2015).
202. Serra, J. P. & Langeani, F. A new *Hasemania* Ellis from the upper rio Paraná basin, with the redescription of *Hasemania crenuchoides* Zarske & Géry (Characiformes: Characidae). *Neotrop. Ichthyol.* **13**, 479–486 (2015).

203. Bertaco, V. A. & Carvalho, F. R. New species of *Hasemania* (Characiformes: Characidae) from Central Brazil, with comments on the endemism of upper rio Tocantins basin, Goiás State. *Neotrop. Ichthyol.* **8**, 27–32 (2010).
204. Mirande, J. M. Phylogeny of the family Characidae (Teleostei: Characiformes): from characters to taxonomy. *Neotrop. Ichthyol.* **8**, 385–568 (2010).
205. Casciotta, J. R., Miquelarena, A., M. & Protogino, L. A New Species of *Odontostilbe* (Teleostei, Characidae) from the Uruguay Basin, with Comments on the Diagnostic Characters of the Genus. *Occasional Papers of the Museum of Natural History, The University of Kansas* **149**, 1–16 (1992).
206. Bertaco, V. A. & Malabarba, L. R. A new species of the characid genus *Hollandichthys* Eigenmann from coastal rivers of southern Brazil (Teleostei: Characiformes) with a discussion on the diagnosis of the genus. *Neotrop. Ichthyol.* **11**, 767–778 (2013).
207. Ohara, W. M., Abrahão, V. P. & Espíndola, V. C. *Hyphessobrycon platyodus* (Teleostei: Characiformes), a new species from the Rio Madeira basin, Brazil, with comments on how multicuspid teeth relate to feeding habits in Characidae. *Journal of Fish Biology* **91**, 835–850 (2017).
208. Netto-Ferreira, A. L., Zanata, A. M., Birindelli, J. L. O. & Sousa, L. M. Two new species of *Jupiaba* (Characiformes: Characidae) from the rio Tapajós and rio Madeira drainages, Brazil, with an identification key to species of the genus. *Zootaxa* **2262**, 53–68 (2009).
209. Malabarba, L. R. & Weitzman, S. H. A new genus and species of inseminating fish (Teleostei: Characidae: Cheirodontinae: Compsurini) from South America with a uniquely derived caudal-fin dermal papillae. *Proceedings of the Biological Society of Washington* **113**, 269–283 (2000).
210. Ferreira, K. M., Menezes, N. A. & Quagio-Grassiotto, I. A new genus and two new species of Stevardiinae (Characiformes: Characidae) with a hypothesis on their relationships based on morphological and histological data. *Neotrop. Ichthyol.* **9**, 281–298 (2011).
211. Vari, R. P., Melo, B. F. & Oliveira, C. *Protocheirodon*, a new genus of Characidae (Teleostei: Characiformes) with the redescription of the poorly known *Protocheirodon pi*. *Neotrop. Ichthyol.* **14**, (2016).
212. Castro, R. M. C., Ribeiro, A. C., Benine, R. C. & Melo, A. L. A. *Lophiobrycon weitzmani*, a new genus and species of glandulocaudine fish (Characiformes: Characidae) from the rio Grande drainage, upper rio Paraná system, southeastern Brazil. *Neotrop. Ichthyol.* **1**, 11–19 (2003).
213. Jerep, F. C. & Malabarba, L. R. Revision of the genus *Macropsobrycon* Eigenmann, 1915 (Characidae: Cheirodontinae: Compsurini). *Neotrop. Ichthyol.* **9**, 299–312 (2011).
214. Baicere-Silva, C. M., Benine, R. C. & Quagio-Grassiotto, I. *Markiana nigripinnis* (Perugia, 1891) as a putative member of the subfamily Stevardiinae (Characiformes: Characidae): spermatoc evidence. *Neotrop. Ichthyol.* **9**, 371–376 (2011).
215. Ohara, W. M., Jerep, F. C. & Cavallaro, M. R. A new species of *Microschemobrycon* (Characiformes: Characidae) from Rio Xingu basin, Brazil. *Zootaxa* **4576**, (2019).
216. Ribeiro, A. C. & Menezes, N. A. Phylogenetic relationships of the species and biogeography of the characid genus *Oligosarcus* Günther, 1864 (Ostariophysi, Characiformes, Characidae). *Zootaxa* **3949**, 41–81 (2015).
217. Bittencourt, P. S., Machado, V. N., Marshall, B. G., Hrbek, T. & Farias, I. P. Phylogenetic relationships of the neon tetras *Paracheirodon* spp. (Characiformes: Characidae: Stethaprioninae), including comments on *Petitella georgiae* and *Hemigrammus bleheri*. *Neotrop. Ichthyol.* **18**, e190109 (2020).
218. Lucena, Z. M. S. D. & Gama, C. D. S. *Phenacogaster apiletostigma*, nova espécie de peixe do Estado do Amapá, Brasil (Characiformes, Characidae). *Rev. Bras. Zool.* **24**, 151–156 (2007).
219. Deprá, G. D. C., Graça, W. J. D., Pavanelli, C. S., Avelino, G. S. & Oliveira, C. Molecular phylogeny of *Planaltina* Böhlke (Characidae: Stevardiinae) and comments on the definition and

geographic distribution of the genus, with description of a new species. *PloS ONE* **13**, e0196291 (2018).

220. Santos, O. & Castro, R. M. C. Taxonomy of *Probolodus* Eigenmann, 1911 (Characiformes: Characidae) with description of two new species, and comments about the phylogenetic relationships and biogeography of the genus. *Neotrop. Ichthyol.* **12**, 403–418 (2014).
221. Bertaco, V. A., Chuctaya, J., Jerep, F. C. & Malabarba, L. R. Revision of *Prodontocharax* and revalidation of *Amblystilbe* (Teleostei: Characidae: Cheirodontinae), with description of a new species. *Neotrop. Ichthyol.* **21**, e230031 (2023).
222. Myers, G. S. *Rhinobrycon negrensis*, a new genus and species of characid fishes from the Rio Negro, Brazil. *Proceedings of the California Academy of Sciences* **23**, 587–590 (1944).
223. Malabarba, L. R. & Jerep, F. C. Review of the species of the genus *Serrapinnus* Malabarba, 1998 (Teleostei: Characidae: Cheirodontinae) from the rio Tocantins-Araguaia basin, with description of three new species. *Zootaxa* **3847**, 57–79 (2014).
224. Weitzman, S. H. & Malabarba, L. R. Systematics of *Spintherobolus* (Teleostei: Characidae: Cheirodontinae) from eastern Brazil. *Ichthyological Explorations of Freshwater* **10**, 1–43 (1999).
225. Moreira, C. R., Bichuette, M. E., Oyakawa, O. T., De Pinna, M. C. C. & Trajano, E. Rediscovery and redescription of the unusual subterranean characiform *Stygichthys typhlops*, with notes on its life history. *Journal of Fish Biology* **76**, 1815–1824 (2010).
226. Silva, G. S. C., Melo, B. F., Oliveira, C. & Benine, R. C. Morphological and molecular evidence for two new species of *Tetragonopterus* (Characiformes: Characidae) from central Brazil. *Journal of Fish Biology* **82**, 1613–1631 (2013).
227. Román-Valencia, C., García-Alzate, C. A., Ruiz-C, R. I., Donald, C. & Taphorn, B. A new species of *Tyttocharax* (Characiformes: Characidae: Stevardiinae) from the Güejar river, Orinoco river Basin, Colombia. *Neotrop. Ichthyol.* **10**, 519–525 (2012).
228. Scharcansky, A. & De Lucena, C. A. S. *Caenotropus 71epidiola*, a new chilodontid fish from the Rio Tapajos drainage, Brazil (Ostariophysi: Characiformes: Chilodontidae). *Zootaxa* **1557**, 59–66 (2007).
229. Weitzman, S. H. & Kanazawa, R. H. *Ammocryptocharax elegans*, a new genus and species of characid fish (Teleostei: Characidae) from South America. *Proceedings of the Biological Society of Washington* **89**, 325–346 (1976).
230. Da Graça, W. J., Pavanelli, C. S. & Buckup, P. A. Two New Species of *Characidium* (Characiformes: Crenuchidae) from Paraguay and Xingu River Basins, State of Mato Grosso, Brazil. *Copeia* **2008**, 326–332 (2008).
231. Eigenmann, C. H. Report on the Expedition to British Guiana of the Indiana University and the Carnegie Museum, 1908. Report No. 1. Some New Genera and Species of Fishes from British Guiana. *Annals of the Carnegie Museum* **6**, 4–54 (1909).
232. Weitzman, S. H. Further notes on the relationships and classification of the South American Characid fishes of the subfamily Gasteropelecinae. *Stanford Ichthyological Bulletin* **7**, 217–239 (1960).
233. Langeani, F. New Species of *Hemiodus* (Ostariophysi, Characiformes, Hemiodontidae) from the Rio Tocantins, Brazil, with Comments on Color Patterns and Tooth Shapes within the Species and Genus. *Copeia* **1999**, 718–722 (1999).
234. Netto-Ferreira, A. L. Three new species of *Lebiasina* (Characiformes: Lebiasinidae) from the Brazilian shield border at Serra do Cachimbo, Pará, Brazil. *Neotrop. Ichthyol.* **10**, 487–498 (2012).
235. Weitzman, S. H. Three New Species of Fishes of the Genus *Nannostomus* from the Brazilian States of Para and Amazonas (Teleostei: Lebiasinidae). *Smithsonian Contributions to Ichthyology* **263**, 1–14 (1978).

236. Pavanelli, C. S. & Britski, H. A. *Apareiodon* Eigenmann, 1916 (Teleostei, Characiformes), from the Tocantins-Araguaia Basin, with Description of Three New Species. *Copeia* **2003**, 337–348 (2003).
237. Ingenito, L. F. S. & Buckup, P. A. A New Species of *Parodon* from the Serra da Mantiqueira, Brazil (Teleostei: Characiformes: Parodontidae). *Copeia* **2005**, 765–771 (2005).
238. Roberts, T. R. Dental polymorphism and systematics in *Saccodon*, a neotropical genus of freshwater fishes (Parodontidae, Characoidei). *Journal of Zoology* **173**, 303–321 (1974).
239. Gosline, W. H. Notes on the characid fishes of the subfamily Serrasalminae. *Proceedings of the California Academy of Sciences* **27**, 17–64 (1951).
240. Sazima, I. Scale-eating in characoids and other fishes. *Environmental Biology of Fishes* **9**, 87–101 (1983).
241. Kolmann, M. A. *et al.* Tooth and consequences: Heterodonty and dental replacement in piranhas and pacus (Serrasalminidae). *Evolution and Development* **21**, 247–262 (2019).
242. Pavanelli, C. S., Ota, R. P. & Petry, P. New species of *Metynnis* Cope, 1878 (Characiformes: Characidae) from the rio Paraguay basin, Mato Grosso State, Brazil. *Neotrop. Ichthyol.* **7**, 141–146 (2009).
243. Jegu, M. Subfamily Serrasalminae (Pacus and piranhas). In *Checklist of the freshwater fishes of South and Central America* 729 (Edipucrs, Porto Alegre, 2003).
244. Jégu, M. & Santos, G. M. D. Revision of the status of *Myleus setiger* Müller & Troschel, 1844 and *Myleus knerii* (Steindachner, 1881) (Teleostei: Characidae: Serrasalminae) with a complementary description of the two species. *Cybium* **26**, 33–57 (2002).
245. Gery, J. Poissons Characoides des Guyanes. I. Generalites, II. Famille des Serrasalminidae. *Zoologische Verhandelingen* **122**, 1–250 (1972).
246. Andrade, M. C., Sousa, L. M., Ota, R. P., Jégu, M. & Giarrizzo, T. Redescription and Geographical Distribution of the Endangered Fish *Ossubtus xinguense* Jégu 1992 (Characiformes, Serrasalminidae) with Comments on Conservation of the Rheophilic Fauna of the Xingu River. *PLoS ONE* **11**, e0161398 (2016).
247. Fink, W. L. Revision of the Piranha Genus *Pygocentrus* (Teleostei, Characiformes). *Copeia* **1993**, 665–687 (1993).
248. Fink, W. L. & Machado-Allison, A. Three new species of piranhas from Brazil and Venezuela (Teleostei: Characiformes). *Ichthyological Explorations of Freshwater* **3**, 55–71 (1992).
249. Jegu, M., Tito De Moraes, L. & Mendes Dos Santos, G. Redescription des types d'*Utiaritichthys sennaebregai* Miranda Ribeiro, 1937 et description d'une nouvelle espece du bassin Amazonien, *U. longidorsalis* (Characiformes, Serrasalminidae). *Cybium* **16**, 105–120 (1992).
250. Miquelarena, A. M. & Casciotta, J. R. Presencia en la Argentina de *Clupeacharax anchoveoides* Pearson, 1924. *Limnobiós* **2**, 333–335 (1982).
251. Malabarba, M. C. Revision of the Neotropical genus *Triportheus* Cope, 1872 (Characiformes: Characidae). *Neotropical Ichthyology* **2**, 167–204 (2004).
252. Oliver, M. K. & Arnegard, M. E. A new genus for *Melanochromis labrosus*, a problematic Lake Malawi cichlid with hypertrophied lips (Teleostei: Cichlidae). *Ichthyol. Explor. Freshwat.* **21**, 209–232 (2010).
253. Ribbink, A. J., Marsh, B. A., Marsh, A. C., Ribbink, A. C. & Sharp, B. J. A preliminary survey of the cichlid fishes of rocky habitats in Lake Malawi. *South African Journal of Zoology* **18**, 149–310 (1983).
254. Trewavas, E. *Tilapiine Fishes of the Genera Sarotherodon, Oreochromis and Danakilia*. (British Museum (Natural History), London, 1983).
255. Froese, R. & Pauly, D. FishBase. www.fishbase.org.

256. Ford, A. G. P. *et al.* Niche divergence facilitated by fine-scale ecological partitioning in a recent cichlid fish adaptive radiation. *Evolution* **70**, 2718–2735 (2016).
257. Poll, M. Les genres des 73epidio d'eau douce de d'Afrique. *Ann. Mus. Roy. Congo Belge* **54**, 1–191 (1957).
258. Takahashi, T. Systematics of Tanganyikan cichlid fishes (Teleostei: Perciformes). *Ichthyological Research* **50**, 367–382 (2003).
259. Takamura, K. Interspecific relationships of aufwuchs-eating fishes in Lake Tanganyika. *Environ Biol Fish* **10**, 225–241 (1984).
260. Eccles, D. H. & Trewavas, E. *Malawian Cichlid Fishes: The Classification of Some Haplochromine Genera*. (Lake Fish Movies, West Germany, 1989).
261. Bills, I. R. & Weyl, O. L. F. A New Species of the Genus *Chetia* (Teleostei: Cichlidae) from the Lecitu River, Buzi System, Mozambique. *Smithiana* **1**, 1–13 (2002).
262. Greenwood, P. H. A review of the serranochromine cichlid fish genera *Pharyngochromis*, *Sargochromis*, *Serranochromis* and *Chetia* (Teleostei: Labroidei). *Bulletin of the British Museum of Natural History (Zoology)* **59**, 33–44 (1993).
263. Skelton, P. H. *A Complete Guide to the Freshwater Fishes of Southern Africa*. (Struik Publishers, Cape Town, South Africa, 2001).
264. Li, S., Konings, A. F. & Stauffer, J. R., Jr. A Revision of the *Pseudotropheus elongatus* species group (Teleostei: Cichlidae) With Description of a New Genus and Seven New Species. *Zootaxa* **4168**, 353–381 (2016).
265. Stauffer, J. R., Bowers, N. J., Kellogg, K. A. & McKaye, K. R. A revision of the blue-black *Pseudotropheus zebra* (Teleostei: Cichlidae) complex from Lake Malawi, Africa, with a description of a new genus and ten new species. *Proceedings of the Academy of Natural Sciences of Philadelphia* **148**, 189–230 (1997).
266. Konings, A. *Ad Konings's Book of Cichlids and All the Other Fishes of Lake Malawi*. (T.F.H. Publications, Inc., Neptune City, NJ, 1990).
267. Teugels, G. G. & Thys van den Audenaerde, D. F. E. Cichlidae. In *Faune des 73epidio d'eaux douce et saumâtres de l'Afrique de l'Ouest* vol. 2 520–600 (Musée Royal de l'Afrique Centrale, Turvuren, Belgium, 2003).
268. Dunz, A. R., Vreven, E. & Schliewen, U. K. Congolapia, a new cichlid genus from the central Congo basin (Perciformes: Cichlidae). *Ichthyol. Explor. Freshwat.* **23**, 155–179 (2012).
269. Dunz, A. R. & Schliewen, U. K. Description of a *Tilapia* (Coptodon) species flock of Lake Ejagham (Cameroon), including a redescription of *Tilapia deckerti* Thys van den Audenaerde, 1967. *Spixiana* **33**, 251–280 (2010).
270. Dunz, A. R. & Schliewen, U. K. Description of a new species of *Tilapia* Smith, 1840 (Teleostei: Cichlidae) from Ghana. *Zootaxa* **2548**, 1–21 (2010).
271. Genner, M. J. *et al.* Revision of the African cichlid fish genus *Ctenochromis* (Teleostei, Cichliformes), including a description of the new genus *Shuja* from Lake Tanganyika and the new species *Ctenochromis scatebra* from northern Tanzania. *EJT* **819**, 23–54 (2022).
272. Haruki, O. Mate monopolization by a dominant male in a multi-male social group of a mouthbrooding cichlid, *Ctenochromis horei*. *Japanese Journal of Ichthyology* **40**, 209–218 (1993).
273. Trewavas, E. VI.—A synopsis of the Cichlid Fishes of Lake Nyasa. *Annals and Magazine of Natural History* **16**, 65–118 (1935).
274. Roberts, T. R. & Kullander, S. O. Endemic cichlid fishes of the Fwa River, Zaire: Systematics and ecology. *Ichthyological Explorations of Freshwater* **5**, 97–154 (1994).
275. Eccles, D. H. & Lewis, D. S. C. A revision of the genus *Docimodus* Boulenger (Pisces: Cichlidae), a group of fishes with unusual feeding habits from Lake Malawi. *Zoological Journal of the Linnean Society* **58**, 165–172 (1976).

276. Regan, C. T. The Cichlid Fishes of Lake Nyassa. *Proceedings of the Zoological Society of London* **1921**, 675–727 (1922).
277. Schliewen, U. K. & Stiassny, M. L. J. Etia nguti, a new genus and species of cichlid fish from the River Mamfue, Upper Cross River basin in Cameroon, West-Central Africa. *Ichthyological Explorations of Freshwater* **14**, 61–71 (2003).
278. Lamboj, A. *The Cichlid Fishes of Western Africa*. (Birgit Schmettkamp Verlag, Germany, 2004).
279. Sparks, J. S. Phylogeny of the Cichlid Subfamily Etroplinae and Taxonomic Revision of the Malagasy Cichlid Genus Paretroplus (Teleostei: Cichlidae). *Bull Mus Nat Hist* **2008**, 1–151 (2008).
280. Sampaio, W., Belei, F., Giongo, P., Dergam, J. & Orsi, M. L. Heterotilapia buttikoferi (Hubrecht, 1881) (Perciformes: Cichlidae), an introduced exotic fish in the upper Parana river basin. *CheckList* **13**, 245–250 (2017).
281. Esmaeili, H. R., Sayyadzadeh, G. & Seehausen, O. Iranocichla persa, a new cichlid species from southern Iran (Teleostei, Cichlidae). *ZK* **636**, 141–161 (2016).
282. Coad, B. W. A new genus and species of cichlid endemic to southern Iran. *Copeia* **1982**, 28–37 (1982).
283. Trewavas, E., Green, J. & Corbet, S. A. Ecological studies on crater lakes in West Cameroon Fishes of Barombi Mbo. *Journal of Zoology* **167**, 41–95 (1972).
284. Brichard, P. *Pierre Brichard's Book of Cichlids and All the Other Fishes of Lake Tanganyika*. (T.F.H. Publications, Inc., Neptune City, NJ, 1989).
285. Seehausen, O., Lippitsch, E., Bouton, N. & Zwennes, H. Mbipi, the rock-dwelling cichlids of Lake Victoria: description of three new genera and fifteen new species. *Ichthyological Explorations of Freshwater* **9**, 129–228 (1998).
286. Stauffer, J. R., Black, K. & Konings, A. F. Descriptions of five new species of Metriaclima (Teleostei: Cichlidae) from Lake Malaŵi, Africa. *Zootaxa* **3647**, 101–136 (2013).
287. Stauffer, J. R. & Konings, A. F. Review of Copadichromis (Teleostei: Cichlidae) with the description of a new genus and six new species. *Ichthyol. Explor. Freshwat.* **17**, 9–42 (2006).
288. Stauffer, J. R., LoVullo, T. J. & McKaye, K. R. Three New Sand-Dwelling Cichlids from Lake Malaŵi, Africa, with a Discussion of the Status of the Genus Copadichromis (Teleostei: Cichlidae). *Copeia* **1993**, 1017–1027 (1993).
289. Greenwood, P. H. & Kullander, S. O. A taxonomic review and redescription of Tilapia polyacanthus and T. stormsi (Teleostei: Cichlidae), with descriptions of two new Schwetzochromis species from the upper Zaïre River drainage. *Ichthyological Exploration of Freshwaters* **5**, 161–180 (1994).
290. De Vos, L. D. G. & Seegers, L. Seven new Orthochromis species (Teleostei: Cichlidae) from the Malagarasi, Luiche and Rugufu basins (Lake Tanganyika drainage), with notes on their reproductive biology. *Ichthyological Exploration of Freshwaters* **9**, 371–420 (1998).
291. Greenwood, P. H. The haplochromine species (Teleostei, Cichlidae) of the Cunene and certain other Angolan rivers. *Bulletin of the British Museum (Natural History) Zoology* **47**, 187–239 (1984).
292. Boulenger, G. A. A revision of the African and Syrian fishes of the family Cichlidae. Part II. *Proceedings of the Zoological Society of London* 98–143 (1899).
293. Mattsson, C. A morphological and molecular analysis of the species diversity of the cichlid genus *Petrochromis* from Lake Tanganyika (Teleostei: Cichlidae). Preprint at <https://doi.org/10.1101/280263> (2018).
294. Yamaoka, K. Feeding behavior and dental morphology of algae scraping cichlids (Pisces: Teleostei) in Lake Tanganyika. *African Study Monographs* **4**, 77–89 (1983).

295. Lundeba, M., Stauffer Jr., J. R. & Konings, A. F. Five new species of the genus *Petrotilapia* (Teleostei: Cichlidae), from Lake Malawi, Africa. *Ichthyological Explorations of Freshwaters* **22**, 149–168 (2011).
296. Greenwood, P. H. A revision and 75epidiolampro of the monotypic cichlid genus *Pharyngochromis* (Teleostei, Labroidei). *Bulletin of the British Museum of Natural History (Zoology)* **58**, 37–52 (1992).
297. Greenwood, P. H. *The Haplochromine Fishes of East African Lakes: Taxonomy, Biology and Evolution*. (Kraus International Publications, Munich, 1981).
298. Trewavas, E. & Stewart, D. J. A new species of *Tilapia* (Pisces, Cichlidae) in the Zambian Zaïre system. *Bulletin of the British Museum (Natural History) Zoology* **28**, 191–197 (1975).
299. Winemiller, K. O. & Kelso-Winemiller, L. C. Food habits of tilapiine cichlids of the Upper Zambezi River and floodplain during the descending phase of the hydrologic cycle. *Journal of Fish Biology* **63**, 120–128 (2003).
300. Bailey, R. M. & Stewart, D. J. Cichlid fishes from Lake Tanganyika: additions to the Zambian fauna including two new species. *Occasional Papers of the Museum of Zoology, University of Michigan* 1–30 (1977).
301. Greenwood, P. H. The genera of pelmatochromine fishes (Teleostei, Cichlidae). A phylogenetic review. *Bulletin of the British Museum of Natural History (Zoology)* **53**, 139–203 (1987).
302. Lamboj, A. Revision des *Chromidotilapia batesii/finleyi*-Komplexes (Teleostei, Perciformes), mit der Beschreibung einer neuen Gattung und dreier neuer Arten. *Verhandlungen der Gesellschaft für Ichthyologie Band 2*, 11–47 (2001).
303. Poll, M. Classification des Cichlidae du lac Tanganika; tribus, genres et espèces. *Acad R Belg Mem Cl Sci* **45**, 1–163 (1986).
304. McKaye, K. R. & Mackenzie, C. *Cyrtocara liemi*, a previously undescribed paedophagus cichlid fish (Teleostei: Cichlidae) from Lake Malawi, Africa. *Proceedings of the Biological Society of Washington* **95**, 398–402 (1982).
305. Stiassny, M. L. J. A phylogenetic overview of the lamprologine cichlids of Africa (Teleostei, Cichlidae): a morphological perspective. *South African Journal of Science* **93**, 513–523 (1997).
306. Kullander, S. O., Karlsson, M., Karlsson, M. & Norén, M. *Chalinochromis cyanophleps*, a new species of cichlid fish (Teleostei: Cichlidae) from Lake Tanganyika. *Zootaxa* **3790**, 425–438 (2014).
307. Van Oijen, M. J. P. & Witte, F. *Taxonomical and Ecological Descriptions of a Species Complex of Zooplanktivorous and Insectivorous Cichlids from Lake Victoria*. (Nationaal Natuurhistorisch Museum, Leiden, 1996).
308. Lamboj, A. *Chromidotilapia melaniae* and *C. nana*, two new cichlid species (Perciformes, Cichlidae) from Gabon, Central Africa. *Zootaxa* **143**, 1–15 (2003).
309. Stiassny, M. L. J. & Schliwen, U. K. *Congochromis*, a New Cichlid Genus (Teleostei: Cichlidae) from Central Africa, with the Description of a New Species from the Upper Congo River, Democratic Republic of Congo. *American Museum Novitates* **3576**, 1–16 (2007).
310. Fryer, G. The trophic interrelationships and ecology of some littoral communities of Lake Nyasa with special reference to the fishes, and a discussion of the evolution of a group of rock-frequenting Cichlidae. *Proceedings of the Zoological Society of London* **132**, 153–282 (1959).
311. Snoeks, J. *The Cichlid Diversity of Lake Malawi/Nyasa/Niassa: Identification, Distribution and Taxonomy*. (Cichlid Press, El Paso, USA, 2004).
312. Stauffer, J. R., Phiri, T. B. & Konings, A. F. Description of two deep-water fishes of the genus *Diplotaxodon* (Teleostei: Cichlidae) from Lake Malaŵi, Africa. *Proceedings of the Biological Society of Washington* **131**, 90–100 (2018).
313. Stiassny, M. L. J., Teugels, G. G. & Hopkins, C. D. *The Fresh and Brackish Water Fishes of Lower Guinea, West-Central Africa*. (Muséum national d'histoire naturelle, Paris, 2007).

314. Lamboj, A. A new dwarf cichlid genus and species (Teleostei, Cichlidae) from Guinea, West Africa. *Zootaxa* **2173**, 41–48 (2009).
315. Fryer, G. A new species of *Gephyrochromis* from Lake Nyasa, with notes on its ecology and affinities. *Revue de Zoologie et de Botanique Africaines* **3**, 347–352 (1957).
316. Yuma, M., Narita, T., Hori, M. & Kondo, T. Food resources of shrimp-eating cichlid fishes in Lake Tanganyika. *Environmental Biology of Fishes* **52**, 371–378 (1998).
317. Takahashi, R., Watanabe, K., Nishida, M. & Hori, M. Evolution of feeding specialization in Tanganyikan scale-eating cichlids: a molecular phylogenetic approach. *BMC Evol Biol* **7**, 1–11 (2007).
318. Loiselle, P. V. An annotated key to the genus *Hemichromis* Peters 1958. *Buntbarsche Bulletin* **148**, 2–19 (1992).
319. Kullander, S. O., Karlsson, M. & Karlsson, M. *Lepidiolamprologus kamambae*, a new species of cichlid fish (Teleostei: Cichlidae) from Lake Tanganyika. *Zootaxa* **3492**, 30–48 (2012).
320. Konings, A. *Tanganyika Cichlids in Their Natural Habitat*. (Cichlid Press, El Paso, USA, 2019).
321. Turner, G. F. *Pallidochromis 76epidiola*, a new genus and species of deep water cichlid (Teleostei: Cichlidae) from Lake Malawai, Africa. *Ichthyological Exploration of Freshwaters* **5**, 377–383 (1994).
322. Lamboj, A. Two new species of *Parananochromis* from Cameroon, Central Africa (Teleostei: Cichlidae). *Ichthyological Exploration of Freshwaters* **25**, 49–57 (2014).
323. Lamboj, A. & Stiassny, M. L. J. Three new *Parananochromis* species (Teleostei, Cichlidae) from Gabon and Cameroon, Central Africa. *Zootaxa* **209**, 1–19 (2003).
324. Legendre, J. Régime alimentaire de la perche 76epidiol. *Comptes-Rendus Hebdomadaires des Séances de l'Académie des Sciences* **170**, 208–211 (1920).
325. Sparks, J. S. *Paretroplus Dambabe*, A New Cichlid Fish (Teleostei : Cichlidae) From Northwestern Madagascar, With A Discussion On The Status Of P-Petiti. *Proceedings of the Biological Society of Washington* **115**, 546–563 (2002).
326. Sparks, J. S. & Reinthal, P. N. *Paretroplus maromandia*, a new cichlid fish from the northwest of Madagascar. *Occasional Papers of the Museum of Zoology, University of Michigan* **727**, 1–18 (1999).
327. Stiassny, M. L. J., Chakrabarty, P. & Loiselle, P. V. Relationships of the Madagascan cichlid genus *Paretroplus*, with description of a new species from the Betsiboka River drainage of northwestern Madagascar. *Ichthyological Exploration of Freshwaters* **12**, 29–40 (2001).
328. Nwadiaro, C. S. The distribution and food habits of the dwarf African cichlid, *Pelvicachromis pulcher* in the River Sombreiro, Nigeria. *Hydrobiologia* **121**, 157–164 (1985).
329. Thys van den Audenaerde, D. F. E. A preliminary contribution to a systematic revision of the genus *Pelmatochromis* Hubrecht sensu lato (Pisces, Cichlidae). *Revue de Zoologie et Botanique Africaines* **77**, 349–391 (1968).
330. Bell-Cross, G. A revision of certain *Haplochromis* species (Pisces: Cichlidae) of central Africa. *Occasional Papers of the National Museums and Monuments of Rhodesia* **5**, 405–464 (1975).
331. Cleaver, R. M., Konings, A. F. & Stauffer Jr., J. R. Two new cave-dwelling cichlids of Lake Malaŵi, Africa. *Ichthyological Exploration of Freshwaters* **20**, 163–178 (2009).
332. Galvez, J. R. *et al.* Trophic Specialization on unique resources despite limited niche divergence in a celebrated example of sympatric speciation. *Ecology of Freshwater Fish* **31**, 675–692 (2022).
333. Stiassny, M. L. J. & Alter, S. E. Phylogenetics of Teleogramma, a riverine clade of African cichlid fishes, with a description of the deepwater molluskivore – *Teleogramma obamaorum* – from the lower reaches of the middle Congo River. *American Museum Novitates* **3831**, 1–18 (2015).

334. Hanssens, M. & Snoeks, J. A new species and geographical variation in the *Telmatochromis temporalis* complex (Teleostei, Cichlidae) from Lake Tanganyika. *Journal of Fish Biology* **63**, 593–616 (2003).
335. Regan, C. T. III. The classification of the fishes of the family Cichlidae.— I. The Tanganyika genera. *Annals and Magazine of Natural History* **5**, 33–53 (1920).
336. Myers, G. S. Report on the fishes collected by H. C. Raven in Lake Tanganyika in 1920. *Proceedings of the United States National Museum* **84**, 1–15 (1938).
337. Walsh, G., Lamboj, A. & Stiassny, M. L. J. Review of the cichlid genus *Thysochromis* (Teleostei: Ovalentaria) with the description of a new species from the Kouilou Province in the Republic of Congo, west–Central Africa. *Journal of Fish Biology* **96**, 1176–1185 (2020).
338. Poll, M. *Poissons Cichlidae. Exploration Hydrobiologique Du Lac Tanganika (1946-1947). Résultats Scientifiques.* (1956).
339. Steinitz, H. & Ben-Tuvia, A. The Cichlid fishes of the genus *Tristramella* Trewavas. *Annals and Magazine of Natural History* **3**, 161–175 (1960).
340. Lamboj, A., Trummer, F. & Metscher, B. D. *Wallaceochromis* gen. nov, a new chromidotilapiine cichlid genus (Pisces: Perciformes) from West Africa. *Zootaxa* **4144**, 124–130 (2016).
341. Pollo, M. & Stewart, D. J. A new cichlid fish of the genus *Xenotilapia* from Lake Tanganyika, Zambia. *Revue zoologique africaine* **89**, 919–924 (1975).
342. Konings, A. A review of the sand-dwelling species of the genus *Aulonocara*, with the description of three new species. *The Cichlids Yearbook* **5**, 26–36 (1995).
343. Meyer, M. K., Riehl, R. & Zetzsche, H. A revision of the cichlid fishes of the genus *Aulonocara* Regan, 1922 from Lake Malawi, with descriptions of six new species (Pisces, Perciformes, Cichlidae). *Courier Forschungsinstitut Senckenberg* **94**, 7–53 (1987).
344. Iles, T. D. A group of zooplankton feeders of the genus *Haplochromis* (Cichlidae) in Lake Nyasa. *Annals and Magazine of Natural History* **2**, 257–289 (1960).
345. Konings, A. Descriptions of three new *Copadichromis* species (Labroidei; Cichlidae) from Lake Malawi, Africa. *Tropical Fish Hobbyist* **47**, 62–84 (1999).
346. Turner, G., Ngatunga, B. & Genner, M. *Astatotilapia* species (Teleostei, Cichlidae) from Malawi, Mozambique and Tanzania, excluding the basin of Lake Victoria. Preprint at <https://doi.org/10.32942/OSF.IO/EU6RX> (2021).
347. Greenwood, P. H. The *Haplochromis* species (Pisces: Cichlidae) of Lake Rudolf, East Africa. *Bulletin of the British Museum (Natural History) Zoology* **27**, 141–165 (1974).
348. de Zeeuw, M. P., Mietes, M., Niemantsverdriet, P., ter Huurne, S. & Witte, F. Seven new species of detritivorous and phytoplanktivorous haplochromines from Lake Victoria. *Zoologische Mededelingen* **84**, 201–250 (2010).
349. Munyandamutsa, P., Jere, W. L., Kassam, D. & Mtethiwa, A. Species specificity and sexual dimorphism in tooth shape among the three sympatric haplochromine species in Lake Kivu cichlids. *Ecology and Evolution* **10**, 5694–5711 (2020).
350. Seegers, L. *The Fishes of the Lake Rukwa Drainage.* (Musée Royal de l'Afrique Centrale, Tervuren, Belgium, 1996).
351. Vranken, N., Van Steenberge, M., Balagizi, A. & Snoeks, J. The synonymy of *Haplochromis pharyngalis* and *Haplochromis 77epidiola* (Cichlidae). *Journal of Fish Biology* **97**, 1554–1559 (2020).
352. Schedel, F. D. B., Friel, J. P. & Schliewen, U. K. A new haplochromine cichlid species from the Great Ruaha River drainage, Rufiji basin, Tanzania. *Spixiana* **37**, 135–149 (2014).
353. Lewis, D. S. C. A revision of the genus *Labidochromis* (Teleostei: Cichlidae) from Lake Malawi. *Zoological Journal of the Linnean Society* **75**, 189–265 (1982).

354. Trewavas, E. X.— A revision of the Cichlid fishes of the genus *Lethrinops*, Regan. *Annals and Magazine of Natural History* **7**, 133–152 (1931).
355. Eccles, D. H. & Lewis, D. S. C. A taxonomic study of the Genus *Lethrinops* Regan (Pisces:Cichlidae) from Lake Malawi. *Ichthyological Bulletin* **38**, 1–20 (1979).
356. Eccles, D. H. & Lewis, D. S. C. A taxonomic study of the genus *Lethrinops* Regan (Pisces: Cichlidae) from Lake Malaŵi. *Ichthyological Bulletin of the J. L. B. Smith Institute of Ichthyology* **36**, 1–12 (1977).
357. Turner, G. & Howarth, J. D. Description of two new species of *Mylochromis* (Teleostei: Cichlidae) from Lake Malawi, Africa. *Ichthyological Explorations of Freshwaters* **12**, 205–212 (2001).
358. Hanssens, M., Snoeks, J. & Verheyen, E. A morphometric revision of the genus *Ophthalmotilapia* (Teleostei, Cichlidae) from Lake Tanganyika (East Africa). *Zoological Journal of the Linnean Society* **125**, 487–512 (1999).
359. Oliver, M. K. Six New Species of the Cichlid Genus *Otopharynx* from Lake Malaŵi (Teleostei: Cichlidae). *Bulletin of the Peabody Museum of Natural History* **59**, 159–197 (2018).
360. Arnegard, M. E. & Snoeks, J. New Three-Spotted Cichlid Species with Hypertrophied Lips (Teleostei: Cichlidae) from the Deep Waters of Lake Malaŵi/Nyasa, Africa. *Copeia* **2001**, 705–717 (2001).
361. Dierickx, K., Hanssens, M., Rusuwa, B. & Snoeks, J. *Trematocranus pachychilus*, a new endemic cichlid from Lake Malawi (Teleostei, Cichlidae). *ZK* **743**, 153–166 (2018).

Chapter 2

Multifaceted impacts of an innovation on dental diversity in an adaptive radiation of cichlid fishes

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Introduction

Evolutionary innovations are thought to generate significant increases of biodiversity. While often resulting in rapid cladogenesis, the evolution of these traits can also shape the tempo and mode of morphological change(1). Innovations bring lineages into new adaptive zones by conferring gains in organismal performance(2,3). However, the range of downstream outcomes on phenotypic diversity is varied(4). Powered flight, which involved major modification of the limbs and sternum, led to the diverse radiation of modern birds and extensive diversification of the flight apparatus. Other innovations may introduce species to a new but restricted adaptive landscape. The unique feeding mechanism of the sling-jaw wrasse (*Epibulus*) facilitates extreme performance in jaw protrusion(5), but there are only two species of *Epibulus* and this novelty has not diversified further. While innovations frequently open up opportunity for further diversification, they may also support impressive gains in performance with little or no subsequent expansion.

What determines these evolutionary paths? The myriad of potential outcomes stem largely from the accessibility of ecological opportunity within the newly invaded adaptive zone

and the potential for substantial reorganization of organismal structures. Innovations that open up rich adaptive zones with ample ecological opportunity could drive rapid diversification(6–9). Intrinsic (e.g., increasing skeletal complexity) innovations could accelerate morphological diversification along novel axes by allowing expansion into new or previously unreachable phenotypic or performance space(10–15). Expanding the range of achievable morphologies could have downstream effects on phenotypic, functional, and ecological diversification(16,17). In contrast, the biomechanical or physiological construction of the innovation may itself limit the potential for subsequent modification, or the adaptive landscape around the innovation may be flat, lacking ecological opportunity(4,18–20). The timing of innovation can also have significant consequences. Because ecological opportunity is expected to decline through time in evolutionary radiations(21), innovations that facilitate novel ecological roles could break barriers of competitive exclusion and biotic filtering, creating opportunities for secondary colonization events(22–24). Competitive pressure from incumbent taxa might inhibit the diversification of secondary colonizers in spatially-restricted systems, despite lacking the same innovation(24,25). The interaction of these intrinsic and extrinsic factors determines the range of morphological outcomes following the evolution of an innovation.

Here, we explore the macroevolutionary outcome of an innovation, complex teeth with multiple cusps(26–28), on dental diversity within the radiation of cichlid fishes in Lake Tanganyika. Teeth are a major component of the fish feeding system, and variation in the arrangement and shape of teeth has important functional implications for prey detachment, capture, and processing(29–33). We first ask in what context complex teeth arose in this radiation and then explore the dynamics of dental evolution among lineages that differ in tooth complexity. The cichlid fishes of Lake Tanganyika (LT) is a compelling system for studies of

dental innovation because the radiation includes a secondary colonization event and multiple independent origins of complex teeth(34,35). The evolution of complex teeth in LT contrasts other rift lakes(28) in that diverse lineages with simple teeth comprise the majority of endemic species. Moreover, the addition of tooth cusps inherently changes the complexity of teeth(30), which may have an intrinsic effect on dental diversification. This combination of features allows us to interpret how both intrinsic and extrinsic factors determine the impact of this innovation on feeding morphology during the adaptive radiation of LT cichlids. We quantified dental diversity along three distinct axes – the overall dentition, attributes of individual teeth, and the shape of tooth cusps – and evaluated whether the trajectory and pace of dental diversification shifts following the evolution of complex teeth. By considering the ecological context in which complex teeth evolved in LT cichlids, we reveal the multifaceted impact this innovation had on the dental system.

Methods

We quantified dental diversity from 352 specimens representing 150 species (1-6 specimens per species) of cichlid fishes from Lake Tanganyika. Specimens were acquired through the aquarium trade or from museum collections (Table S3). Our sampling includes representatives from all twelve tribes and all but two genera, both monotypic (*Baileychromis* and *Tangachromis*), and encompasses 60% of endemic species in the radiation. We also included *Astatotilapia burtoni*, a haplochromine species widespread in Lake Tanganyika's marginal habitats.

Data Collection

To characterize aspects of the overall dentition, we counted the number of outer row teeth and number of inner tooth rows for each specimen, separately for the premaxilla and dentary. We computed the variance in tooth height (see below) per jaw, per specimen, as a proxy for heterodonty and homodonty(31). We refer to this dataset as “dentition”. We define simple teeth as those having a single tooth cusp, and complex teeth as those having two or more tooth cusps following Peoples et al. (28) (Fig. 1a).

To quantify functional aspects of individual teeth, we extracted the first 5 outer row teeth on the premaxilla and dentary, beginning at the symphysis and moving posteriorly. Extracted teeth were photographed under a compound microscope in both lateral and labial view. We excluded teeth that were broken or had clear signs of significant wear. We measured and computed 7 morphological traits with functional significance (Table S1) including tooth height, tooth curvature, tooth base length, cusp height, cusp cross-section aspect ratio, enameloid ratio, and cusp taper using the software ToupView (ToupTek Photonics, Inc) (Fig. S1a, b). We converted measurements to log-shape ratios(36) by dividing each by the geometric mean of (standard length [SL] x premaxilla length x jaw width [JW]) for premaxillary teeth and by the geometric mean of (SL x dentary length x JW) for dentary teeth, then using a log-transformation, to account for effects of size. Ratios generated from linear measurements were log-transformed without size correction. Because ratios may not account for allometric trends, we also retained the residuals of a log-log regression of each ratio against SL as an alternate method that removes effects of size. We found that values from both approaches were highly correlated ($R^2=0.978-0.998$) (Fig. S2) and use log-ratios in all analyses. Our final dataset included 3,231 extracted teeth across the premaxilla and dentary. We refer to this dataset as “linear morphometrics”. To

obtain species-level information for phylogenetic comparative analyses, we first quantified the mean value of each trait per specimen, then the mean values per specimen to get species mean values. Each species' mean value was thus based off measurements from 5-15 teeth. Teeth from the premaxilla and dentary were analyzed separately; results in the main text represent dentary teeth. Patterns of dental diversity did not vary between jaws.

Finally, we used geometric morphometrics to quantify the shape of individual tooth cusps. To reduce noise and non-biological variation, we chose a single tooth from each specimen (n=352 teeth for each jaw). We used a combination of fixed landmarks and sliding semi-landmarks, which can accurately capture variation in cusp morphology without introducing non-biological variation(37,38). We placed two landmarks at the enameloid-dentine junction, and a third at the tip of the major cusp – for simple teeth, this was simply the tip of the tooth (Fig. S1c). We then placed 40 sliding semi-landmarks around two curves (n=20 landmarks each) traced around the lateral edge of the cusp using tpsDig2 v1.1. We aligned and scaled all landmarks using a generalized Procrustes analysis (GPA) in ‘*geomorph*’, sliding semilandmarks based on minimizing bending energy. We computed species means of Procrustes-aligned coordinates; species means were used for all further analyses.

Disparity and morphospace occupation

We used the time-calibrated phylogeny of Ronco et al.(34), pruned to include the 150 species in our study, for all phylogenetic comparative analyses of dental diversity. This timetree was built using 547 cichlid genomes and includes both cichlid and other teleost fossil calibrations. All analyses were replicated across the three datasets (dentition, linear morphometrics, and geometric morphometrics).

To visualize the major axes of dental variation, we performed principal component analyses on the correlation matrix of the dentition and linear morphometrics dataset using ‘*prcomp*’ in the ‘*stats*’ R package, and on the covariance matrix of the geometric morphometrics dataset using the ‘*gm.prcomp*’ function in ‘*geomorph*’. To compare dental diversity between species with simple and complex teeth, as well as herbivores and non-herbivores, we calculated disparity for each group using ‘*morphol.disparity*’ in ‘*geomorph*’(39,40), using the size-corrected values for the dentition and linear morphometric datasets and Procrustes coordinates for the geometric morphometric dataset as inputs. We employed two methods to test for differences in morphospace occupation between species with simple and complex teeth. First, we used phylogenetic multivariate analysis of variance (p-MANOVA) under a Brownian motion model in the R package ‘*geiger*’(41); we also used this approach to compare dental traits between herbivores and non-herbivores. To compare the degree of morphospace overlap and uniqueness between groups, we created n -dimensional hypervolumes for our dentition ($n=3$, 100% variance), linear morphometrics ($n=7$; 100% variance) and geometric morphometrics ($n=5$; 94.5% variance) datasets using the first three, seven, and five Principal Component (PC) axes, respectively, with the R package ‘*hypervolume*’ v3.1.4(42,43). The first five PC axes of the geometric morphometrics dataset were chosen through visual examination of scree plots. We created a sample of 100 permuted hypervolumes for each group, generated a null distribution of overlap statistics under the null hypothesis that the two groups occupy the same volumes, estimated fraction of space unique to each group as well as Jaccard and Sorensen similarity, and calculated the P -values for the observed statistics with respect to the generated null distributions.

We visualized how the accumulation of disparity through time differed from that expected under a null Brownian model with the ‘*dti*’ function in the R package ‘*geiger*’(41,44).

For each dataset, we estimated the morphological disparity index (MDI), which quantifies the average difference in disparity of a clade compared to a null Brownian model. Positive values indicate that disparity is distributed within rather than among subclades, suggesting species within each subclade are exploring a greater range of dental space than expected under BM.

Integration

We compared the strength of evolutionary phenotypic integration between species with simple and complex teeth. For each pairwise trait combination, we estimated the strength of morphological integration (*r-pls*) separately for simple and complex species with the ‘*phylo.integration*’ function (10,000 permutations). We used the first five principal component axes from the geometric morphometric analysis of cusp shape. We assessed significance with the ‘*compare.pls*’ function. We used analysis of variance (ANOVA) tests to compare the distribution of *r-pls* values between simple and complex species across all comparisons (n=95) and for only those trait comparisons that differ significantly in the strength of integration between groups (n=19).

Rates of evolution

We used four approaches to estimate multivariate rates of trait evolution across our three datasets: (1) a single, constant-rate Brownian (BM) rate for each group, (2) state-dependent BM rates, (3) branch-specific BM rates, and (4) multivariate rates under an Ornstein-Uhlenbeck model. (1) For all three datasets, we compared multivariate Brownian rates of evolution between simple and complex lineages, as well as herbivores and non-herbivores, using the ‘*compare.evol.rates*’ function in ‘*geomorph*’, with 10,000 permutations to assess significance. However, this simple model attributes all rate variation to the observed groups, even when

variation in rate is independent of the group identity. To account for this background rate variation, we employed the (2) MuSSCRat model implemented in RevBayes(45,46). This expanded Brownian model jointly estimates the evolution of the discrete and continuous characters, separating the effect of the discrete character from other sources of rate variation. We modeled background rate variation using an uncorrelated log-normal (UCLN) clock, which places an independent rate parameter on each branch, and set a prior of 20 discrete character transitions. The Markov chain Monte Carlo (MCMC) was run for 200,000 generations with 10% burn-in and convergence checked with Tracer v1.7.2(47). (3) To estimate branch-specific and species-specific rates (i.e., tip rates), we used a relaxed-clock multivariate Brownian model implemented in RevBayes following Burress et al.(48), with a prior of 20 rate shifts. The MCMC was run for 500,000 generations with 10% burn-in and convergence was checked with Tracer v1.7.2. We plotted these rates through time using a custom R script that summarizes the rate across branches of the phylogeny in user-specified time slices. Finally, to evaluate if the evolution of dental traits is subject to unique selective regimes between simple and complex lineages (represented by an Ornstein-Uhlenbeck [OU] model of evolution), we fit several multivariate BM and OU models with the R package '*mvMORPH*'(49). We fit these models separately for each dataset. We included OU models that had single (OU1) or multiple (OUM) selective regimes, and BM models with either a single rate (*BMI*) or separate rates for each group (*BMM*). We fit each model 100 times over a distribution of 100 stochastic character maps to account for uncertainty in the ancestral history of complex teeth and compared models using the mean AIC of 100 fitted models. For the best-fit OU model, we averaged all fits by their Akaike weights to evaluate the adaptive values (θ) of each trait.

Evolution of complex teeth and diet

To investigate whether the origin of complex teeth occurred within LT or in surrounding riverine lineages, we used the tooth complexity classifications of Peoples et al.(28) and the full phylogeny (n=265 species) of Ronco et al.(34), which includes both endemic LT species and Haplochromine outgroups, to generate an ancestral state reconstruction of tooth complexity using stochastic character mapping. We compared the fit of an equal rates (ER) and unequal rates (ARD) character evolution model, fitted with the *'fitMk'* function in *'phytools'*(50), using AIC. We then generated 100 stochastic character maps with *'make.simmap'*, with the root state fixed to simple teeth. We summarized all 100 simmaps using *'densitymap'* to plot the posterior probability of complex teeth on each branch.

To quantify the proportion of lineages occupying different diet categories through time, we used the diet classifications of Peoples et al.(28), pruned to match the 150 species in our study. We generated 100 stochastic character maps under an ARD model and summarized the proportions of each diet through time in equal-time-length bins averaged across all 100 simmaps. Using the same diet classifications, we grouped species as herbivores and non-herbivores in the PCA biplots of the three dental diversity datasets to visualize how dental diversity reflects diet (Fig. S3b-d). To supplement our discrete diet classifications, we used the stable isotope data from Ronco et al.(34) as a continuous proxy for trophic level. We calculated species mean $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values, and visualized the log-transformed phylogenetic distribution of these values through time with the *'contMap'* function in *'phytools'*(50).

Results

Riverine origins and ecological consequences of complex teeth

The adaptive radiation of cichlid fishes in Lake Tanganyika (LT) occurred in two stages. After the initial radiation, multiple lineages dispersed out of LT(35). Riverine lineages then re-entered the lake ~5-7 Ma, resulting in a successful secondary colonization event and subsequent diversification of the Tropheini (~40 species) in parallel to an established radiation(34,35,51). Ancestral state reconstructions show that complex teeth first evolved outside LT in riverine lineages ancestral to the modern Haplochromines and were absent from the initial radiating lineages within LT (Fig. 1b). This indicates that the re-entry into LT by riverine species and subsequent radiation of the Tropheini in rocky littoral habitats was accomplished by lineages with complex teeth, likely under intense competition with established species(34,35,51). Within the lake, there are four independent origins (in *Asprotilapia leptura*, *Ophthalmotilapia boops*, *Cunningtonia longiventralis*, and *Cyathopharynx foae*) and a single reversion in *Ctenochromis horei* (Fig. 1b) (ARD; $\Delta\text{AIC}=-0.89$, $\text{AIC}_w=0.61$; Table S2), contrasting the exceptional lability of tooth complexity seen in Lakes Malawi and Victoria(28). The evolution of complex teeth and herbivory is a coupled process in cichlids(28). Ancestral state reconstructions of diet, $\delta^{15}\text{N}$, and $\delta^{13}\text{C}$ reveal that herbivorous niches were not occupied by cichlids at the onset of the radiation and only began being filled after the increase in lineages with complex teeth (Fig. 1c-d; S3a). Collectively, these results suggest that the riverine origin of complex teeth played a role in the successful incursion of riverine species into LT and subsequent in-situ radiation of the Tropheini by increasing ecological opportunity through low trophic level niches, despite the presence of considerable ecological and morphological diversity(34).

Morphospace occupation

Species with simple and complex teeth segregate in dental morphospace across all three morphological axes, and there is high dental diversity across the LT cichlid radiation (Fig. 2a-c).

Among dentitions (tooth number, row number, variance in tooth height), the primary axis of variation (PC1, 46.3%) separates morphologically homodont dentitions of few teeth in a single row (low PC1, e.g. *Perissodus*) from heterodont dentitions comprising many teeth in multiple rows (high PC1, e.g. *Lepidolamprologus*) (Fig. 1a). Species with simple teeth have 2.45x greater disparity ($P<0.01$) (Fig. 3a) and occupy different morphospace regions (phylogenetic MANOVA; $P<0.05$) that are largely unique from area occupied by complex species, despite the appearance of significant overlap in two-dimensions (Jaccard similarity [JS]=0.069, $P<0.0001$; fraction of unique morphospace [F_{simple}]=0.915, $P<0.0001$). Species with simple teeth occupy extreme morphospace regions while species with complex teeth are restricted to central regions with a lower proportion of unique occupation ($F_{\text{complex}}=0.724$) (Fig. 2a). These patterns show that species with simple teeth occupy a greater amount and have explored more extreme regions of morphospace, while complex-toothed species have similar numbers of teeth and tooth rows and largely homodont dentitions. Functional aspects of individual teeth, captured with seven linear measurements, vary considerably across species. While simple teeth show great diversity and range from short cones (low PC1, e.g. *Trematocara*) to stout and highly recurved in scale-eaters (high PC1, e.g. *Plecodus*), complex teeth are more limited in morphology despite having similar amounts of disparity (Fig. 2b, 3a). Complex teeth are larger, with bases elongated along the antero-posterior axis, and have broad cusps with higher cross-sectional aspect ratios (Fig. 2b). This combination of functional traits is largely unique to complex lineages ($F_{\text{complex}}=0.85$) with little overlap between simple lineages ($JS=0.012$, $P<0.0001$; $F_{\text{simple}}=0.987$) (Fig. 2b). Complex teeth are thus largely distinct from but more limited than simple teeth, even without a-priori classification of their complexity. Through the addition of cusps, complex teeth may be able to intrinsically generate a greater diversity of cusp shapes because of their increased complexity.

We find that complex species, which can have teeth with two or three cusps, have 3.7X greater disparity in the shapes of tooth cusps than simple species ($P < 0.001$) (Fig. 2c, 3a). The cusp shapes of complex teeth have nearly no overlap with simple cusps ($JS = 0.0038$, $P < 0.0001$) and occupy an almost entirely distinct multidimensional shape space ($F_{\text{complex}} = 0.996$, $P < 0.0001$) (Fig. 2c). While simple teeth are largely restricted to cone-shapes, the addition of tooth cusps generates a remarkable range of cusp shapes.

Comparing the dental diversity of herbivores with that of non-herbivores yielded largely similar results. Herbivores have different dental characteristics than non-herbivores across all three axes of dental diversity (phylogenetic MANOVA; $P_{\text{dentition}} < 0.01$, $P_{\text{linear}} < 0.001$, $P_{\text{geometric}} < 0.005$) and occupy a similar region of morphospace as species with complex teeth (Fig. S3b-d). Disparity followed a similar pattern; herbivores have greater disparity for the geometric morphometric (3.38x, $P < 0.001$) and linear morphometric (1.16x, $P = 0.5$) datasets, but non-herbivores have greater disparity of dentition traits (1.6x, $P = 0.069$).

Disparity-through-time (DTT) analyses indicate positive but non-significant morphological disparity index (MDI) values and an increase in disparity above the expectations under Brownian motion (above 95% CI) beginning ~3 Ma for all three axes of dental diversity ($MDI_{\text{dentition}} = 0.056$, $MDI_{\text{linear}} = 0.161$, $P = 0.997$; $P = 0.691$; $MDI_{\text{geometric}} = 0.074$, $P = 0.867$) (Fig. 2d-f). However, we acknowledge that these increases in disparity near the present could also be caused by measurement error or incomplete sampling. This rise coincides with the proportional increase of complex lineages, herbivores and lower trophic level feeding in the lake (Fig. 1b-d). During this time, the principal clades with complex teeth (*Tropheus* and *Petrochromis*) experienced rapid speciation and parallel evolution of ecomorphologies (34,52). These results suggest that the

rise of lineages with complex teeth may have been a significant generator of dental novelty compared to when the radiation contained primarily species with simple teeth.

The tempo of dental evolution

We employed multiple approaches, including branch-specific(53), state-dependent(46) and Ornstein-Uhlenbeck(54) models, to characterize differences in the tempo and mode of multivariate dental evolution between species with simple and complex teeth. Lineages with complex teeth have faster rates of morphological evolution across all three axes of dental diversity, a congruent trend across models (Fig. 3a). Under a single-rate Brownian motion (BM) model, evolutionary rates are 1.04x (dentition, $P=0.8$), 1.57x (linear morphometrics, $P<0.05$), and 4.8x (geometric morphometrics, $P<0.001$) faster for lineages with complex teeth. When including only linear morphometrics of tooth cusps, we find that species with complex teeth have 1.55x faster rates of cusp shape evolution ($P<0.05$), which agrees with our geometric morphometric analysis of cusp shape. We find a similar pattern when comparing herbivores with non-herbivores; rates are 1.34x (linear morphometrics, $P<0.001$), 1.33x (dentition, $P=0.09$), and 3.35x (geometric morphometrics, $P<0.001$) faster for herbivorous species. Tip rates, which are estimated under a model that allows for rate variation both across branches and through time, are 1.32x (dentition, phylogenetic Analysis of Variance [p-ANOVA]; $P=0.2$), 1.28x (linear morphometrics, p-ANOVA; $P=0.6$) and 4.91x (geometric morphometrics, p-ANOVA; $P<0.001$) faster for lineages with complex teeth (Fig. 3a). When accounting for rate variation independent of the observed character using the Multiple State-Specific Rates of continuous character evolution (MuSSCRat)(46) model in RevBayes, we found that only rates of geometric cusp shape evolution are state dependent (posterior probability [PP]=1), with complex lineages having 18.8x higher relative rates (Fig. 3a, b). Rates of evolution of dentition (PP=0.311) and linear

morphometric traits (PP=0.263) are not state dependent, and estimated relative rates largely overlap (Fig. 3b), indicating that the evolution of many aspects of the dental system does not depend strictly on whether teeth are simple or complex under a BM model. Branches with transitions to complex teeth were often associated with high rates of evolution, including at the base of the Tropheini and leading to all four independent origins within LT, and rates across the tree increase following the rise of complex lineages in the lake ~3 Ma (Fig. S4).

We fit and compared several Ornstein-Uhlenbeck (OU) models of adaptive evolution in the R package '*mvMorph*' (49,54) to test if the dental systems of simple and complex species are evolving towards distinct adaptive peaks. OU models with separate adaptive optima (θ) for lineages with simple and complex teeth had strong support when fit to the dentition (OUM; $\Delta AIC=18.66$; Table 1) and linear morphometrics (OUM; $\Delta AIC=82.08$; Table 2) datasets, but a BM model with distinct rates for simple and complex lineages best describes the evolution of geometric cusp shapes (BMM; $\Delta AIC=16.24$; Table 3). We estimated trait optima for OUM models, averaged across 100 fitted models by their Akaike weights. Optimal complex dentitions are comprised of more equally sized teeth, reflecting homodonty, and many inner tooth rows (Fig. 4a). Teeth are taller, with more elongated bases and have cusps that are broad and blunt, with a higher cross sectional aspect ratio and a lower amount of enameloid-covered surface (Fig. 4b). These evolutionary optima are similar to the observed trait values of *Tropheus*. Optimal values for tooth number and curvature are similar between groups (Fig. 4a, b). These results indicate that lineages with simple and complex teeth are evolving towards distinct evolutionary optima for many traits within their dental system which may reflect adaptive evolution of the dental system in response to ecological pressures.

Integration

Directional morphological change may be a result of selection driving strong evolutionary integration between traits(55,56). We evaluated the levels of evolutionary covariation within the dental system between simple and complex species by quantifying the strength of evolutionary integration between dental traits. The strength of covariation between traits varied greatly within both simple and complex species. While the range of *r-pls* values was similar between groups ($\text{range}_{\text{complex}}=0.8224$, $\text{range}_{\text{simple}}=0.8072$), the highest values were found in complex species, and the mean strength of integration between all pairwise trait combinations ($n=95$) is 1.5x greater within species with complex teeth (Analysis of Variance [ANOVA]: $F=12.6$, $P<0.0005$) (Fig. 4c). Mean *r-pls* values were 0.295 for species with complex teeth and 0.196 for species with simple teeth. When only considering trait combinations for which there is a significant difference in the strength of integration between groups (pairwise P -value <0.05 , $n=19$ comparisons), integration was 1.8x greater within species with complex teeth ($r\text{-}pls_{\text{complex}}=0.419$, $r\text{-}pls_{\text{simple}}=0.229$; ANOVA: $F=8.017$, $P<0.01$) (Fig. 4c).

Discussion

Evolutionary innovations are often seen to promote expansion into new ecological niches or habitats(1,2). By doing so, innovations can have varied effects on the evolution of form and function by subjecting species to novel ecological and evolutionary forces associated with ecological opportunity(6,10–12,18,57). Here, we highlight the myriad of effects that the evolution of complex teeth has on the fish dental system, driven by both intrinsic properties of the innovation and extrinsic properties of the environment. Complex teeth facilitated the successful secondary colonization of and subsequent radiation within LT by the Tropheini by opening opportunity in low trophic level niches. Using a comprehensive toolkit of phylogenetic

comparative methods, we show that complex teeth brought species into novel but restricted morphospace regions while accelerating diversification along a new axis. These novel diversification patterns, imposed by the functional demands of herbivory, likely broke barriers of competitive exclusion to facilitate diversification within herbivory but not beyond it. Our results suggest that innovations can be an important catalyst of morphological change, but their effect is determined by both intrinsic properties, such as how the innovation is accommodated within the functional system and the extrinsic ecological context.

The ecological context of Lake Tanganyika

Adaptive radiations may often unfold in stages, a pattern that leads to depleted ecological opportunity through time(34,58,59). This could establish barriers to secondary colonization by requiring adaptive traits for unexploited resources. Our results show that during the initial radiation in LT, lineages lacked an ecologically versatile dental innovation that enables diet transitions to herbivory and omnivory(28). As a result, higher trophic level niches were occupied first (Fig. 1b, c; S3a) which left opportunity available at low trophic levels. Moreover, there are few non-cichlid herbivores in LT, largely limited to river mouths (e.g. *Labeo*)(60). Following the evolution of complex teeth in rivers, this novelty was introduced to LT with the arrival of the Tropheini and rapidly increased in frequency as this lineage expanded (Fig. 1b) to become the dominant lineage in shallow-water environments. Our findings suggest complex teeth allowed lineages to overcome barriers of competitive exclusion and biotic filtering(22–24), as these species overlap little with incumbent lineages in morphospace (Fig. 2a-c). In contrast, species with simple teeth repeatedly evolved dentitions with more extreme tooth sizes, numbers, or variation in tooth size (Fig. 2a, b) that meet the demands of other ecological niches (e.g., large slender fangs in *Bathybates*, a piscivore). These specialized morphologies for non-herbivorous

niches, which likely existed during the early diversification of the Tropheini, may have limited further ecological diversification of complex lineages despite complex teeth being ecologically versatile.

The ancestors of the Tropheini are not the only secondary colonizers of LT with complex teeth. *Oreochromis tanganyicae* (Oreochromini) and *Coptodon rendalli* (Coptodonini) are two herbivorous tilapiine cichlids that secondarily colonized LT, while *O. niloticus* maintains a small population in marginal habitats but has not colonized the lacustrine zone(34,61,62). Despite these species having complex teeth, their young age suggests considerable overlap with the established Tropheini, which dominate shallow-water herbivorous niches and likely stymied intralacustrine diversification. These contrasting diversification patterns highlight the importance of ecological context in determining the outcome of innovation.

Complex teeth and herbivory

Complex teeth with multiple cusps are an adaptation for herbivory across vertebrates(26–28). In fishes, the multiple angled cusps of complex teeth may be beneficial when cropping or scraping algae or removing loose food items from an algal matrix. Herbivory was an open niche during the early history of LT, and the majority of current herbivores are found within the Tropheini (Fig. 1b, c). Herbivores occupy similar dental morphospace as species with complex teeth (Fig. 2a-c; S3b-d) and comparisons of disparity, dental traits, and evolutionary rates between herbivores and non-herbivores mirror comparisons between species with simple and complex teeth. Moreover, 84% (21/15 species) of species with complex teeth in our study are herbivorous, indicating a tight association between complex teeth and herbivory in LT. Herbivorous fishes may be under strong selection due to the constraints and requirements to feed on plant material. These functional demands have led to herbivores evolving similar

morphologies, such as larger teeth and shorter, more robust jaws, which often occupy novel morphospace regions(63–68). Our results show that species with complex teeth have a more integrated dental system (Fig. 4c), occupy unique, often extreme regions of dental morphospace (Fig. 2b, c), and are evolving towards distinct optimal trait values (Fig. 4a, b). High integration is known to generate more extreme traits combinations as a result of strong selection for ecological performance, promoting expansion into unexplored regions of morphospace(69). Together, these results suggest that the functional demands of herbivory drive coordinated change in the dental system in fishes(63) (Fig. S3b-d). Though herbivores have a restricted niche breadth, they often have the highest rates of morphological evolution(70,71), a pattern supported by our results. By enabling transitions to available low trophic level niches, complex teeth subjected the dental system to new demands, leading to coordinated and accelerated change (Figs. 1d-f, 2a, 3a-c).

Our results suggest that tooth complexity alone is not sufficient for specialized herbivory. Indeed, many adaptive changes in feeding morphology coincide with macroevolutionary transitions to herbivory in fishes (68,72,73). In the dental system, change in the aspect ratio of the cusp and height of teeth, an increase in the number of inner tooth rows, and a reduction in the variance in tooth height is also necessary when transitioning to a primarily herbivorous diet (Fig. 4a, b). Complex lineages that are not herbivorous are omnivorous or have evolved further feeding adaptations while retaining a complex dentition (e.g. hypertrophied lips in *Lobochilotes labiatus*). Given its functional demands and specialized morphology, herbivory may often be absent during the onset of radiations(67,71), leaving ecological opportunity open in low-level niches for new lineages to exploit.

Innovations shape phenotypic evolution

The occupation of new ecological zones is fundamental to understanding how innovations influence macroevolution(1–3). By introducing new functional demands associated with niche performance or increasing the amount of accessible phenotypic space, innovations can shape the trajectory and pace of morphological change(16,17). Our results demonstrate that the trajectory of dental diversification following the evolution of complex teeth was shaped by both intrinsic and extrinsic factors. Complex teeth brought cichlids into herbivorous niches, which placed new functional demands on the dental system that accelerated evolution to unique morphospace regions (Fig. 2a-c; 3a). However, the limited ecological opportunity outside herbivory likely restricted further diversification of morphology and ecology. By opening a new axis to diversify along through the addition of tooth cusps, complex teeth could generate more diverse interactions with food items(30). These diverse cusp shapes likely increased opportunities for niche partitioning within herbivory, enabling diversification within but not between ecological niches(74–76). These varied outcomes highlight the interplay of intrinsic and extrinsic factors operating on the dental system following the evolution of complex teeth.

Appraising whether innovations have largely intrinsic or extrinsic effects can provide insight into the expected impacts on morphology. Intrinsic innovations expand the achievable phenotypic space by promoting major structural or mechanical reorganization(16), which often triggers pulses of accelerated change. For example, change to melanosome morphology in African starlings (*Sturnidae*)(10), modification to chromatin structure in eukaryotes(13,77), the intramandibular joint and mobile premaxilla of coral reef fish(4,11,14,78), and the latch-spring mechanism of trap-jaw ants(15) all enabled diversification of new forms. When combined with sufficient ecological opportunity and a broad adaptive landscape, these intrinsic innovations may promote diversification of the organismal system of which they are contained within.

Alternatively, other innovations primarily bring lineages into new ecological zones where extrinsic factors determine the trajectory of morphological change and the innovation itself does not diversify. Antifreeze proteins brought icefishes (Notothenioidei) into extreme thermal environments(79,80), but high ecological opportunity promoted their diversification(81,82). Adhesive toepads limit morphological diversification in lizards because arboreal habitats introduce new constraints on morphology(83–85); the secondary loss of toepads accelerates diversification of locomotory traits(19). These innovations subject existing functional systems to new demands and may only function in a narrow range of parameter space. In these cases, extrinsic niche requirements or environmental conditions determine how morphology diversifies further. Distinguishing between the intrinsic and extrinsic effects of innovations, while appreciating the interaction between them, should be an emphasis for future studies that address how innovations have shaped morphological diversification across phylogenetic scales.

Conclusion

Our results suggest that by exposing lineages to new evolutionary and ecological pressures associated with herbivory, the evolution of complex teeth drove diversification of the cichlid dental system to novel but restricted regions of morphospace. The complex evolutionary dynamics of the dental system following the evolution of complex teeth was thus shaped by both intrinsic and extrinsic factors. Despite these varied impacts on morphology, complex teeth were a crucial innovation for the secondary incursion of riverine lineages into Lake Tanganyika and subsequent diversification of the Tropheini in shallow-water, algal rich habitats by breaking barriers of competitive exclusion. Innovations can have profound effects on the trajectory and pace of morphological change, the directionality of which is determined by properties of both the innovation and the new ecological zone. Understanding the interaction of these factors provides

insight into why the outcome of innovation is varied and highlights the macroevolutionary diversity of innovations themselves.

Figures and Tables

Table 1 – Comparison of *mvMorph* trait evolution models fit to the dentition dataset. The AIC range reports the lower and upper limits of the range. The proportion preferred reports the proportion of SIMMAPs for which that model had the lowest AIC.

Model	Parameters	AIC	AIC range	Δ AIC	Proportion preferred
BM1	single σ^2	799.8123	NA	39.2632	-
BMM	multiple σ^2	795.6558	787.8328, 804.1055	35.1067	-
OU1	single θ	779.2126	NA	18.6635	-
OUM	multiple θ	760.5491	755.806, 767.1262	0	1.0

Table 2 – Comparison of *mvMorph* trait evolution models fit to the linear morphometrics dataset. The AIC range reports the lower and upper limits of the range. The proportion preferred reports the proportion of SIMMAPs for which that model had the lowest AIC.

Model	Parameters	AIC	AIC range	Δ AIC	Proportion preferred
BM1	single σ^2	-1050.681	NA	212.941	-
BMM	multiple σ^2	-1113.692	-1228.355, -1020.606	149.93	0.04
OU1	single θ	-1181.544	NA	82.078	-
OUM	multiple θ	-1263.622	-1272.119, -1211.329	0	0.96

Table 3 – Comparison of *mvMorph* trait evolution models fit to the geometric morphometrics dataset. The AIC range reports the lower and upper limits of the range. The proportion preferred reports the proportion of SIMMAPs for which that model had the lowest AIC.

Model	Parameters	AIC	AIC range	ΔAIC	Proportion preferred
BM1	single σ^2	-895.5905	NA	261.7735	-
BMM	multiple σ^2	-1157.364	-1186.088, -975.9417	0	0.64
OU1	single θ	-956.5445	NA	200.8195	-
OUM	multiple θ	-1141.126	-1157.489, -1080.739	16.238	0.36

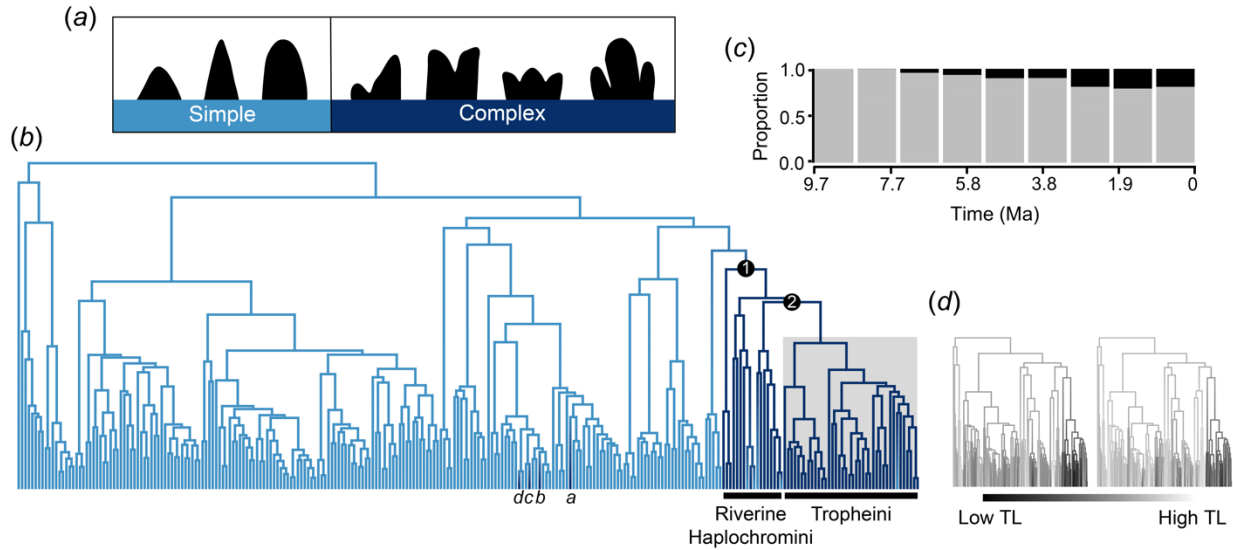


Figure 1 | The evolution of complex teeth and trophic diversity in Lake Tanganyika

cichlids. **(a)** Silhouettes depicting a range of simple and complex tooth shapes found in Lake Tanganyika (LT) cichlids. **(b)** Ancestral state reconstruction of complex teeth (dark blue) in the LT radiation and riverine outgroups. The Tropheini are highlighted in grey. Black points correspond to (1) the riverine origin of complex teeth and (2) the split between Haplochromini and Tropheini. Four intralacustrine origins of complex teeth in *Asprotilapia leptura* (a), *Ophthalmotilapia boops* (b), *Cunningtonia longiventralis* (c), and *Cyathopharynx fuae* (d). **(c)** The proportion of herbivorous and omnivorous lineages (black) through time in ~1 My intervals. Grey bars include zoobenthivores, zooplanktivores, piscivores, and lepidophages. **(d)** Ancestral state estimation of $\delta^{15}\text{N}$ (nitrogen, left) and $\delta^{13}\text{C}$ (carbon, right) stable isotope signatures, a proxy for trophic level (TL), across the LT radiation (n=232 species).

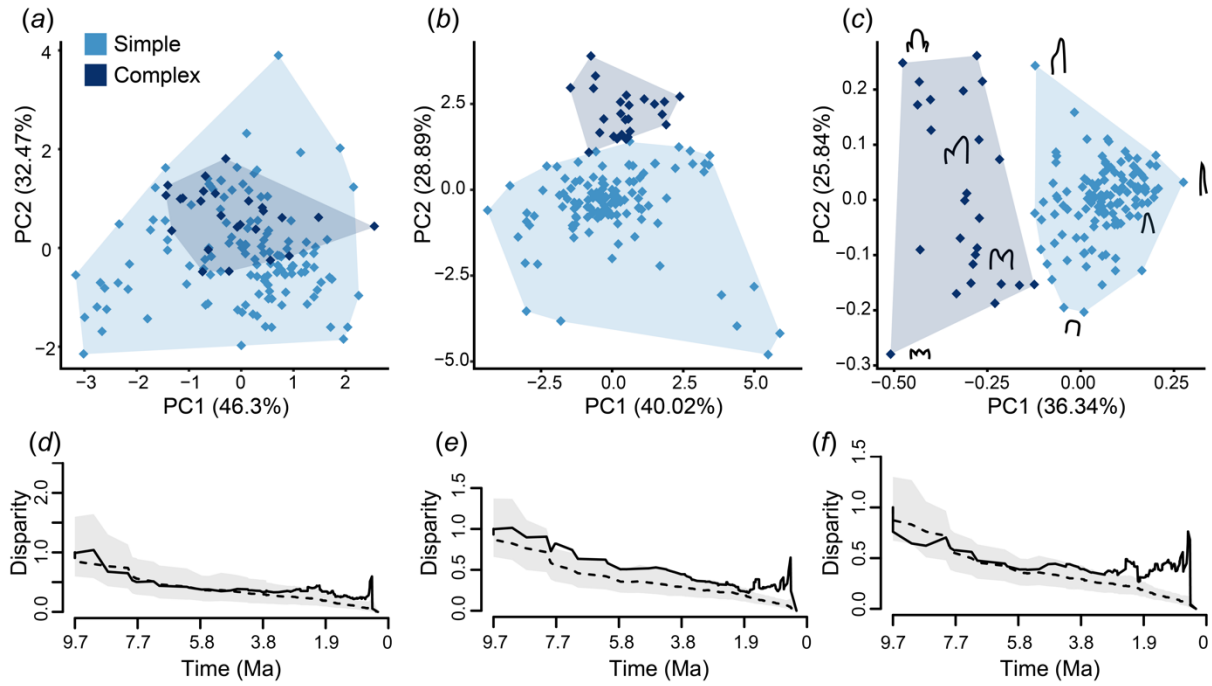


Figure 2 | The dental diversity of Lake Tanganyika cichlids. Principal component biplots of the dentition (*a*), linear morphometric (*b*), and geometric morphometric (*c*) datasets.

Representative cusp shapes are included in (*c*). Species with simple teeth are light blue and species with complex teeth are dark blue. Morphological disparity-through-time (DTT) plots for the dentition (*d*), linear morphometric I, and geometric morphometric (*f*) datasets. The grey area represents the 95% confidence interval, the dotted line the expectation under Brownian motion, and the solid black line the observed data.

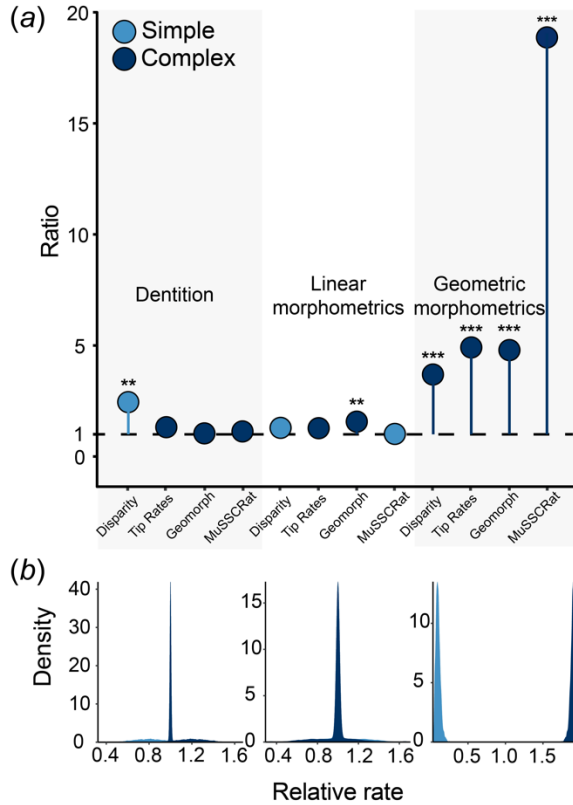


Figure 3 | The tempo and mode of dental evolution in cichlid species with simple and complex teeth. (a) Ratios of morphological disparity and rates between species with simple (light blue) and complex (dark blue) teeth, for each dataset (dentition, linear morphometrics, geometric morphometrics). For each comparison, the group with ratio >1 is shown. **(b)** Posterior distribution of multivariate relative rates estimated using the MuSSCRat model in RevBayes between species with simple and complex teeth for the dentition (left), linear morphometric (middle) and geometric morphometric (right) datasets. Relative rates for the dentition and linear morphometric datasets almost completely overlap.

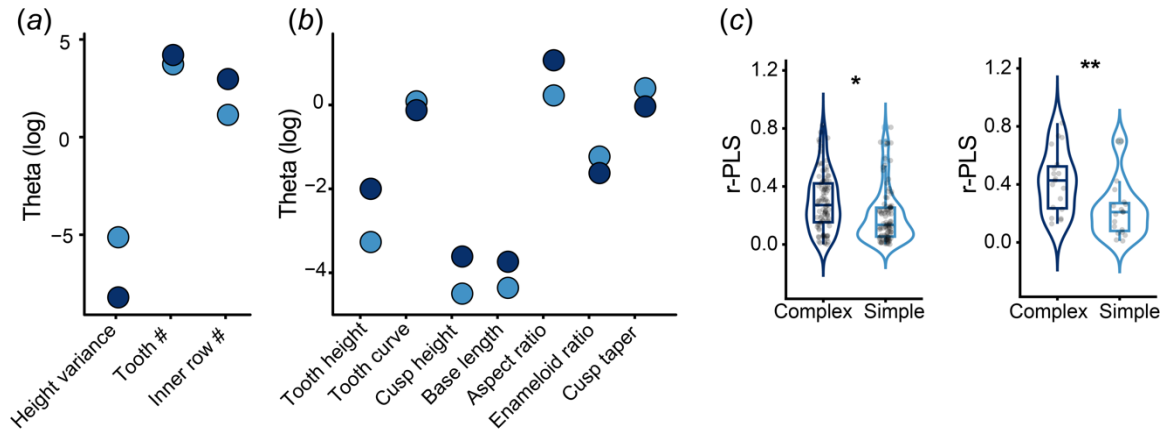


Figure 4 | Coordinated directional evolution of complex lineages. Adaptive optima trait values for dentition (**a**) and linear morphometric (**b**) traits estimated under an OUM model in *mvMorph*. All traits were log-transformed before estimation. Colors represent simple (light blue) and complex (dark blue) species. (**c**) Violin plots comparing the strength of integration (*r*-PLS) between species with simple and complex teeth for all pairwise trait combinations (n=95, left) and only comparisons for which the difference in integration between groups was significant (n=19, right).

References

1. Miller AH, Stroud JT, Losos JB. The ecology and evolution of key innovations. *Trends in Ecology & Evolution*. 2023 Feb;38(2):122–31.
2. Simpson GG. The major features of evolution. New York: Columbia University Press; 1953.
3. Miller, Alden H. Some ecological and morphologic considerations in the evolution of higher taxonomic categories. In: *Ornithologie als Biologische Wissenschaft*. 1949. P. 84–8.
4. Wainwright PC, Price SA. The Impact of Organismal Innovation on Functional and Ecological Diversification. *Integr Comp Biol*. 2016 Sept;56(3):479–88.
5. Westneat MW, Wainwright PC. Feeding mechanism of *Epibulus insidiator* (Labridae; Teleostei): Evolution of a novel functional system. *Journal of Morphology*. 1989 Nov;202(2):129–50.
6. Evans KM, Larouche O, Gartner SM, Faucher RE, Dee SG, Westneat MW. Beaks promote rapid morphological diversification along distinct evolutionary trajectories in labrid fishes (Eupercaria: Labridae). Stayton T, Connallon T, editors. *Evolution*. 2023 Sept 1;77(9):2000–14.
7. Liem KF. Evolutionary Strategies and Morphological Innovations: Cichlid Pharyngeal Jaws. *Systematic Zoology*. 1973 Dec;22(4):425–41.
8. Marki PZ, Kennedy JD, Cooney CR, Rahbek C, Fjeldså J. Adaptive radiation and the evolution of nectarivory in a large songbird clade. *Evolution*. 2019 June;73(6):1226–40.
9. Garcia-Porta J, Ord TJ. Key innovations and island colonization as engines of evolutionary diversification: a comparative test with the Australasian diplodactyloid geckos. *J of Evolutionary Biology*. 2013 Dec;26(12):2662–80.
10. Maia R, Rubenstein DR, Shawkey MD. Key ornamental innovations facilitate diversification in an avian radiation. *Proc Natl Acad Sci USA*. 2013 June 25;110(26):10687–92.
11. Price SA, Wainwright PC, Bellwood DR, Kazancioglu E, Collar DC, Near TJ. Functional Innovations and Morphological Diversification in Parrotfish. *Evolution*. 2010 Aug 19;64(10):3057–68.
12. Troyer EM, Evans KM, Goatley CHR, Friedman M, Carnevale G, Nicholas B, et al. Evolutionary innovation accelerates morphological diversification in pufferfishes and their relatives. Sherratt E, Morlon H, editors. *Evolution*. 2024 Oct 28;78(11):1869–82.
13. Hajheidari M, Koncz C, Bucher M. Chromatin Evolution-Key Innovations Underpinning Morphological Complexity. *Front Plant Sci* [Internet]. 2019 Apr 12 [cited 2025 Feb 21];10(454). Available from: <https://www.frontiersin.org/article/10.3389/fpls.2019.00454/full>
14. Konow N, Bellwood DR, Wainwright PC, Kerr AM. Evolution of novel jaw joints promote trophic diversity in coral reef fishes. *Biological Journal of the Linnean Society*. 2008 Feb 4;93(3):545–55.
15. Booher DB, Gibson JC, Liu C, Longino JT, Fisher BL, Janda M, et al. Functional innovation promotes diversification of form in the evolution of an ultrafast trap-jaw mechanism in ants. Khila A, editor. *PloS Biol*. 2021 Mar 2;19(3):e3001031.
16. Wainwright PC. Functional Versus Morphological Diversity in Macroevolution. *Annual Review of Ecology, Evolution, and Systematics*. 2007;38(1):381–401.
17. Erwin DH. Novelty and Innovation in the History of Life. *Current Biology*. 2015 Oct;25(19):R930–40.
18. Ord TJ, Garcia-Porta J, Querejeta M, Collar DC. Gliding Dragons and Flying Squirrels: Diversifying versus Stabilizing Selection on Morphology following the Evolution of an Innovation. *The American Naturalist*. 2020 Feb;195(2):E51–66.
19. Higham TE, Birn-Jeffery AV, Collins CE, Hulsey CD, Russell AP. Adaptive simplification and the evolution of gecko locomotion: Morphological and biomechanical consequences of losing adhesion. *Proc Natl Acad Sci USA*. 2015 Jan 20;112(3):809–14.

20. De Alencar LRV, Martins M, Burin G, Quental TB. Arboreality constrains morphological evolution but not species diversification in vipers. *Proc R Soc B*. 2017 Dec 20;284(1869):20171775.
21. Mahler DL, Revell LJ, Glor RE, Losos JB. ECOLOGICAL OPPORTUNITY AND THE RATE OF MORPHOLOGICAL EVOLUTION IN THE DIVERSIFICATION OF GREATER ANTILLEAN ANOLES: OPPORTUNITY AND RATE IN ANOLIS LIZARDS. *Evolution*. 2010 Sept;64(9):2731–45.
22. Patton AH, Harmon LJ, Del Rosario Castañeda M, Frank HK, Donihue CM, Herrel A, et al. When adaptive radiations collide: Different evolutionary trajectories between and within island and mainland lizard clades. *Proc Natl Acad Sci USA*. 2021 Oct 19;118(42):e2024451118.
23. Rowsey DM, Heaney LR, Jansa SA. Tempo and mode of mandibular shape and size evolution reveal mixed support for incumbency effects in two clades of island-endemic rodents (Muridae: Murinae)*. *Evolution*. 2019 July;73(7):1411–27.
24. Emerson BC, Gillespie RG. Phylogenetic analysis of community assembly and structure over space and time. *Trends in Ecology & Evolution*. 2008 Nov;23(11):619–30.
25. Schenk JJ, Rowe KC, Steppan SJ. Ecological Opportunity and Incumbency in the Diversification of Repeated Continental Colonizations by Muroid Rodents. *Systematic Biology*. 2013 Nov 1;62(6):837–64.
26. Hunter JP, Jernvall J. The hypocone as a key innovation in mammalian evolution. *Proc Natl Acad Sci USA*. 1995 Nov 7;92(23):10718–22.
27. Lafuma F, Corfe IJ, Clavel J, Di-Poi N. Multiple evolutionary origins and losses of tooth complexity in squamates. *Nat Commun*. 2021 Oct 14;12(1):6001.
28. Peoples N, Burns MD, Mihalitsis M, Wainwright PC. Evolutionary lability of a key innovation spurs rapid diversification. *Nature*. 2025 Feb 26;639(8056):962–7.
29. Pollock TI, Deakin WJ, Chatar N, Milla Carmona PS, Rovinsky DS, Panagiotopoulou O, et al. Functional optimality underpins the repeated evolution of the extreme “saber-tooth” morphology. *Current Biology*. 2025 Jan;S0960982224016269.
30. Crofts SB, Smith SM, Anderson PSL. Beyond Description: The Many Facets of Dental Biomechanics. *Integrative and Comparative Biology*. 2020 Sept 1;60(3):594–607.
31. Cohen KE, Weller HI, Summers AP. Not your father’s homodonty—stress, tooth shape, and the functional homodont. *Journal of Anatomy*. 2020 Nov;237(5):837–48.
32. Mihalitsis M, Bellwood D. Functional implications of dentition-based morphotypes in piscivorous fishes. *R Soc open sci* [Internet]. 2019 Sept 27 [cited 2024 Jan 17];6(9). Available from: <https://royalsocietypublishing.org/doi/10.1098/rsos.190040>
33. Segall M, Houssin C, Delapr   A, Cornette R, Herrel A, Milgram J, et al. Armed to the teeth: The underestimated diversity in tooth shape in snakes and its relation to feeding behavior and diet. *Ecology and Evolution*. 2023 Apr;13(4):e10011.
34. Ronco F, Matschiner M, B  hne A, Boila A, B  scher HH, El Taher A, et al. Drivers and dynamics of a massive adaptive radiation in cichlid fishes. *Nature*. 2021 Jan 7;589(7840):76–81.
35. Salzburger W, Mack T, Verheyen E, Meyer A. Out of Tanganyika: Genesis, explosive speciation, key-innovations and phylogeography of the haplochromine cichlid fishes. *BMC Evol Biol*. 2005;5(1):17.
36. Claude J. Log-Shape Ratios, Procrustes Superimposition, Elliptic Fourier Analysis: Three Worked Examples in R. *Hystrix, the Italian Journal of Mammology*. 2013 July 1;24(1):94–102.
37. Dieleman J, Van Bocxlaer B, Manntsckhe C, Nyingi DW, Adriaens D, Verschuren D. Tracing functional adaptation in African cichlid fishes through morphometric analysis of fossil teeth: exploring the methods. *Hydrobiologia*. 2015 Aug;755(1):73–88.
38. Dieleman J, Van Bocxlaer B, Nyingi WD, Lyaruu A, Verschuren D. Recurrent changes in cichlid dentition linked to climate-driven lake-level fluctuations. *Ecosphere*. 2019 Apr;10(4):e02664.

39. Baken EK, Collyer ML, Kaliontzopoulou A, Adams DC. Geomorph v4.0 and gmShiny: Enhanced analytics and a new graphical interface for a comprehensive morphometric experience. *Methods Ecol Evol.* 2021 Dec;12(12):2355–63.
40. Adams DC, Collyer ML, Kaliontzopoulou A, Baken EK. Geomorph: Software for geometric morphometric analyses [Internet]. 2025. Available from: <https://cran.r-project.org/package=geomorph>
41. Pennell MW, Eastman JM, Slater GJ, Brown JW, Uyeda JC, FitzJohn RG, et al. 108epidi v2.0: an expanded suite of methods for fitting macroevolutionary models to phylogenetic trees. *Bioinformatics.* 2014 Aug 1;30(15):2216–8.
42. Blonder B, Lamanna C, Violle C, Enquist BJ. The n-dimensional hypervolume. *Global Ecology and Biogeography.* 2014;23:595–609.
43. Blonder B, Morrow CB, Maitner B, Harris DJ, Lamanna C, Violle C, et al. New approaches for delineating n-dimensional hypervolumes. *Methods in Ecology and Evolution.* 2018;9:305–19.
44. Harmon LJ, Schulte JA, Larson A, Losos JB. Tempo and Mode of Evolutionary Radiation in Iguanian Lizards. *Science.* 2003 Aug 15;301(5635):961–4.
45. Höhna S, Landis MJ, Heath TA, Boussau B, Lartillot N, Moore BR, et al. RevBayes: Bayesian Phylogenetic Inference Using Graphical Models and an Interactive Model-Specification Language. *Syst Biol.* 2016 July;65(4):726–36.
46. May MR, Moore BR. A Bayesian Approach for Inferring the Impact of a Discrete Character on Rates of Continuous-Character Evolution in the Presence of Background-Rate Variation. Holder M, editor. *Systematic Biology.* 2020 May 1;69(3):530–44.
47. Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. Susko E, editor. *Systematic Biology.* 2018 Sept 1;67(5):901–4.
48. Burress ED, Martinez CM, Wainwright PC. Decoupled jaws promote trophic diversity in cichlid fishes. *Evolution.* 2020 May;74(5):950–61.
49. Clavel J, Escarguel G, Merceron G. mv MORPH : an R package for fitting multivariate evolutionary models to morphometric data. Poisot T, editor. *Methods Ecol Evol.* 2015 Nov;6(11):1311–9.
50. Revell LJ. Phytools: an R package for phylogenetic comparative biology (and other things). *Methods Ecol Evol.* 2012 Apr;3(2):217–23.
51. Irisarri I, Singh P, Koblmüller S, Torres-Dowdall J, Henning F, Franchini P, et al. Phylogenomics uncovers early hybridization and adaptive loci shaping the radiation of Lake Tanganyika cichlid fishes. *Nat Commun.* 2018 Aug 8;9(1):3159.
52. Koblmüller S, Egger B, Sturmbauer C, Sefc KM. Rapid radiation, ancient incomplete lineage sorting and ancient hybridization in the endemic Lake Tanganyika cichlid tribe Tropheini. *Molecular Phylogenetics and Evolution.* 2010 Apr;55(1):318–34.
53. Eastman JM, Alfaro ME, Joyce P, Hipp AL, Harmon LJ. A NOVEL COMPARATIVE METHOD FOR IDENTIFYING SHIFTS IN THE RATE OF CHARACTER EVOLUTION ON TREES: IDENTIFYING RATE HETEROGENEITY IN CHARACTER EVOLUTION. *Evolution.* 2011 Dec;65(12):3578–89.
54. Beaulieu JM, Jhweung DC, Boettiger C, O'Meara BC. Modeling Stabilizing Selection: Expanding The Ornstein-Uhlenbeck Model of Adaptive Evolution. *Evolution.* 2012 Aug;66(8):2369–83.
55. Armbruster WS, Schwaegerle KE. Causes of covariation of phenotypic traits among populations. *J of Evolutionary Biology.* 1996 May;9(3):261–76.
56. Klingenberg CP. Studying morphological integration and modularity at multiple levels: concepts and analysis. *Phil Trans R Soc B.* 2014 Aug 19;369(1649):20130249.

57. Roberts-Hughes AS, Martinez CM, Corn KA, Wainwright PC. A classic key innovation constrains oral jaw functional diversification in fishes. *Evolution Letters*. 2024 Oct 8;qrae046.
58. Gavrillets S, Losos JB. Adaptive Radiation: Contrasting Theory with Data. *Science*. 2009 Feb 6;323(5915):732–7.
59. Todd Streelman J, Danley PD. The stages of vertebrate evolutionary radiation. *Trends in Ecology & Evolution*. 2003 Mar;18(3):126–31.
60. Worthington EB, Ricardo OK. The Fish of Lake Tanganyika (other than Cichlidæ). *Proceedings of the Zoological Society of London*. 1936 Dec;106(4):1061–112.
61. Bbole I, Zhao JL, Katongo C, Tang SJ. Escapes from aquaculture facilities in freshwater ecosystems; conservation concerns for *Oreochromis tanganicae*, an endemic species of Lake Tanganyika. *Egyptian Journal of Aquatic Research*. 2023 Dec;49(4):542–8.
62. Ronco F, Büscher HH, Indermaur A, Salzburger W. The taxonomic diversity of the cichlid fish fauna of ancient Lake Tanganyika, East Africa. *Journal of Great Lakes Research*. 2020 Oct;46(5):1067–78.
63. Davis AM, Unmack PJ, Vari RP, Betancur-R. R. Herbivory Promotes Dental Disparification and Macroevolutionary Dynamics in Grunters (Teleostei: Terapontidae), a Freshwater Adaptive Radiation. *The American Naturalist*. 2016 Mar;187(3):320–33.
64. Kolmann MA, Poulin E, Rosen J, Hemraj-Naraine D, Burns MD. Phenotypic Convergence Is Stronger and More Frequent in Herbivorous Fishes. *Integrative And Comparative Biology*. 2024 Nov 21;64(5):1467–83.
65. Ford AGP, Rüber L, Newton J, Dasmahapatra KK, Balarin JD, Bruun K, et al. Niche divergence facilitated by fine-scale ecological partitioning in a recent cichlid fish adaptive radiation. *Evolution*. 2016 Dec;70(12):2718–35.
66. Burns MD, Satterfield DR, Peoples N, Chan H, Barley AJ, Yuan ML, et al. Complexity and weak integration promote the diversity of reef fish oral jaws. *Commun Biol*. 2024 Nov 4;7(1):1433.
67. Bellwood DR. Origins and escalation of herbivory in fishes: a functional perspective. *Paleobiology*. 2003;29(1):71–83.
68. Davis AM, Betancur-R R. Widespread ecomorphological convergence in multiple fish families spanning the marine–freshwater interface. *Proc R Soc B*. 2017 May 17;284(1854):20170565.
69. Felice RN, Randau M, Goswami A. A fly in a tube: Macroevolutionary expectations for integrated phenotypes. *Evolution*. 2018 Dec;72(12):2580–94.
70. Borstein SR, Fordyce JA, O’Meara BC, Wainwright PC, McGee MD. Reef fish functional traits evolve fastest at trophic extremes. *Nat Ecol Evol*. 2018 Nov 26;3(2):191–9.
71. Corn KA, Friedman ST, Burrell ED, Martinez CM, Larouche O, Price SA, et al. The rise of biting during the Cenozoic fueled reef fish body shape diversification. *Proc Natl Acad Sci USA*. 2022 Aug 2;119(31):e2119828119.
72. Burns MD. Adaptation to herbivory and detritivory drives the convergent evolution of large abdominal cavities in a diverse freshwater fish radiation (Otophysi: Characiformes). *Evolution*. 2021 Mar;75(3):688–705.
73. Cohen KE, Lucanus O, Summers AP, Kolmann MA. Lip service: Histological phenotypes correlate with diet and feeding ecology in herbivorous pacus. *The Anatomical Record*. 2023 Feb;306(2):326–42.
74. Hata H, Shibata J, Omori K, Kohda M, Hori M. Depth segregation and diet disparity revealed by stable isotope analyses in sympatric herbivorous cichlids in Lake Tanganyika. *Zoological Lett*. 2015 Dec;1(1):15.
75. Sturmbauer C, Mark W, Dallinger R. Ecophysiology of Aufwuchs-eating cichlids in Lake Tanganyika: niche separation by trophic specialization. *Environ Biol Fish*. 1992 Nov;35(3):283–90.

76. Sturmbauer C, Hainz U, Baric S, Verheyen E, Salzburger W. Evolution of the tribe Tropheini from Lake Tanganyika: synchronized explosive speciation producing multiple evolutionary parallelism. *Hydrobiologia*. 2003 June;500(1–3):51–64.
77. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. *Cell Res*. 2011 Mar;21(3):381–95.
78. Wainwright PC, Longo SJ. Functional Innovations and the Conquest of the Oceans by Acanthomorph Fishes. *Current Biology*. 2017 June;27(11):R550–7.
79. Near TJ, Dornburg A, Kuhn KL, Eastman JT, Pennington JN, Patarnello T, et al. Ancient climate change, antifreeze, and the evolutionary diversification of Antarctic fishes. *Proc Natl Acad Sci USA*. 2012 Feb 28;109(9):3434–9.
80. Fletcher GL, Hew CL, Davies PL. Antifreeze Proteins of Teleost Fishes. *Annu Rev Physiol*. 2001 Mar;63(1):359–90.
81. Rives N. Diverse Origins of Near-Identical Antifreeze Proteins in Unrelated Fish Lineages Provide Insights Into Evolutionary Mechanisms of New Gene Birth and Protein Sequence Convergence. *Molecular Biology and Evolution*. 2024;41(9):msae182.
82. Burns MD, Friedman ST, Corn KA, Larouche O, Price SA, Wainwright PC, et al. High-latitude ocean habitats are a crucible of fish body shape diversification. *Evolution Letters*. 2024 Sept 25;8(5):669–79.
83. Miller AH, Stroud JT. Novel Tests of the Key Innovation Hypothesis: Adhesive Toepads in Arboreal Lizards. Uyeda J, editor. *Systematic Biology*. 2021 Dec 16;71(1):139–52.
84. Collar DC, Schulte JA, O'Meara BC, Losos JB. Habitat use affects morphological diversification in dragon lizards. *J of Evolutionary Biology*. 2010 May;23(5):1033–49.
85. Russell AP. Parallelism and Integrated Design in the Foot Structure of Gekkonine and Diplodactyline Geckos. *Copeia*. 1979 Feb 20;1979(1):1–21.
86. Peoples N, Wainwright PC. Data from: Multifaceted impacts of an innovation on dental diversity in an adaptive radiation of cichlid fishes [Internet]. Dryad; 2025. Available from: <https://doi.org/10.5061/dryad.wstqjq30j>

Supplementary Information

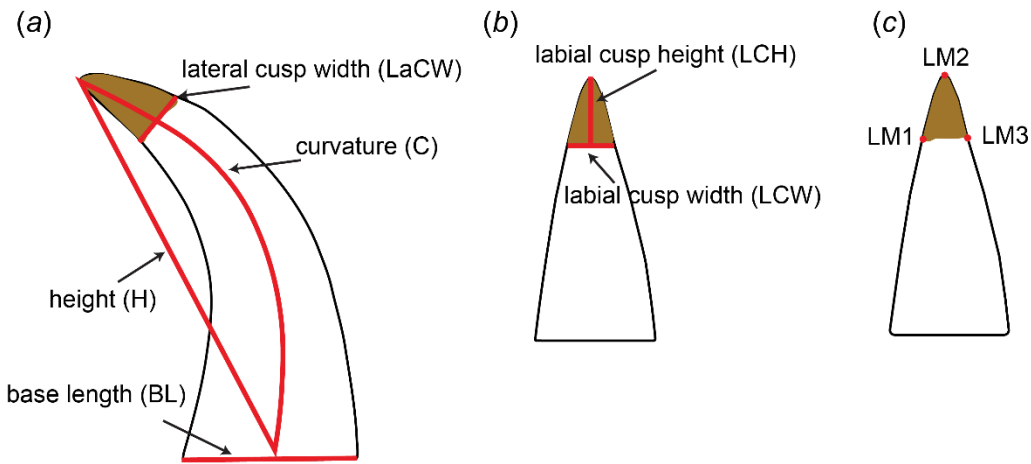


Figure S1 | Linear measurements of teeth. Linear measurements used to describe functional aspects of individual teeth, taken from lateral (*a*) and labial (*b*) view images. (*c*) Location of fixed landmarks (LM) for geometric morphometric analysis of cusp shape. The variables used in analyses include tooth height (H), tooth curvature (C/H), base length (BL), cusp cross sectional aspect ratio (LCW/LaCW), cusp height (LCH), ratio of enameloid (LCH/H), and taper (LCH/LCW).

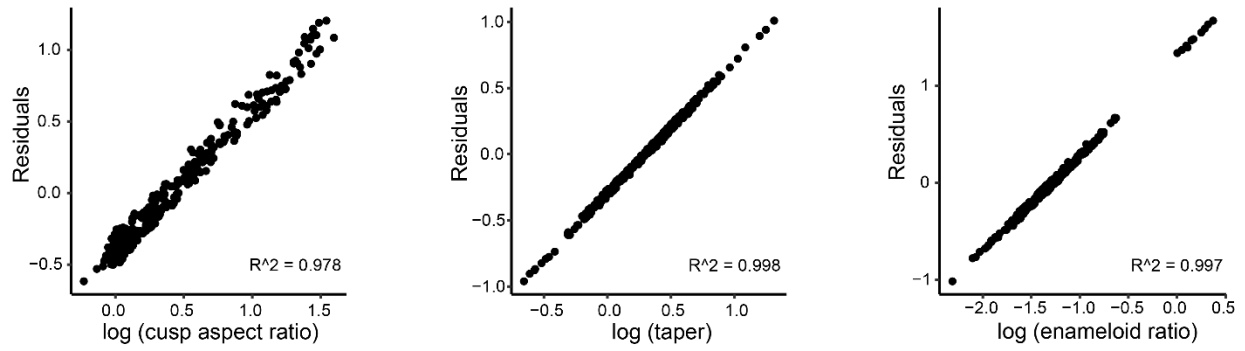


Figure S2 | Comparison of ratio data from two approaches to size correction. Residuals from log-log regressions of each ratio against standard length, plotted against the log-transformed ratio values.

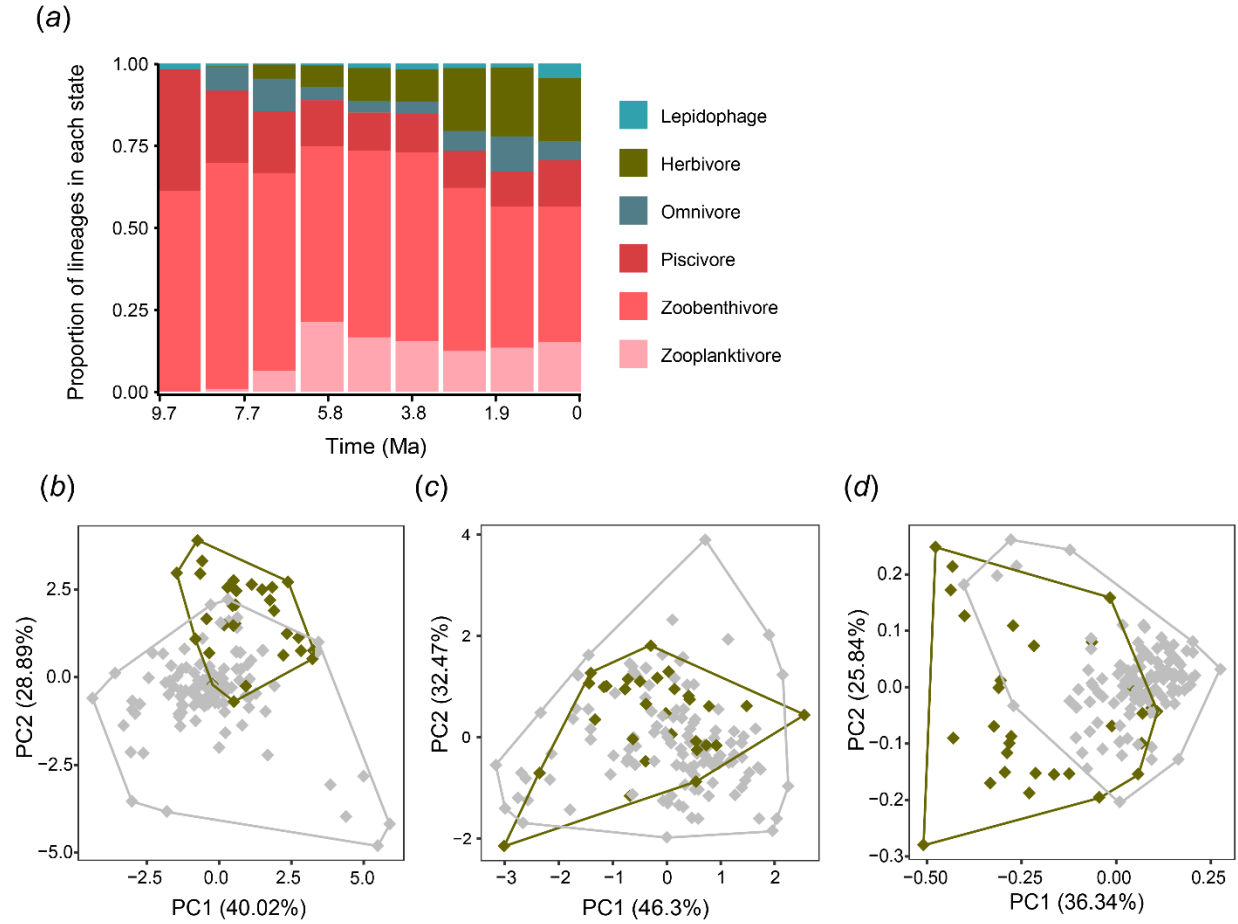


Figure S3 | Diet and dental diversity. (a) Proportion of discrete diets through time, in 1-My intervals. Principal component biplots of dentition (b), linear morphometric, (c), and geometric morphometric (d) datasets. Colors correspond to the diet categories of (a), and all non-herbivores are colored grey.

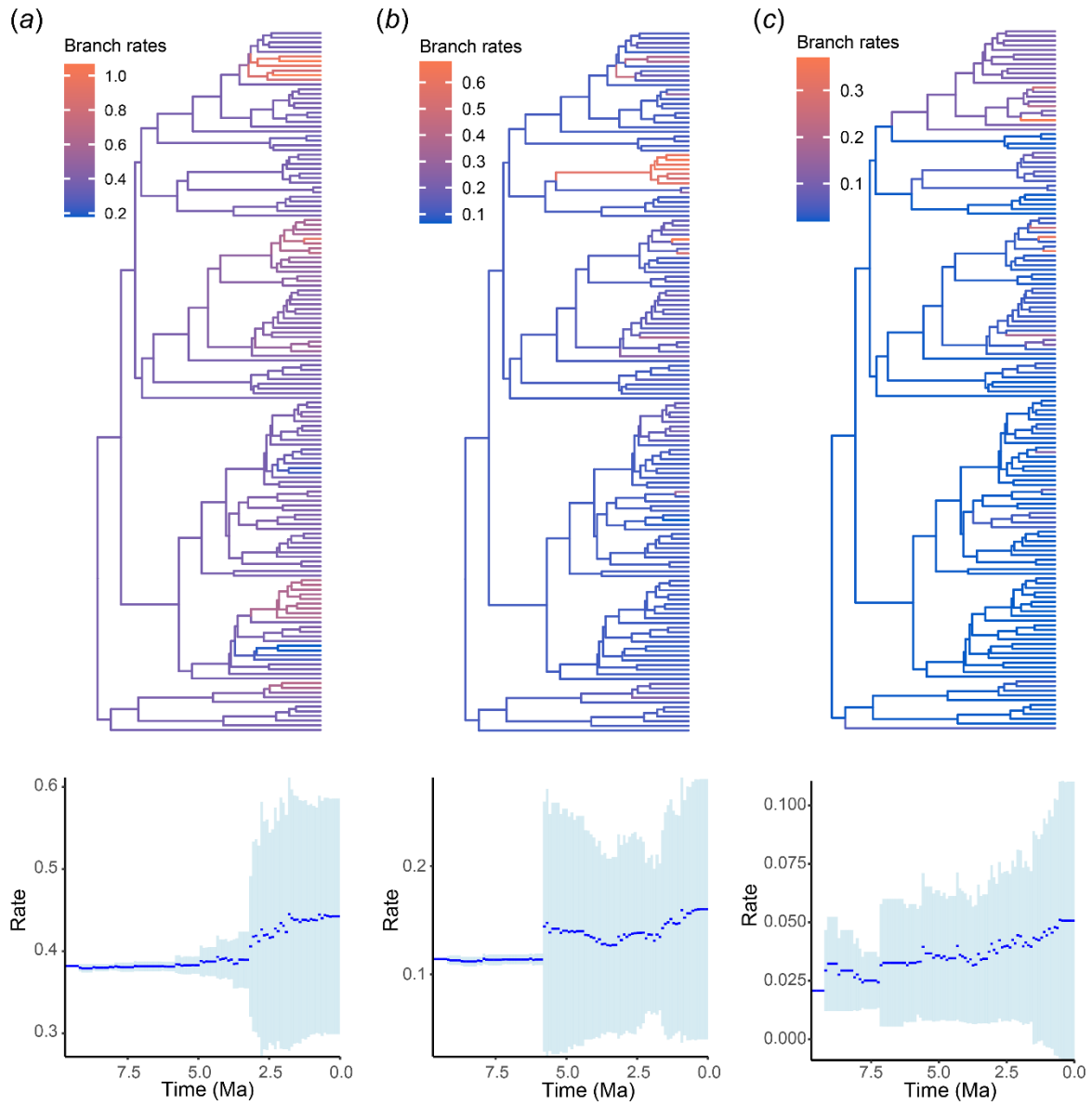


Figure S4 | The tempo of dental evolution. Branch-specific multivariate rates of trait evolution for the dentition (*a*), linear morphometric (*b*), and geometric morphometric (*c*) datasets.

Branches are colored according to the estimated rate. The corresponding mean rate (blue line) and standard deviation (shading) through time, in 100 equal-length intervals, is plotted below each phylogeny.

Table S1 – Functional basis of tooth traits.

Trait	Functional inference	References
Number of teeth	Prey capture; damage infliction	(1–3)
Number of rows	Grip	(1,4,5)
Variance in tooth height	Heterodonty-homodonty	(6,7)
Tooth height	Jaw protrusion; damage infliction; breakage resistance	(2,3,8,9)
Tooth base length	Breakage resistance	(8)
Tooth curvature	Grip; puncture	(10–12)
Cross-sectional aspect ratio	Puncture; sharpness; slicing	(13–17)
Taper	Puncture; damage infliction	(8,13,15,18–22)
Cusp height	Damage inflection	(2)
Enameloid ratio	Hardness; stiffness	(22–25)
Cusp shape (geometric morphometrics)	Cutting; piercing; crushing	(5,17,26–29)
Cusp complexity (>1 cusp)	Cutting; trapping	(27,29)

Table S2 | Comparison of discrete character evolution models.

Model	AIC	ΔAIC	AICω
ER	99.206	0.898	0.39
ARD	98.308	-	0.61

Table S3 | List of specimens included in this study. UMMZ (University of Michigan Museum of Zoology), MCZ (Harvard Museum of Comparative Zoology), SMNH (Swedish Museum of Natural History), MRAC (Royal Museum for Central Africa).

Species		SL (mm)	Museum	Collection ID
<i>Altolamprologus calvus</i>	N=3	60-72	Own collection	
<i>Altolamprologus compressiceps</i>	N=3	73-96	UMMZ	218320
<i>Altolamprologus</i> sp. “ <i>compressiceps</i> shell”	N=1	39	Aquarium trade	
<i>Asprotilapia leptura</i>	N=2	61-76	Own collection	
<i>Astatotilapia burtoni</i>	N=3	73-87	MCZ	49249
<i>Aulonocranus dewindti</i>	N=3	77-97	UMMZ	199865
<i>Bathybates leo</i>	N=3	107-154	MCZ	49293
<i>Bathybates ferox</i>	N=3	158-165	UMMZ	199759
<i>Bathybates minor</i>	N=3	112-138	MCZ	199805
<i>Bathybates graueri</i>	N=3	143-147	UMMZ	199802
<i>Benthochromis melanoides</i>	N=3	126-128	SMNH	12781
<i>Benthochromis tricoti</i>	N=3	127-130	MCZ	50834
<i>Boulengerochromis microlepis</i>	N=1	63	Own collection	
<i>Boulengerochromis microlepis</i>	N=5	47-109	MRAC	1984.044.P.0173-0322
<i>Callochromis pleurospilus</i>	N=3	46-47	MCZ	49308
<i>Callochromis macrops</i>	N=3	80-86	UMMZ	199866
<i>Callochromis melanostigma</i>	N=2	38-40	Aquarium trade	
<i>Cardiopharynx schoutedeni</i>	N=3	87-99	UMMZ	196025
<i>Chalinochromis brichardi</i>	N=2	54-101	UMMZ	196155
<i>Chalinochromis cyanophleps</i>	N=2	42-47	Aquarium trade	
<i>Chalinochromis brichardi</i>	N=1	93	Own collection	
<i>Chalinochromis</i> sp. “ <i>bifrenatus</i> ”	N=2	39-48	Aquarium trade	
<i>Ctenochromis horei</i>	N=1	127	UMMZ	199867
<i>Ctenochromis horei</i>	N=3	79-86	MCZ	32635
<i>Cunningtonia longiventralis</i>	N=2	72-79	MCZ	49243

<i>Cyathopharynx furcifer</i>	N=2	113-116	UMMZ	199761
<i>Cyathopharynx foae</i>	N=3	32-37	Aquarium trade	
<i>Cyphotilapia frontosa</i>	N=3	77-104	MCZ	50837
<i>Cyprichromis leptosoma</i>	N=2	80-81	MCZ	174088
<i>Cyprichromis leptosoma</i>	N=2	68-75	Own collection	
<i>Cyprichromis coloratus</i>	N=1	93	Own collection	
<i>Cyprichromis microlepidotus</i>	N=2	73-88	MRAC	1994.069.P.1592-1662
<i>Cyprichromis zonatus</i>	N=3	43-52	Aquarium trade	
<i>Ectodus descampsii</i>	N=2	82-87	UMMZ	196158
<i>Enantiopus melanogenys</i>	N=2	94-96	UMMZ	196176
<i>Eretmodus cyanostictus</i>	N=3	69-72	UMMZ	199942
<i>Gnathochromis permaxillaris</i>	N=2	95-112	MCZ	49244, 49253
<i>Gnathochromis permaxillaris</i>	N=1	56	Own collection	
<i>Gnathochromis pfefferi</i>	N=3	67-80	MCZ	49258
<i>Grammatotria lemairei</i>	N=3	99-112	MCZ	49277
<i>Greenwoodochromis bellcrossi</i>	N=1	117	Own collection	
<i>Greenwoodochromis bellcrossi</i>	N=2	125-147	UMMZ	196117, 195992
<i>Greenwoodochromis christyi</i>	N=1	108	UMMZ	196120
<i>Haplotaxodon microelpis</i>	N=3	164-177	UMMZ	199765
<i>Hemibates stenosoma</i>	N=3	165-212	UMMZ	199792
<i>Julidochromis marlieri</i>	N=1	40	Own collection	
<i>Julidochromis transcriptus</i>	N=3	44-54	Aquarium trade	
<i>Julidochromis marksmithi</i>	N=3	44-46	Aquarium trade	
<i>Julidochromis dickfeldi</i>	N=1	57	Own collection	
<i>Julidochromis regani</i>	N=2	51-60	MCZ	49217
<i>Julidochromis ornatus</i>	N=3	43-44	MRAC	P.130619-130653
<i>Lamprologus hecqui</i>	N=1	39	MCZ	32620
<i>Lamprologus pleuromaculatus</i>	N=3	87-93	MCZ	49265
<i>Lamprologus callipterus</i>	N=3	98-105	UMMZ	199766
<i>Lamprologus lemairei</i>	N=1	101	UMMZ	199770
<i>Lamprologus lemairei</i>	N=1	41	Own collection	

<i>Lamprologus speciosus</i>	N=1	35	Own collection	
<i>Lamprologus signatus</i>	N=1	31	Own collection	
<i>Lamprologus ornatipinnis</i>	N=1	31	MCZ	60970
<i>Lestradea stappersii</i>	N=3	59-62	MCZ	32593
<i>Lestradea perspicax</i>	N=3	69-86	MRAC	P.115331-115340
<i>Lepidiolamprologus attenuatus</i>	N=3	82-102	MCZ	195967
<i>Lepidiolamprologus elongatus</i>	N=3	118-143	UMMZ	199768
<i>Lepidiolamprologus profundicola</i>	N=2	149-192	UMMZ	195983
<i>Lepidiolamprologus mimicus</i>	N=3	114-123	Own collection	
<i>Lepidiolamprologus p. "meeli Kipili"</i>	N=3	64-67	Own collection	
<i>Lepidiolamprologus nkambe</i>	N=2	53-56	Own collection	
<i>Limnotilapia dardennii</i>	N=3	144-161	MCZ	49286
<i>Limnochromis abeelei</i>	N=1	156	UMMZ	196119
<i>Limnochromis auritus</i>	N=1	117	UMMZ	196139
<i>Limnochromis auritus</i>	N=2	63-78	MCZ	50835
<i>Lobochilotes labiatus</i>	N=3	93-160	MRAC	P.129641-129650
<i>Lobochilotes labiatus</i>	N=3	34-39	MCZ	49307
<i>Microdontochromis rotundiventralis</i>	N=1	55	MRAC	2001.094.P.1205-1211
<i>Microdontochromis tenuidentata</i>	N=1	52	MRAC	1995.069.P.2275-2282
<i>Neolamprologus petricola</i>	N=2	69-94	MCZ	49242
<i>Neolamprologus tetrocephalus</i>	N=1	61	MCZ	60971
<i>Neolamprologus brevis</i>	N=2	36-37	MCZ	60965
<i>Neolamprologus modestus</i>	N=1	67	MCZ	60972
<i>Neolamprologus fasciatus</i>	N=3	91-105	UMMZ	195978
<i>Neolamprologus leleupi</i>	N=1	32	Aquarium trade	
<i>Neolamprologus cylindricus</i>	N=3	41-51	Aquarium trade	
<i>Neolamprologus brichardi</i>	N=2	54	Own collection	
<i>Neolamprologus helianthus</i>	N=2	36-42	Aquarium trade	
<i>Neolamprologus longior</i>	N=2	50-56	Own collection	
<i>Neolamprologus prochilus</i>	N=2	56-58	Own collection	

<i>Neolamprologus savoryi</i>	N=2	42-54	Own collection	
<i>Neolamprologus multifasciatus</i>	N=1	39	Own collection	
<i>Neolamprologus boulengeri</i>	N=2	59-61	Own collection	
<i>Neolamprologus cunningtoni</i>	N=3	89-116	MCZ	49284
<i>Neolamprologus longicaudatus</i>	N=2	42-61	Own collection	
<i>Neolamprologus hecqui</i>	N=1	52	Own collection	
<i>Neolamprologus obscurus</i>	N=2	34-26	Own collection	
<i>Neolamprologus splendens</i>	N=1	64	Own collection	
<i>Neolamprologus tetracanthus</i>	N=2	40-51	Own collection	
<i>Neolamprologus nigriventris</i>	N=1	51	Own collection	
<i>Neolamprologus bifasciatus</i>	N=3	40-47	Own collection	
<i>Neolamprologus maruguensis</i>	N=1	35	Own collection	
<i>Neolamprologus mondabu</i>	N=1	34	Own collection	
<i>Neolamprologus caudopunctatus</i>	N=3	43-47	MRAC	1984.023.P.0321-0347
<i>Neolamprologus walteri</i>	N=1	34	Own collection	
<i>Neolamprologus furcifer</i>	N=1	43	Own collection	
<i>Neolamprologus leloupi</i>	N=1	49	Own collection	
<i>Neolamprologus buescheri</i>	N=2	35-48	Aquarium trade	
<i>Neolamprologus signatus</i>	N=3	41-43	Aquarium trade	
<i>Neolamprologus gracilis</i>	N=3	37-43	Aquarium trade	
<i>Neolamprologus falcicula</i>	N=2	62-80	Aquarium trade	
<i>Ophthalmotilapia heterodonta</i>	N=3	71-78	MRAC	2001.104.P.0017-0026
<i>Ophthalmotilapia nasuta</i>	N=3	114-137	MCZ	50843
<i>Ophthalmotilapia ventralis</i>	N=3	57-61	Aquarium trade	
<i>Ophthalmotilapia boops</i>	N=3	61-73	Aquarium trade	
<i>Paracyprichromis brienii</i>	N=3	74-83	MRAC	2001.094.P.0980-0993
<i>Petrochromis orthognathus</i>	N=3	92-99	MRAC	P.129600-129630
<i>Petrochromis fasciolatus</i>	N=2	112-114	UMMZ	199779
<i>Petrochromis trewavasae</i>	N=3	29-31	Aquarium trade	
<i>Petrochromis</i> sp. "red"	N=3	39-44	Aquarium trade	

<i>Petrochromis polyodon</i>	N=3	97-148	UMMZ	199952
<i>Perissodus microlepis</i>	N=1	72	MCZ	49332
<i>Plecodus straeleni</i>	N=3	96-105	UMMZ	199781
<i>Plecodus elaviae</i>	N=1	206	UMMZ	199810
<i>Plecodus elaviae</i>	N=1	128	MCZ	49331
<i>Plecodus paradoxus</i>	N=1	131	MCZ	32594
<i>Plecodus multidentatus</i>	N=1	128	MCZ	49543
<i>Pseudosimochromis babaulti</i>	N=3	42-44	MCZ	49302
<i>Pseudosimochromis curvifrons</i>	N=3	88-107	UMMZ	199882
<i>Reganochromis calliurus</i>	N=3	66-86	UMMZ	196166
<i>Simochromis diagramma</i>	N=3	94-129	UMMZ	199883
<i>Spathodus marlieri</i>	N=3	34-43	UMMZ	196169
<i>Spathodus erythron</i>	N=3	62-74	MRAC	2010.012.P.1143-1146
<i>Tanganicodus irsacae</i>	N=1	57	MRAC	P.115511-115515
<i>Telmatochromis temporalis</i>	N=3	36-53	UMMZ	199954
<i>Telmatochromis dhonti</i>	N=3	49-73	MCZ	50693
<i>Telmatochromis brichardi</i>	N=1	48	Own collection	
<i>Telmatochromis</i> sp. “shell”	N=2	33-37	Aquarium trade	
<i>Trematocara macrostoma</i>	N=3	83-94	UMMZ	199107
<i>Trematocara marginatum</i>	N=1	46	MCZ	32626
<i>Trematocara variabile</i>	N=3	40-46	MCZ	50697
<i>Trematocara stigmaticum</i>	N=3	39-48	MCZ	50701
<i>Trematocara unimaculatum</i>	N=3	96-102	MCZ	49262
<i>Triglachromis otostigma</i>	N=3	77-79	MCZ	49275
<i>Tropheus moorii</i>	N=3	82-97	UMMZ	218335
<i>Tropheus duboisi</i>	N=3	45-49	Aquarium trade	
<i>Tropheus</i> sp. “brichardi Kipili”	N=3	30-35	Aquarium trade	
<i>Tropheus</i> sp. “Mpimbwe”	N=1	35	Aquarium trade	
<i>Tropheus</i> sp. “black”	N=3	26-29	Aquarium trade	
<i>Tropheus</i> sp. “red”	N=3	36-42	Aquarium trade	

<i>Tropheus brichardi</i>	N=3	52-60	Own collection	
<i>Tropheus polli</i>	N=1	63	Own collection	
<i>Tropheus annectens</i>	N=3	72-82	MRAC	1984.023.P.0258-0284
<i>Variabilichromis moorii</i>	N=3	61-71	UMMZ	199771
<i>Xenochromis hecqui</i>	N=3	149-154	MCZ	49333
<i>Xenotilapia boulengeri</i>	N=3	96-121	UMMZ	196074
<i>Xenotilapia longispinis</i>	N=3	103-107	UMMZ	196079
<i>Xenotilapia caudafasciata</i>	N=2	88-89	MCZ	49279
<i>Xenotilapia nigrolabiata</i>	N=1	72	MCZ	63312
<i>Xenotilapia ornatipinnis</i>	N=3	64-68	MCZ	50849
<i>Xenotilapia ochrogenys</i>	N=3	76-83	MCZ	49263
<i>Xenotilapia sima</i>	N=3	89-93	MCZ	49269
<i>Xenotilapia papilio</i>	N=3	84-87	M. Bjørneskov	
<i>Xenotilapia singularis</i>	N=3	54-64	Aquarium trade	
<i>Xenotilapia bathyphilus</i>	N=3	41-42	Aquarium trade	
<i>Xenotilapia flavipinnis</i>	N=3	44-52	Aquarium trade	

Supplementary References

1. Mihalitsis M, Bellwood D. Functional implications of dentition-based morphotypes in piscivorous fishes. R Soc open sci [Internet]. 2019 Sept 27 [cited 2024 Jan 17];6(9). Available from: <https://royalsocietypublishing.org/doi/10.1098/rsos.190040>
2. Muruga P, Bellwood DR, Mihalitsis M. Forensic odontology: Assessing bite wounds to determine the role of teeth in piscivorous fishes. Integrative Organismal Biology. 2022 Apr 29;4(1):obac011.
3. Carr EM, Motta PJ. Tooth length and occlusion in four species of piscivorous fishes: getting a grip on prey. Environ Biol Fish. 2020 Aug;103(8):903–12.
4. Venesky MD, Wassersug RJ, Parris MJ. How Does a Change in Labial Tooth Row Number Affect Feeding Kinematics and Foraging Performance of a Ranid Tadpole (*Lithobates sphenoccephalus*)? The Biological Bulletin. 2010 Apr;218(2):160–8.
5. Berkovitz B, Shellis P. The Teeth of Non-Mammalian Vertebrates. London: Academic Press; 2017. 354 p.
6. Cohen KE, Weller HI, Summers AP. Not your father’s homodonty—stress, tooth shape, and the functional homodont. Journal of Anatomy. 2020 Nov;237(5):837–48.
7. Cohen KE, Weller HI, Westneat MW, Summers AP. The Evolutionary Continuum of Functional Homodonty to Heterodonty in the Dentition of *Halichoeres* Wrasses. Integrative And Comparative Biology. 2023 July 31;63(1):176–87.
8. Pollock TI, Deakin WJ, Chatar N, Milla Carmona PS, Rovinsky DS, Panagiotopoulou O, et al. Functional optimality underpins the repeated evolution of the extreme “saber-tooth” morphology. Current Biology. 2025 Jan;S0960982224016269.
9. Peoples N, Mihalitsis M, Wainwright PC. Incompatibility between two major innovations shaped the diversification of fish feeding mechanisms. Hedenström A, editor. PloS Biol. 2025 June 24;23(6):e3003225.
10. Segall M, Houssin C, Delapré A, Cornette R, Herrel A, Milgram J, et al. Armed to the teeth: The underestimated diversity in tooth shape in snakes and its relation to feeding behavior and diet. Ecology and Evolution. 2023 Apr;13(4):e10011.
11. Zhang B, Baskota B, Chabain JJ, Anderson PSL. Curving expectations: The minimal impact of structural curvature in biological puncture mechanics. Sci Adv [Internet]. 2024 Aug 16 [cited 2025 July 10];10(33). Available from: <https://www.science.org/doi/10.1126/sciadv.adp8157>
12. Galloway KA, Anderson PSL, Wilga CD, Summers AP. Performance of teeth of lingcod, *Ophiodon elongatus*, over ontogeny: TEETH PERFORMANCE OF LINGCOD, *O PHIODON ELONGATUS*. J Exp Zool. 2016 Feb;325(2):99–105.
13. Freeman PW, Lemen CA. The trade-off between tooth strength and tooth penetration: predicting optimal shape of canine teeth. Journal of Zoology. 2007;273(2007):273–80.
14. Popowics TE, Fortelius M. On the cutting edge: Tooth blade sharpness in herbivorous and faunivorous mammals. Annales Zoologici Fennici. 2025;34(2):73–88.
15. Ferguson AR, Huber DR, Lajeunesse MJ, Motta PJ. Feeding performance of king Mackerel, *Scomberomorus cavalla*. J Exp Zool. 2015 Aug;323(7):399–413.
16. Grubich JR, Rice AN, Westneat MW. Functional morphology of bite mechanics in the great barracuda (*Sphyræna barracuda*). Zoology. 2008 Jan;111(1):16–29.

17. Velasco-Hogan A, Huang W, Serrano C, Kisailus D, Meyers MA. Tooth structure, mechanical properties, and diet specialization of Piranha and Pacu (Serrasalminidae): A comparative study. *Acta Biomaterialia*. 2021 Oct;134:531–45.
18. Anderson PSL. Making a point: shared mechanics underlying the diversity of biological puncture. *Journal of Experimental Biology*. 2018 Nov 15;221(22):jeb187294.
19. Anderson PSL, Crofts SB, Kim JT, Chamorro LP. Taking a Stab at Quantifying the Energetics of Biological Puncture. *Integrative and Comparative Biology*. 2019 Dec 1;59(6):1586–96.
20. Crofts SB, Lai Y, Hu Y, Anderson PSL. How do morphological sharpness measures relate to puncture performance in viperid snake fangs? *Biol Lett*. 2019 Apr;15(4):20180905.
21. Zhang B, Anderson PSL. Investigation of the rate-mediated form-function relationship in biological puncture. *Sci Rep [Internet]*. 2023 July 26 [cited 2025 July 10];13(1). Available from: <https://www.nature.com/articles/s41598-023-39092-8>
22. Song J, Ortiz C, Boyce MC. Threat-protection mechanics of an armored fish. *Journal of the Mechanical Behavior of Biomedical Materials*. 2011 July;4(5):699–712.
23. Chen PY, Schirer J, Simpson A, Nay R, Lin YS, Yang W, et al. Predation versus protection: Fish teeth and scales evaluated by nanoindentation. *J Mater Res*. 2012 Jan 14;27(1):100–12.
24. Sasagawa I, Ishiyama M, Yokosuka H, Mikami M, Uchida T. Tooth enamel and enameloid in actinopterygian fish. *Front Mater Sci China*. 2009 June;3(2):174–82.
25. Van Casteren A, Crofts SB. The Materials of Mastication: Material Science of the Humble Tooth. *Integrative and Comparative Biology*. 2019 July 18;icb129.
26. Crofts SB, Smith SM, Anderson PSL. Beyond Description: The Many Facets of Dental Biomechanics. *Integrative and Comparative Biology*. 2020 Sept 1;60(3):594–607.
27. Anderson PSL, LaBarbera M. Functional consequences of tooth design: effects of blade shape on energetics of cutting. *Journal of Experimental Biology*. 2008 Nov 15;211(22):3619–26.
28. Lucas PW. *Dental Functional Morphology*. Cambridge University Press; 2004. 355 p.
29. Anderson PSL. The effects of trapping and blade angle of notched dentitions on fracture of biological tissues. *Journal of Experimental Biology*. 2009 Nov 15;212(22):3627–32.

Chapter 3

Incompatibility between two major innovations shaped the diversification of fish feeding mechanisms

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Introduction

Innovations, or evolutionary novelties that confer substantial gains in organismal performance, are thought to be a major generator of biodiversity. By allowing species to interact with the environment in new ways, these traits often have profound effects on patterns of ecological, phenotypic, and lineage diversification (1–3). New adaptive zones and axes of diversification may be unlocked by increased performance, a famous example being the modified pharyngeal jaw of some fishes allowing them to process tougher prey items (4). However, the evolution of functional traits is subject to both biomechanical limits and performance trade-offs, which themselves can have impacts on macroevolutionary patterns (5–9). Also, the effect of nested innovations may magnify positive effects or lead to conflicts. For example, teeth are a major vertebrate innovation that had extensive consequences for trophic diversity, but the evolution of powered flight in Aves placed a premium on the body being lightweight; this conflict may underlie the loss of teeth in birds (10,11). Despite the potential for interactions between separate innovations being complex and having a significant effect on macroevolutionary patterns, these interactions are rarely studied.

Ray-finned fishes (Actinopterygii) are the largest vertebrate group in aquatic environments, representing about half of all vertebrates. With over 30,000 species, their success is often attributed to a suite of innovations in the feeding apparatus that have evolved over 400 million years to overcome the challenges of feeding in a fluid (12–15). Since the Devonian, the evolution of large teeth has provided a multitude of functional benefits that allow fishes to feed across the span of the food web by fracturing tough prey, impaling and restraining evasive prey, or removing prey from a substrate (16–20). For example, within predatory fishes, species with raptorial teeth often capture prey by grabbing, inflicting damage through laceration and puncture (21,22). Large teeth with multiple cusps are also characteristic of many early herbivores (23), suggesting that large teeth can reflect feeding specializations across multiple trophic levels.

However, irrespective of prey type, capturing food resources in an aquatic environment requires consumers to be near their prey. Fishes close this gap using a combination of high-speed bursts of forward swimming (body ram), rapid forward projection of their jaws (jaw ram), and hydrodynamic forces (suction) (24,25). This diversity of prey capture modes reflects further innovations in the feeding apparatus that have improved the efficiency of aquatic feeding through time (13–15,26). The emergence of suction feeding was a major advance in fish feeding (15,27). Suction feeding draws-in elusive prey items through a swift flow of water into the mouth, which is generated by rapid expansion of the mouth and oral cavity (28,29). While many fishes can produce small amounts of suction, the evolution of a protrusible upper jaw was a second major innovation in the feeding system that has since provided multiple benefits to fishes (13–15). By mechanically increasing hydrodynamic suction forces and swiftly closing the distance to the prey, rapid protrusion of the jaws enhances performance in suction feeding predators (30,31). Additionally, jaw protrusion enabled fishes to feed on cryptic prey associated

with the benthos or elusive prey with fast escape mechanisms (26). Upper jaw protrusion has evolved independently multiple times with different mechanisms but shows convergent effects on function (14,32,33). Protrusible jaws and large teeth are thus two innovations that both improved the efficiency of feeding modes and fundamentally expanded the diversity of prey capture strategies in fishes, which may have contributed to their dominance in the aquatic realm (12–14,20,26,27). However, the functional and evolutionary relationship between these two innovations remains unclear.

In this study, we examined the relationship between tooth size and the kinematic diversity of feeding strikes across a diverse array of ray-finned fishes (Actinopterygii) to understand the functional and evolutionary dynamics between these two major advances in aquatic feeding. We captured high-speed feeding videos of 161 actinopterygian species spanning 23 orders and 66 families to measure the contributions of body ram, jaw protrusion, and suction to prey capture and assessed these relationships with tooth size in a phylogenetic framework. We fit continuous trait evolution models to describe macroevolutionary patterns of tooth size evolution. If fishes can capitalize on both innovations – large teeth and jaw protrusion – we expect to find species with large teeth using high amounts of jaw ram during prey capture. Our results provide key insight into the functional and evolutionary relationships between two major innovations that drove success within the largest group of aquatic feeding vertebrates.

Results and Discussion

Tooth size and the kinematic diversity of feeding

We found extensive variation in relative tooth size in the oral jaws of 161 species of ray-finned fishes, spanning 23 orders and 66 families (Table S1; Fig. 1a). Tooth size varied 160-fold

across the phylogeny and ranged from being absent (e.g., *Neolissochilus stracheyi*, blue mahseer) or significantly reduced (e.g., *Lates niloticus*, Nile perch) to long piercing fangs (e.g., *Hydrolycus armatus*, vampire tetra) and forceps-like incisors (e.g., *Zanclus cornutus*, Moorish idol). The largest and smallest teeth are found in old lineages (Fig. 1a) and there is high phylogenetic signal of tooth size across the phylogeny (Pagel's $\lambda=0.94$; $P<0.0001$), suggesting that long evolutionary periods are required for significant change in tooth size.

We quantified the diversity of prey capture strategies using high-speed video to estimate the relative contribution of body ram, jaw ram, and suction to closing the gap between the fish and its prey. The kinematic diversity of feeding strikes ranged from the exclusive use of body ram (e.g., *Boulengerella maculata*; body ram relative contribution $[P_{br}]=0.998$, jaw ram relative contribution $[P_{jr}]=0.001$, suction relative contribution $[P_s]=0.001$) to a primary contribution of jaw ram (e.g., *Aulostomus maculatus*; $P_{br}=0.021$, $P_{jr}=0.938$, $P_s=0.041$) or suction (e.g., *Odontanthias borbonius*; $P_{br}=0.121$, $P_{jr}=0.428$, $P_s=0.451$) (Fig. 1a). The majority (76%) of species close the distance to the prey predominantly through forward swimming (body ram). We used phylogenetic generalized least-squares (PGLS) regression to determine relationships between tooth size and the proportions of body ram, jaw ram, and suction used during prey capture while accounting for phylogenetic relatedness. Tooth size has a significant relationship with the relative contributions of body and jaw ram but not suction (Fig. 2a). There is a strong positive relationship between tooth size and the proportion of body ram used during feeding (PGLS; $t=3.707$, $P<0.001$). As tooth size increases, species are restricted to using greater amounts of body ram to capture prey, and species with the largest teeth are almost exclusively body ram feeders (Fig. 2a). Conversely, we find a significant negative relationship between tooth size and the contribution of jaw ram (PGLS; $t=-4.999$, $P<0.001$). A reduction in tooth size

appears to be necessary for the highest use of jaw ram during prey capture, but species with reduced tooth size can still use high amounts of body ram (Fig. 2a). Extreme cases of these reductions in tooth size include the complete loss of teeth within toothed families (e.g., *Emmelichthys atlanticus* [Haemulidae]; $P_{jr}=0.678$) (34) or the evolution of edentulism (i.e., absence of teeth) in coordination with a novel form of jaw protrusion as seen in carps and minnows (Cyprinidae) (14,35). To examine the relationship between jaw protrusion and tooth size at a broader phylogenetic scale, we compared tooth size between two large clades in our study that differ in their capacity for jaw ram. Though characiformes (tetras, piranhas, headstanders), a clade that largely lacks premaxillary jaw protrusion (36,37), have 1.6x larger teeth than cichlids, which all have protrusible jaws, this difference is not significant when accounting for unequal group variances (Welch's Analysis of Variance [ANOVA]; $F=2.32$, $P=0.146$) (Fig. S2b). We find no relationship between tooth size and the relative contribution of suction (PGLS; $t=0.287$, $P=0.775$) (Fig. 2a). Though produced by most ray-finned fishes, suction often has a minor contribution to closing the predator-prey distance, relative to jaw ram and body ram. In our data, only three species (2% of all species analyzed) had a suction contribution of >50%, and 37% of species had a negligible (<10%) contribution of suction, confirming body ram-jaw ram as the primary axis of feeding diversity in fishes (24) (Fig. 1a,b). We tested the sensitivity of these results to the large sample of cichlids ($n=59$ species) in our dataset and found concordant patterns when all cichlids were removed before analysis (PGLS no cichlids; $P_{body\ ram}<0.001$, $P_{jaw\ ram}<0.001$, $P_{suction}=0.053$) (Fig. S1).

While PGLS can account for phylogenetic relatedness, proportional data is often non-linear (38). To supplement our PGLS analyses, we fit Dirichlet regression models that can effectively model non-linear relationships between proportional data with more than two

categories (38–40). Model comparison using maximum likelihood confirmed that tooth size can predict the relative contribution of body ram and jaw ram in a strike, but not of suction ($\Delta\text{AICc}=2.12$) (Table S2). As tooth size increases, the proportion of body ram used to capture prey increases while the contribution of both suction and jaw ram decreases (Fig. 3a), which follows the patterns of the PGLS results. Overall, our results show an overarching trade-off axis between different feeding modes being used by ray-finned fishes with different tooth sizes.

The functional diversity of prey capture for lineages with large teeth may be limited if large teeth constrain species to jaw ram feeding. To quantify the diversity of prey capture for multiple ranges of tooth size, we measured the variance in body ram, jaw ram, and suction contributions for 10 equal-frequency tooth-size bins. Linear regression models reveal the variances of all three components have significantly negative relationships with tooth size (body ram: $\text{df}=8$, $F=34.68$, $P<0.001$, $r^2=0.79$; jaw ram: $\text{df}=8$, $F=24.37$, $P<0.01$, $r^2=0.72$; suction: $\text{df}=8$, $F=9.283$, $P<0.05$, $r^2=0.48$) (Fig. 2b). Variance in the contribution of these components to prey capture is 3.7–8.4x lower between species with the largest and smallest teeth. These consistent linear decreases in variance across species show that the diversity of prey capture kinematics decreases as teeth become larger. We recover this strong relationship between feeding kinematics and tooth size in spite of extensive ecological diversity among large-toothed species. These include grabbing piscivores (*Hydrolycus armatus*), zooplanktivores (*Ecsenius midas*), mobile invertivores (*Rhinecanthus rectangulus*), and herbivores (*Zebrasoma flavescens*). Although having large teeth in the jaws may constrain species to body ram feeding, small teeth permit species to capture prey using a greater diversity of kinematic strategies by utilizing varied amounts of jaw ram and suction.

Models of tooth size evolution

As species that use extensive jaw protrusion to capture prey are constrained to have small teeth, we hypothesized that adaptive evolution ‘pushes’ jaw ram feeders to have smaller teeth than body ram feeders. We tested this by fitting continuous trait evolution models of tooth size evolution, after classifying species as either jaw ram or body ram feeders. Model comparison reveals that tooth size evolves under an Ornstein-Uhlenbeck (OU) process with distinct optimal tooth sizes for body and jaw ram feeders, but with the same evolutionary rate (σ^2) and strength of selection (α) ($\Delta AIC=0.74$) (Table S3; Fig. 3b). The small size of our dataset reduces the power to distinguish between models that allow σ^2 and α to vary, however there was strong support for all models that allowed distinct evolutionary optima for jaw and body ram feeders when compared to Brownian models and a single-optimum OU model (OU1; Table S3). We find a relationship where the optimum tooth size for jaw ram feeders (body size-independent tooth size [shape ratio] =0.011) is 4.1x smaller than that of body ram feeders (shape ratio=0.046) (Fig. 3b). These optima are similar to the observed tooth sizes of *Monocirrhus polyacanthus* (Amazon leaf-fish, 59.6% jaw ram) and *Gymnothorax griseus* (geometric moray eel, 99.8% body ram). We also estimated the evolutionary correlation I between jaw ram feeding and tooth size under a threshold model, in which the state of a discrete character (i.e., jaw ram feeding) changes depending on the value of a secondary character (i.e., tooth size) (41). We find a negative evolutionary correlation between increasing tooth size and feeding through jaw ram ($r=-0.436$), indicating that an antagonistic interaction defines the evolution of these two traits (Fig. S2a). Overall, our results on the evolutionary dynamics between tooth size and feeding mode reveal that tooth size affects the use of jaw protrusion during feeding. Large teeth constrain prey capture kinematics to primarily body ram feeding while the combination of small teeth and jaw protrusion represents a separate adaptive peak.

Large teeth and highly protrusible jaws are incompatible

Our results reveal that jaw protrusion and large teeth, two notable innovations in the fish feeding system, are functionally and evolutionarily incompatible with each other. Independently, these innovations have greatly expanded the trophic opportunities of fishes in their habitats (14,20,26). However, they cannot simultaneously be maximized, pointing towards a major trade-off axis and implicating a biomechanical relationship in governing the diversity of prey capture techniques in fishes. Evolution towards large teeth constrains the diversity of prey capture kinematics, requiring species to use increasingly greater proportions of body ram and thus close the gap between themselves and the prey through swimming. Our results point to adaptive evolution towards reduced tooth sizes as a crucial component in the evolution of fishes with highly protrusible jaws. However, without having large teeth, jaw ram feeders are limited in the way they can detach, manipulate, and damage food resources. This restriction may have evolutionary implications for jaw ram feeders and follows a well-known tradeoff in fishes between force and velocity-modified jaws that is strongly related to ecology (7,42–44). Species with velocity-modified jaws often use high protrusion and consume prey whole in rapid strikes with little oral manipulation or processing (21,24). In contrast, species with little to no jaw protrusion often target attached, non-evasive prey that require removal from a substrate or elusive prey that requires further restraint or damage by raptorial teeth. Considerable change in dentition and skeletal elements of the jaw system is likely required for jaw ram feeders with small teeth to undergo dietary transitions towards resources that require detachment or post-capture processing (42).

Biomechanical limits on both feeding performance and the evolution of feeding structures is a widespread feature of vertebrate evolution (45–48), suggesting there may be a

biomechanical limit on tooth size. Intraosseous (i.e., within the bone) tooth replacement is a derived state in teleosts and the predominant replacement mode among fishes in our study (e.g., Perciformes, Cichliformes, Characiformes, Acanthuriformes) (49) (Table S1). By requiring replacement teeth to develop in a crypt within the dentigerous bone (49), this process may impose a limit on tooth size. As replacement teeth cease developing, they erupt and replace the functional tooth in a “one for one” manner, joining the existing functional tooth row (49–51). However, the dentigerous bone must have enough space to accommodate these full-size replacements. This presents a dilemma for many acanthomorph species with premaxillary protrusion. Fishes with high jaw protrusion that capture prey through jaw ram typically have slender, mobile jaws adapted for rapid motions (42,52), and therefore do not have as much space for teeth to develop within the jaw bone. We suggest that the jaws of species with high jaw protrusion may impose a structural limit on tooth size based on the space available for tooth development in the dentigerous bones. In addition to this constraint, tooth size may be minimized in jaw ram feeders to avoid interference with suction forces or reduce drag during high-velocity strikes (53).

Unique adaptations to accommodate large teeth

Our results demonstrate that increased tooth sizes limit species to predominantly body ram feeding. While large teeth (in our study, the upper 10% of the tooth size distribution) may limit the kinematic diversity of feeding, they have unlocked a wide range of unique ecological niches by providing unique abilities to damage, detach, cut, and collect food resources. For fishes feeding on non-evasive resources, long teeth are a more recent (~40 mya) adaptation that unlocked new benthic resources including coral mucus and detritus (20,54). Large teeth have given rise to other specialized feeding modes such as herbivory (e.g., Siganidae, Acanthuridae)

(55), wood-eating (e.g., Loricariidae) (56), frugivory (e.g., Serrasalminae) (57,58), and exceptional feeding behaviors such as entrapping prey in a dental cage, seen in ‘wolftrap’ anglerfishes (Ceratioidei) in the deep sea (59). Despite our results pointing towards a biomechanical limit on relative tooth size, species with exceptionally large teeth (Table S1) are united by unique, independent adaptations to the modes of tooth attachment and replacement as well as modifications to the teeth themselves (Fig. 4a-d). These modifications allow large dental tools to be positioned in a comparatively small set of jaws. For example, the modified tooth replacement process of pufferfishes (Tetraodontidae) and parrotfishes (Labridae) gives rise to a beak, functionally equivalent to a single large tooth. In parrotfishes, superimposed rows of rounded teeth are cemented together in a bone-like layer (Fig. 4a) while in pufferfishes, teeth are continuously stacked together to form a compact, highly-mineralized osteodentine mass (60–62) (Fig. 4b). These modifications, which circumvent the common “one for one” replacement in fishes, enabled these species to unlock new ecological roles within their environments (63). Modification to the location of tooth attachment enables the positioning of larger teeth in the jaw. The procumbent implantation of teeth in *Zanclus cornutus* (Moorish idol) and associated horizontal tooth crypts within the jaw illustrate modifications to the orientation of tooth crypts used to accommodate larger tooth sizes (Fig. 4c). In triggerfishes (Balistidae), tooth attachment occurs between the lingual aspect of the tooth and labial aspect of the jaw with modified connective tissue (64), which provides a larger attachment surface (Fig. 4d). An extreme case of modification to the structure of teeth themselves is found in some butterflyfishes (Chaetodontidae), which have highly flexible tooth shafts, a feature similar to the teeth of some loricariid catfishes (65). Overall, while the reduction of tooth size may be achieved relatively simplistically through conserved developmental pathways (66), coordinated changes within the

tooth developmental program, as well as major changes to the bone morphology of the jaws and skull, may be required for extreme increases in tooth size (Fig. 4a-d).

Conclusion

Our results demonstrate a fundamental incompatibility between two functional innovations in fish feeding, which separately contributed to the success of fishes in aquatic environments by enabling new prey capture strategies and consequently, altered ecosystem processes (12,20,26). We use high-speed videos to describe the kinematic diversity of fish feeding, finding strong relationships between tooth size and the major axis of prey capture diversity in fishes (24). Evolutionary model fitting reveals that adaptive evolution towards small teeth is a feature of jaw ram feeders with negative implications for resource acquisition abilities (42,67). Enlarged teeth facilitate novel trophic habits but limit kinematic diversity while small teeth permit extensive kinematic diversity yet limit resource processing abilities. The evolution of large teeth may highlight major transitions in the history of fishes where despite restricting kinematic diversity and requiring major modifications, it allowed them to explore novel niches and previously inaccessible resources in competitive habitats, such as coral reefs. While feeding innovations may profoundly expand the trophic opportunities of fishes, underlying trade-offs can impose functional and evolutionary incompatibilities between these traits, preventing simultaneous expression of multiple innovations.

Methods

Ethics Statement

All experiments were carried out in accordance with the University of California, Davis Institutional Animal Care and Use Committee, protocol numbers 22206 and 23818.

High-speed video

During feeding events, fish close the distance between themselves and prey through the use of three mechanisms: 1) they draw the prey towards their mouth using suction, 2) they move their body toward the prey by swimming (body ram), or 3) they extend their mouth toward the prey using jaw protrusion or flexion of the body (jaw ram) (24,25). We quantified the proportions of these three movements (body ram, jaw ram, suction) to closing the total distance between predator and prey during feeding events of 161 species of ray-finned fishes (Actinopterygii) across 66 families (Table S1). 140 species were filmed for this study and data for 21 species was taken from Longo et al., (2016). Individuals were fed a variety of foods to elicit strikes, including brine shrimp, live black worms, or live feeder fish (*Gambusia affinis*); we acknowledge that incorporating a variety of prey types may introduce some variation in the relative use of ram and suction during the strike. Fish were filmed in 110–210-liter aquaria, depending on their size.

Individuals were recorded feeding using digital high-speed cameras (Fastec Imaging, High Spec 2G or Photron FastCam Mini) at 1000-2000 frames per second (fps). Multiple strikes were recorded for each individual, and the single best strike (i.e., limited movement of the prey and lateral positioning of the fish relative to the camera throughout the strike) was used for further analysis. All sequences were chosen by a single observer (N.P.) to minimize bias in the

choice of strike for analysis. Following filming, each specimen was euthanized with overexposure to a solution of MS-222 and fixed in buffered formalin. For each sequence, we extracted two frames using the software ImageJ (68); (1) the onset of the strike, and (2) the time at which the prey's estimated center of mass passed through the mouth of the predator. We determined the onset of the strike to be at the start of depression of the buccal cavity, cranial elevation, or acceleration towards the prey, which varied between species.

Quantifying kinematic proportions

To quantify the proportions of each kinematic component used in each strike, we rotated and aligned the two frames for each strike in Adobe Illustrator (Adobe Inc.), so that the strike occurred along the x-axis of the aligned frames. To quantify body ram, we measured the distance between the center of the eye between frames. Jaw ram was quantified as the length of the protruded jaw in the 2nd frame, measured from the tip of the maxilla, which was often the point of insertion of the premaxillary ascending process, to the anteriormost point of the upper jaw. This measurement includes a negligible constant (compared to the contribution of other kinematic components) for each species, which represents the distance between these points in a closed jaw system. Suction was measured as the horizontal distance travelled toward the predator by the center of mass of the prey item between the two frames. Each measurement was then converted to a proportion of the total movement (body ram + jaw ram + suction).

Measuring tooth size

We extracted the single largest tooth of each individual under a dissecting scope, mounted each tooth flat on a slide with double sided tape, and photographed it with a microscope camera (Hangzhou ToupTek Photonics Co., Ltd). We measured tooth size as the linear distance

between the midpoint of the base of the tooth and the tip of the cusp using the software ToupView. To capture the size of the individual, we measured standard length (SL), upper jaw length (JL; measured from the tip of the premaxilla to the end of the maxilla), and jaw width (JW; between ends of the maxilla) using dial calipers. We size-corrected tooth length using log shape ratios, using the geometric mean of SL, JL, and JW to represent body size (69). We $+0.1$ transformed all raw measurements of tooth length before calculating the geometric mean to accommodate edentulate species (tooth length = 0mm). This method of size correction accounts for the wide range of body shapes and relative jaw sizes in our dataset. Two substitutions were made based on specimen availability: *Rhinecanthus verrucosus* (tooth size measured) for *Rhinecanthus rectangulus* (filmed), and *Pseudobalistes flavimarginatus* (tooth size measured) for *Pseudobalistes fuscus* (filmed).

Quantification and statistical analysis

We conducted all analyses in R v4.2.2 (R Core Team, 2022). For all phylogenetic analyses, we used the DNA sequence based phylogeny of Rabosky et al. (2018), pruned to include the species in our study (n=161 species). We substituted *Dormitator latifrons* for *Dormitator lebretonsis* (filmed in this study), as *D. lebretonsis* was absent from the molecular phylogeny. First, we applied an arcsine transformation to the proportions of body ram, jaw ram, and suction to improve normality. To examine the relationship between the contribution of each component and tooth size, we used phylogenetic generalized least squares regression (PGLS) implemented with the ‘*gls*’ function in the package ‘*nlme*’ (v.3.1-160) (71). We accounted for phylogenetic relatedness by setting a correlation structure under Brownian motion (‘*corBrownian*’ in the package ‘*ape*’ (v.5.8) (72)). For each model, we estimated the variance explained by tooth size by calculating r^2 for predication (r^2_{pred}) in the package ‘*rr2*’ (v.1.1.1)

(73,74). To test the sensitivity of our results to our sampling bias towards Cichlidae (n=59 species), we repeated these analyses after excluding all cichlids.

We supplemented our PGLS analyses with Dirichlet regression using the ‘*DirichletReg*’ package (v.0.7-1) (40). Although this approach does not account for phylogenetic relationships, it can model non-linear relationships between tooth size and all three kinematic proportions together, providing an advantage when used together with PGLS. We compared the fits of six models using AICc scores (for a description of the models, see Table S2). The significance of the PGLS analyses suggests that while tooth size alone may have high phylogenetic signal, its relationship with body and jaw ram is much less dependent on phylogenetic relationships. Therefore, we would not expect the general patterns or significance of the Dirichlet regression results to change if phylogeny was included. To test for a relationship between the diversity of kinematic proportions used during feeding and tooth size, we first discretized species into 10 equal-frequency (n=16) bins (one bin n=17) based on tooth size using the package ‘*discretize*’ function in the ‘*arules*’ package (v.1.7.8) (75). For each bin, we calculated the mean tooth size. We then fit linear regression models with the ‘*lm*’ function in the ‘*stats*’ package (v.4.2.2), where variance is a function of tooth size. We then calculated r^2_{pred} for each regression to estimate the variance explained.

To understand how tooth size evolves across ray-finned fishes, we fit both Brownian motion (BM) and Ornstein-Uhlenbeck (OU) models of trait evolution in ‘*Ouwie*’ (v.2.10) (76). We categorized species as either body or jaw ram feeders (a binary trait) based on the highest contribution, ignoring suction, given our initial results. For example, if a species’ contributions were br=0.25, jr=0.35, and s=0.4, that species was categorized as a jaw ram feeder. We removed suction for this categorization as we found no relationship between tooth size and suction, and

only a small number of species fed predominantly through suction. We generated 100 stochastic character maps under an equal-rates (ER) transition model using ‘*make.simap*’ in ‘*phytools*’ (v.2.1.1) (77) to account for uncertainty in the history of body and jaw ram feeding. We then fit seven trait evolution models (Table S3) across all 100 simmaps and compared models using the mean AIC scores. The small size of our dataset reduces the power to distinguish between models that allow the evolutionary rate (σ^2) and strength of selection (α) to vary, however there was strong support for all models that allowed distinct evolutionary optima for jaw and body ram feeders when compared to Brownian models and a single-optimum OU model (OU1; Table S3). We fit an additional threshold model of trait evolution in a Bayesian framework using the ‘*threshBayes*’ function in ‘*phytools*’ to evaluate the strength of evolutionary covariation [®] between tooth size and jaw ram feeding (41). The Markov chain Monte Carlo (MCMC) was run for 500,000 generations, sampling every 500 generations, and we removed the first 10% of samples as burn-in. We estimated phylogenetic signal in our tooth size data using ‘*phylosig*’ in ‘*phytools*’ (method= “lambda”). We compared tooth size between two large clades in our study, cichlids (n=59 species) and characiformes (n=16 species) using Welch’s ANOVA in the R package ‘*rstatix*’ (v.0.7.2) (78) to account for unequal sample variances (Levene’s test; df=74, F=10.036, $P<0.01$).

Figures and Tables

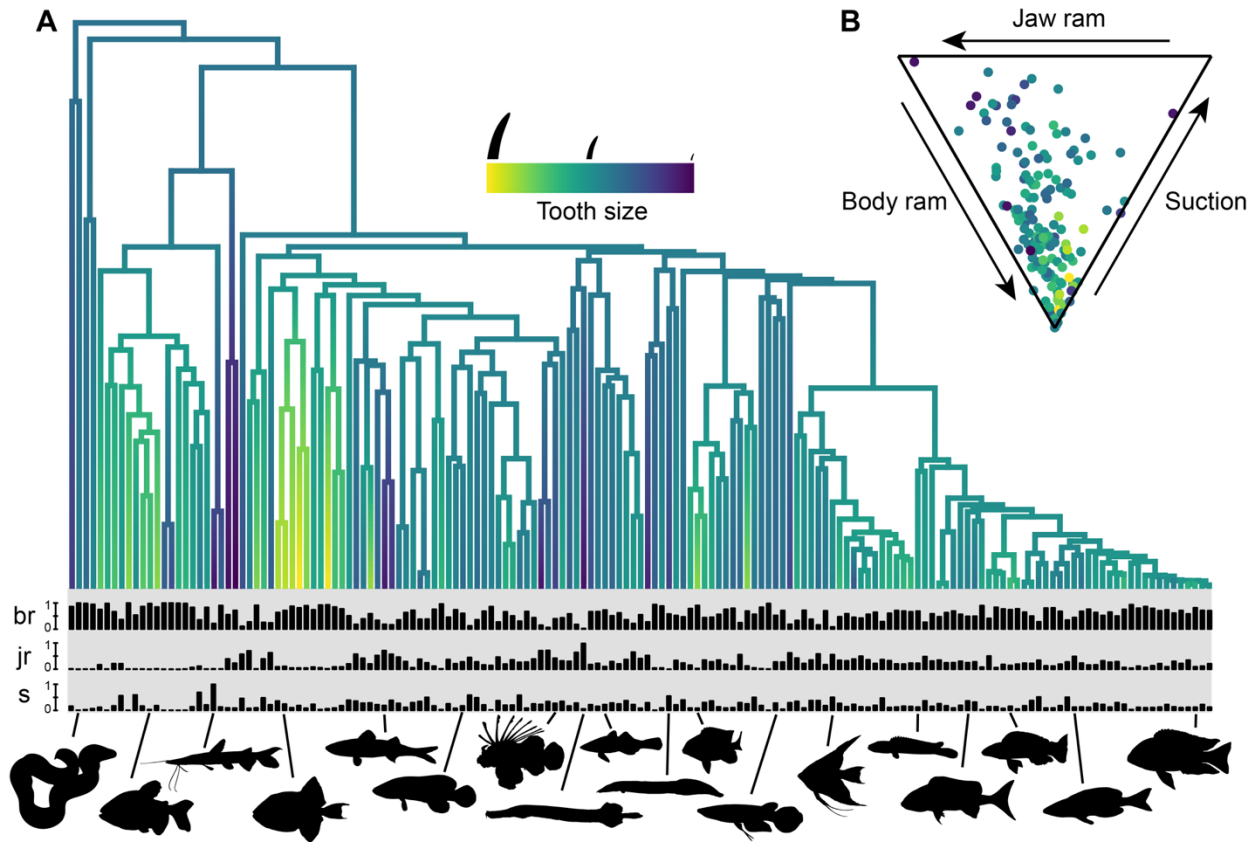


Figure 1 – The diversity of tooth sizes and feeding kinematics across ray-finned fishes.

The phylogenetic distribution of tooth size across 161 species of ray-finned fishes. Barplots at the tips correspond to the proportion of body ram (br), jaw ram (jr), and suction (s) for each species. Species (from L to R): *Gymnothorax griseus*, *Piaractus brachipomus*, *Sorubim lima*, *Pseudobalistes fuscus*, *Emmelichthyops atlanticus*, *Rypticus maculatus*, *Pterois volitans*, *Aulostomus maculatus*, *Butis butis*, *Mastacembelus armatus*, *Microspathodon chrysurus*, *Aplocheilichthys lineatus*, *Pterophyllum scalare*, *Teleogramma brichardi*, *Boulengerichromis microlepis*, *Julidochromis dickfeldi*, *Cyprichromis leptosoma*, *Chilotilapia rhoadesii*. **B)** Ternary plot depicting the combined proportions of body ram, jaw ram, and suction; each point represents

a species, and the color of the points corresponds to tooth size. The data underlying this figure can be found in S1 Data and S2 Data.

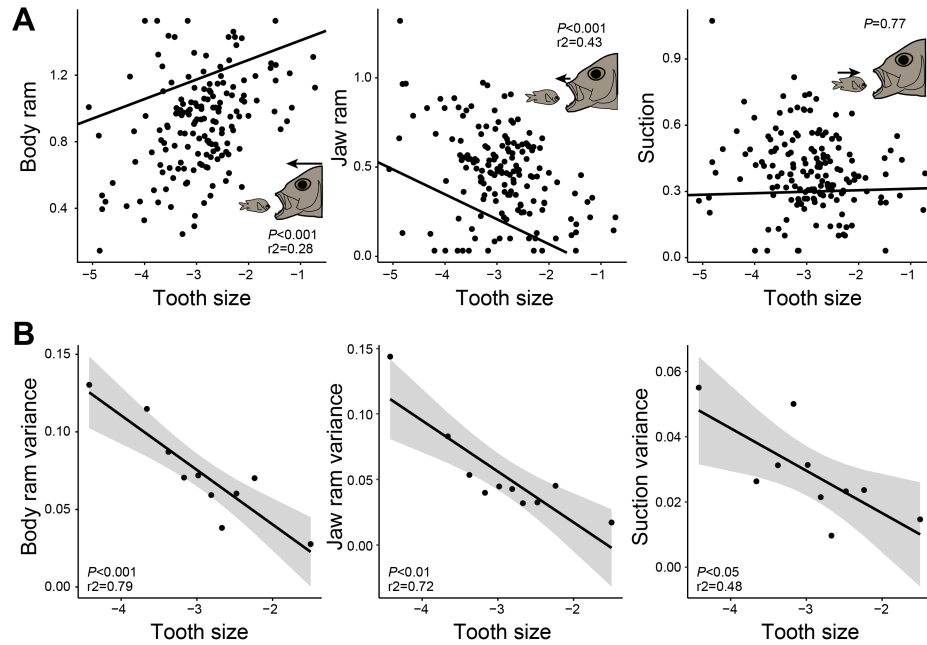


Figure 2 – The relationship between prey capture kinematics and tooth size in ray-finned fishes. A) Phylogenetic generalized least-squares (PGLS) regressions between tooth size, quantified as a log-shape ratio, and the arcsine-transformed proportions of body ram, jaw ram, and suction. P -values and r^2_{Pred} are reported for each regression. **B)** Linear regressions between kinematic variance and tooth size, for body ram, jaw ram, and suction. 95% confidence intervals are depicted in grey. Each point corresponds to the variance quantified from 10 equal-frequency bins, based on tooth size. The data underlying this figure can be found in S1 Data and S2 Data.

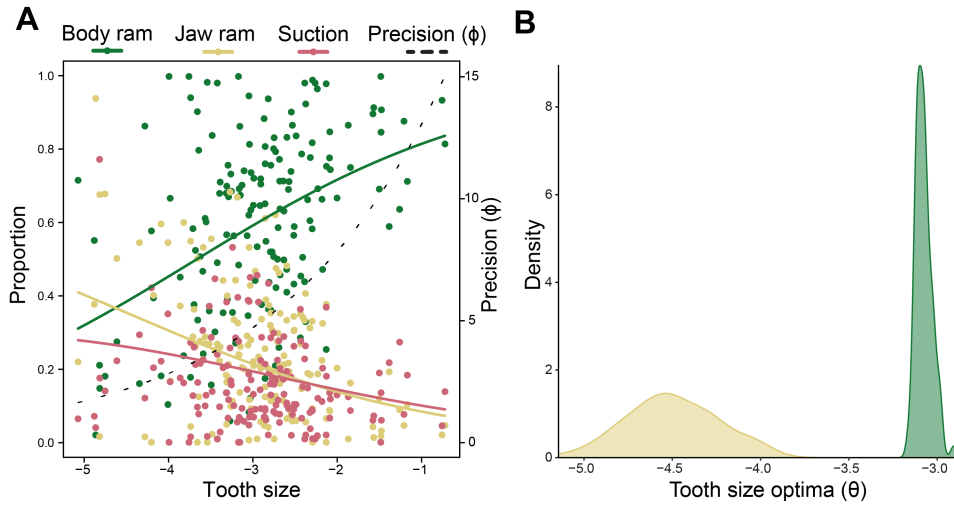


Figure 3 – Large teeth and highly protrusible jaws are evolutionarily incompatible.

Dirichlet regression between the proportions of body ram (green), jaw ram (yellow), and suction (red) used in a strike, and tooth size, quantified as a log-shape ratio. Precision (ϕ) is plotted on the second y-axis (dotted line). **B)** Distribution of optimal values for log-transformed tooth size (θ) between body ram (green) and jaw ram (yellow) feeders under a multiple optima Ornstein-Uhlenbeck model with single σ^2 and α parameters (OUM), based off 100 model fits. The data underlying this figure can be found in S1 Data and S3 Data.

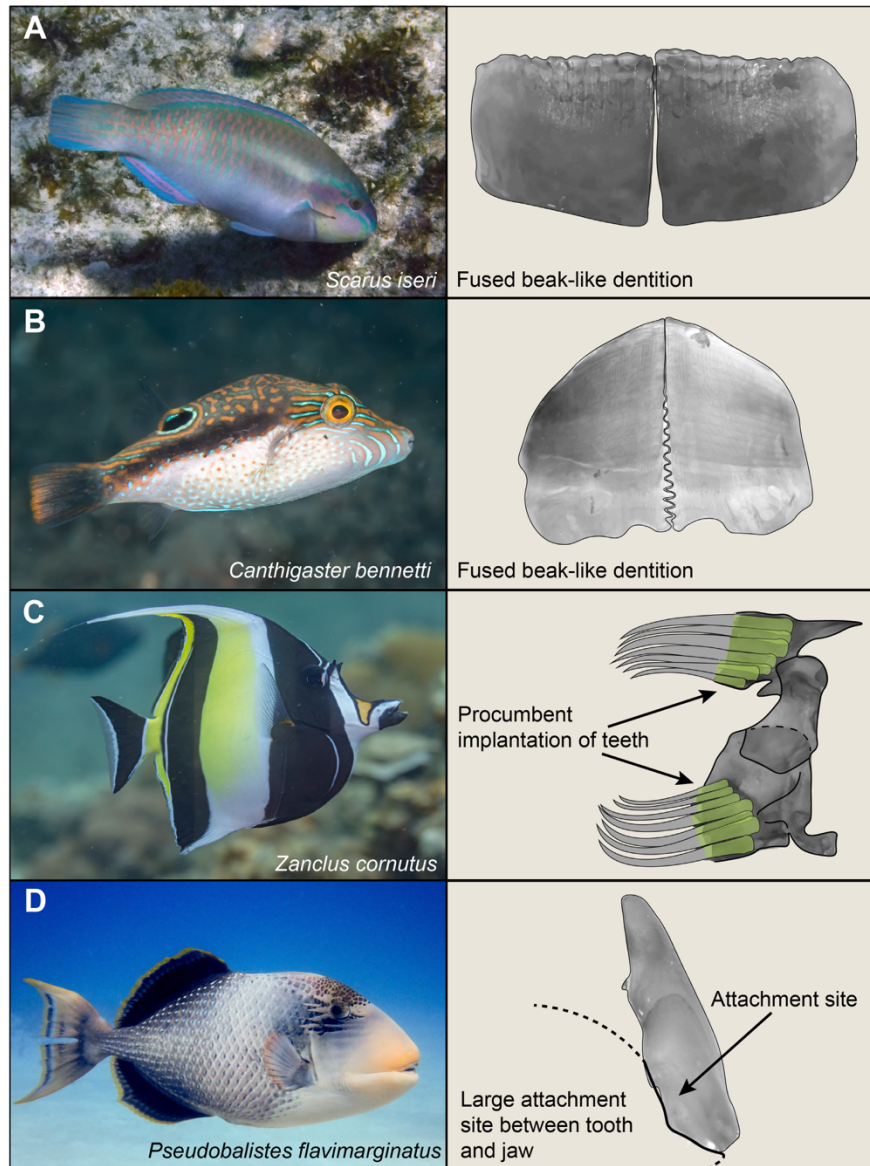
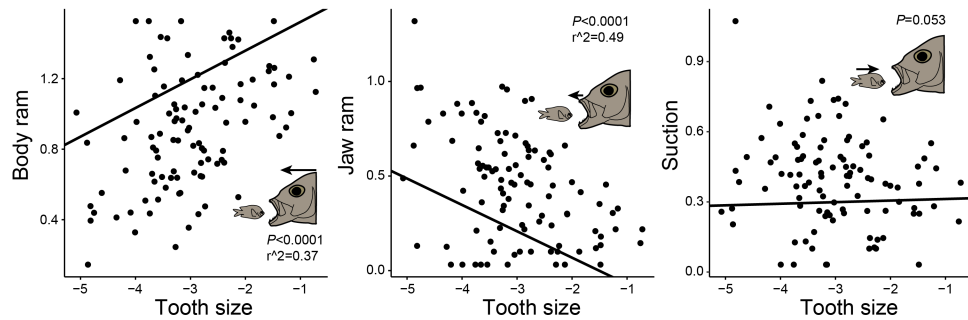


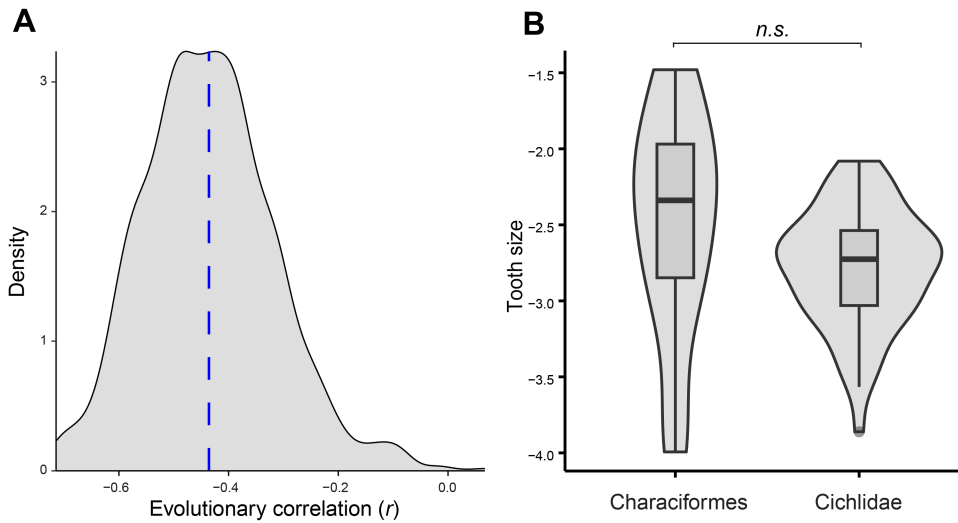
Figure 4 – Extreme morphological adaptations to accommodate large teeth in fish jaws.

Depicted are species from the upper 10% of our dataset on tooth size, which have all evolved unique modifications to accommodate large teeth in their jaws. **A)** Upper jaw of *Scarus iseri*, in labial view. Photo credit Louis Imbeau, [iNaturalist](#) (CC BY 4.0). **B)** Lower jaw of *Canthigaster bennetti*, in labial view, showing the fused beak. Photo credit Rickard Zerpe, [Flickr](#) (CC BY 2.0). **C)** Lateral view of *Zanclus cornutus* upper jaw showing the procumbent implantation of teeth. The arrow indicates the location of a horizontal tooth crypt. Photo credit Laszlo Ilyes, [Flickr](#) (CC

[BY 2.0](#)). **D)** Lateral view of teeth from the lower jaw of *Pseudobalistes flavimarginatus*. The arrows indicate large attachment sites on the lingual aspect of the tooth. Photo credit Rickard Zerpe, [Flickr](#) (CC BY 2.0).



S1 Fig. The relationship between prey capture kinematics and tooth size in ray-finned fishes, after removing all Cichlidae species. Phylogenetic generalized least-squares (PGLS) regressions between tooth size, quantified as a log-shape ratio, and the arcsine-transformed relative contributions of body ram, jaw ram, and suction, for 102 species of ray-finned fishes, after removing all 59 species of cichlids. P -values and r^2_{Pred} are reported for each regression. The data underlying this figure can be found in S1 Data and S2 Data.



S2 Fig. Evolutionary relationships between tooth size and jaw protrusion. **A)** Posterior distribution of the evolutionary correlation coefficient r between tooth size and body and jaw ram feeding, estimated under a threshold model. The blue dotted line indicates the mean of the distribution. **B)** Clade comparisons of tooth size between Characiformes (no premaxillary protrusion) and Cichlidae (premaxillary protrusion). Significance is reported from Welch's Analysis of Variance (ANOVA) test. The data underlying this figure can be found in S1 Data and S4 Data.

S1 Table. List of taxa included in this study. A complete list of taxa included in this study.

Species with exceptionally large teeth, defined as the upper 10% of the tooth size distribution, are marked with a (*).

Order	Family	Species
Acanthuriformes	Acanthuridae	<i>Acanthurus lineatus</i>
		<i>Zebrasoma flavescens</i>
	Chaetodontidae	<i>Chaetodon lunula</i> *
		<i>Chelmon rostratus</i>
	Haemulidae	<i>Emmelichthyops atlanticus</i>
		<i>Haemulon vitattum</i>
	Lobotidae	<i>Datnoides microlepis</i>
	Malacanthidae	<i>Hoplolatilus purpureus</i>
	Scatophagidae	<i>Scatophagus argus</i>
	Siganidae	<i>Siganus vulpinus</i>
	Zanclidae	<i>Zanclus cornutus</i> *
Anabantiformes	Osphronemidae	<i>Osphronemus laticlavius</i>
Anguilliformes	Muraenidae	<i>Gymnothorax griseus</i>
Atheriniformes	Melanotaeniidae	<i>Melanotaenia goldiei</i>
Batrachoidiformes	Batrachoididae	<i>Opsanus beta</i>
Beloniformes	Belonidae	<i>Xenentodon cancila</i>
Blenniiformes	Blenniidae	<i>Ecsenius midas</i> *
	Chaenopsidae	<i>Chaenopsis alepidota</i>
		<i>Plesiops coeruleolineatus</i>
	Plesiopidae	<i>Paraplesiops poweri</i>

	Pomacentridae	<i>Chromis cyanea</i>
		<i>Chromis viridis</i>
		<i>Hypsypops rubidundus</i>
		<i>Abudefduf saxatilis</i>
		<i>Stegastes fuscus</i>
		<i>Microspathodon chrysurus*</i>
Carangiformes	Carangidae	<i>Trachinotus blochii</i>
	Latidae	<i>Lates niloticus</i>
	Sphyraenidae	<i>Sphyraena barracuda</i>
Centrarchiformes	Centrarchidae	<i>Lepomis macrochirus</i>
		<i>Lepomis gibbosus</i>
	Cirrhitidae	<i>Oxycirrhites typus</i>
		<i>Cirrhitis pinnulatus</i>
		<i>Paracirrhites arcatus</i>
	Kyphosidae	<i>Kyphosus sectatrix</i>
	Terapontidae	<i>Terapon jarbua</i>
Cypriniformes	Cyprinidae	<i>Neolissochilus stracheyi</i>
		<i>Leptobarbus hoevenii</i>
Cyprinodontiformes	Aplocheilidae	<i>Aplocheilus lineatus</i>
	Poeciliidae	<i>Poecilia latipinna</i>
Cichliformes	Cichlidae	<i>Altolamprologus compressiceps</i>
		<i>Aristochromis christyi</i>
		<i>Astatotilapia burtoni</i>
		<i>Astatoreochromis alluaudi</i>
		<i>Astronotus ocellatus</i>
		<i>Asprotilapia leptura</i>

	<i>Bathybates minor</i>
	<i>Buccochromis nototaenia</i>
	<i>Caquetaia krausii</i>
	<i>Chalinochromis brichardi</i>
	<i>Chilotilapia rhoadesii</i>
	<i>Cincolichthys bocourti</i>
	<i>Copadichromis borleyi</i>
	<i>Crenicichla regani</i>
	<i>Cyprichromis leptosoma</i>
	<i>Dimidiochromis compressiceps</i>
	<i>Ectodus descampsii</i>
	<i>Enterochromis paropus</i>
	<i>Etroplus maculatus</i>
	<i>Geophagus abalios</i>
	<i>Gnathochromis pfefferi</i>
	<i>Greenwoodochromis bellcrossi</i>
	<i>Harpagochromis "orange rock hunter"</i>
	<i>Heros severus</i>
	<i>Hemichromis elongatus</i>
	<i>Herichthys minckleyi</i>
	<i>Julidochromis dickfeldi*</i>
	<i>Labidochromis caeruleus</i>
	<i>Labeotropheus trewavasae</i>
	<i>Lepidiolamprologus elongatus</i>
	<i>Lepidiolamprologus nkambe</i>
	<i>Lepidiolamprologus mimicus</i>
	<i>Limnotilapia dardenii</i>
	<i>Limnochromis auritus</i>
	<i>Limnochromis abeelei</i>
	<i>Maylandia callainos</i>
	<i>Myaka myaka</i>

		<i>Neochromis rufocaudalis</i>
		<i>Nimbochromis venustus</i>
		<i>Orthochromis stormsi</i>
		<i>Parachromis managuensis</i>
		<i>Paratilapia polleni</i>
		<i>Paraneetroplus gibbiceps</i>
		<i>Parachromis dovii</i>
		<i>Pelvicachromis pulcher</i>
		<i>Petenia splendida</i>
		<i>Perissodus microelpis</i>
		<i>Pterophyllum scalare</i>
		<i>Pundamilia pundamilia</i>
		<i>Pyxichromis orthostoma</i>
		<i>Rhamphochromis longiceps</i>
		<i>Simochromis babaulti</i>
		<i>Teleogramma brichardi</i>
		<i>Trematocara variabile</i>
		<i>Trichromis salvini</i>
		<i>Tropheus moorii</i>
		<i>Tyrannochromis nigriventer</i>
		<i>Xenotilapia rotundiventralis</i>
		<i>Monocirrhus polyacanthus</i>
Characiformes	Acestroryhnchidae	<i>Acestrorynchus microelpis</i>
	Alestidae	<i>Hydrocynus vittatus*</i>
	Anostomidae	<i>Leporinus fasciatus*</i>
	Characidae	<i>Charax gibbosus</i>
		<i>Exodon paradoxus</i>
		<i>Gymnocorymbus ternetzi</i>
	Chilodontidae	<i>Chilodus punctatus</i>
	Chalceidae	<i>Chalceus erythrurus</i>

	Ctenoluciidae	<i>Ctenolucius hujeta</i>
		<i>Boulengerella maculata</i>
	Cynodontidae	<i>Hydrolycus armatus*</i>
	Distichodontidae	<i>Distichodus sexfasciatus*</i>
	Hemiodontidae	<i>Hemiodus gracilis</i>
	Hepsetidae	<i>Hepsetus odoe</i>
	Serrasalminae	<i>Piaractus brachipomus*</i>
		<i>Metynnis argenteus</i>
Elopiiformes	Megalopidae	<i>Megalops cyprinoides</i>
Gobiiformes	Apogonidae	<i>Glossamia aprion</i>
	Butidae	<i>Butis butis</i>
		<i>Oxyleotris marmorata</i>
	Eleotridae	<i>Dormitator lebretonsis</i>
	Gobiidae	<i>Stigmatogobius pleurostigma</i>
	Microdesmidae	<i>Ptereleotris microlepis</i>
		<i>Ptereleotris evides</i>
		<i>Nemateleotris magnifica</i>
Labriiformes	Labridae	<i>Epibulus insidiator</i>
		<i>Coris formosa</i>
		<i>Scarus iseri*</i>
Lophiiformes	Antennariidae	<i>Antennarius commerson</i>
Osteoglossiformes	Mormyridae	<i>Mormyrus longirostris</i>
	Osteoglossidae	<i>Osteoglossum bicirrhosum</i>
Perciformes	Anthiidae	<i>Odontanthias borbonius</i>
		<i>Pseudanthias dispar</i>

	Caesionidae	<i>Pterocaesio pisang</i>
	Epinephelidae	<i>Paranthias furcifer</i>
		<i>Cephalopholis urodeta</i>
		<i>Epinephelus ongus</i>
		<i>Cromileptes altivelis</i>
		<i>Cephalopholis miniata</i>
		<i>Plectropomus laevis</i>
	Gasterosteidae	<i>Gasterosteus aculeatus</i>
	Grammistidae	<i>Rypticus maculatus</i>
	Liopropomatidae	<i>Liopropoma rubre</i>
	Serranidae	<i>Serranus annularis</i>
		<i>Hypoplectrus puella</i>
		<i>Serranus tigrinus</i>
	Scorpaenidae	<i>Inimicus didactylus</i>
		<i>Synanceia verrucosa</i>
		<i>Pterois volitans</i>
Siluriformes	Pimelodidae	<i>Sorubim lima</i>
		<i>Pimelodus pictus</i>
Synbranchiformes	Mastecembelidae	<i>Mastecembelus armatus</i>
Syngnathiformes	Aulostomidae	<i>Aulostomus maculatus</i>
	Callionymidae	<i>Dactylopus dactylopus</i>
	Mullidae	<i>Parupeneus cyclostomus</i>
Tetraodontiformes	Balistidae	<i>Pseudobalistes fuscus*</i>
		<i>Rhinecanthus rectangularis*</i>
	Tetraodontidae	<i>Canthigaster bennetti*</i>
		<i>Sphoeroides spengleri*</i>

S2 Table. Dirichlet regression model fitting results. Dirichlet regression model fitting using the ‘*DirichReg*’ function in the R package ‘*DirichletReg*’. ‘dr’ is the compositional data of the proportions of body ram, jaw ram, and suction, and is tooth size, corrected for body size. AIC scores, corrected for small sample size, are reported. The best-fit model is bold.

Model	Formula	Parameterization	AICc
model 1 (null)	$\text{dr} \sim 1 \mid 1 \mid 1$	common	-397.1422
model 2	$\text{dr} \sim s$	common	-441.4276
model 3	$\text{dr} \sim s \mid 1$	alternative	-414.4703
model 4	$\text{dr} \sim s + s^2$	alternative	-410.6331
model 5	$\text{dr} \sim s + s^2 \mid s + s^2 \mid s + s^2$	common	-439.3474
model 6	$\text{dr} \sim 1 \mid s$	alternative	-401.8757

S3 Table. Ornstein-Uhlenbeck model fitting results. Results of continuous trait evolution model fitting with ‘*Ouwie*’. AIC scores, corrected for small sample size, are reported as the mean of 100 fitted models. The best-fit model (OUM) is identified in bold. Parameters refer to the Brownian rate parameter (σ^2), strength of selection (α) and location of the adaptive peak (θ).

Model	Parameters	AICc
BM1	single σ^2	357.52
BMS	multiple σ^2	359.17
OU1	single σ^2 , single α , single θ	342.25
OUM	single σ^2, single α, multiple θ	335.32
OUMV	multiple σ^2 , single α , multiple θ	336.84
OUMA	single σ^2 , multiple α , multiple θ	336.06
OUMVA	multiple σ^2 , multiple α , multiple θ	337.27

References

1. Miller AH, Stroud JT, Losos JB. The ecology and evolution of key innovations. *Trends in Ecology & Evolution*. 2023 Feb;38(2):122–31.
2. Hunter JP. Key innovations and the ecology of macroevolution. *Trends in Ecology & Evolution*. 1998 Jan;13(1):31–6.
3. Peoples N, Burns MD, Mihalitsis M, Wainwright PC. Evolutionary lability of a key innovation spurs rapid diversification. *Nature*. 2025 Feb 26;639(8056):962–7.
4. Liem KF. Evolutionary Strategies and Morphological Innovations: Cichlid Pharyngeal Jaws. *Systematic Zoology*. 1973 Dec;22(4):425–41.
5. Roberts-Hughes AS, Martinez CM, Corn KA, Wainwright PC. A classic key innovation constrains oral jaw functional diversification in fishes. *Evolution Letters*. 2024 Oct 8;qrae046.
6. Wainwright PC, Price SA. The Impact of Organismal Innovation on Functional and Ecological Diversification. *Integr Comp Biol*. 2016 Sep;56(3):479–88.
7. Burress ED, Muñoz MM. Functional Trade-Offs Asymmetrically Promote Phenotypic Evolution. Thomson R, editor. *Systematic Biology*. 2023 May 19;72(1):150–60.
8. Schelp MI, Burress ED. Biomechanical Specialization Acts as an Asymmetrical Constraint on the Phenotype. *Integrative Organismal Biology*. 2025 Jan 21;7(1):obaf013.
9. Muñoz MM, Hu Y, Anderson PSL, Patek S. Strong biomechanical relationships bias the tempo and mode of morphological evolution. *eLife*. 2018 Aug 9;7:e37621.
10. Zhou Z, Li FZZ. A new Lower Cretaceous bird from China and tooth reduction in early avian evolution. *Proc R Soc B*. 2010 Jan 22;277(1679):219–27.
11. Louchart A, Viriot L. From snout to beak: the loss of teeth in birds. *Trends in Ecology & Evolution*. 2011 Dec;26(12):663–73.
12. Wainwright PC, Longo SJ. Functional Innovations and the Conquest of the Oceans by Acanthomorph Fishes. *Current Biology*. 2017 Jun;27(11):R550–7.
13. Schaeffer B, Rosen DE. Major adaptive levels in the evolution of the actinopterygian feeding system. *Am Zool*. 1961 May;1(2):187–204.
14. Motta PJ. Mechanics and Functions of Jaw Protrusion in Teleost Fishes: A Review. *Copeia*. 1984 Feb 23;1984(1):1–18.
15. Lauder GV. Patterns of Evolution in the Feeding Mechanism of Actinopterygian Fishes. *Am Zool*. 1982 May;22(2):275–85.
16. Mihalitsis M, Bellwood D. Functional implications of dentition-based morphotypes in piscivorous fishes. *R Soc open sci* [Internet]. 2019 Sep 27 [cited 2024 Jan 17];6(9). Available from: <https://royalsocietypublishing.org/doi/10.1098/rsos.190040>
17. Ebert M, Kölbl-Ebert M, Lane JA. Fauna and Predator-Prey Relationships of Ettling, an Actinopterygian Fish-Dominated Konservat-Lagerstätte from the Late Jurassic of Southern Germany. Friedman M, editor. *PloS ONE*. 2015 Jan 28;10(1):e0116140.
18. Botella H, Blom H, Dorka M, Ahlberg PE, Janvier P. Jaws and teeth of the earliest bony fishes. *Nature*. 2007 Aug;448(7153):583–6.
19. Anderson PSL, Westneat MW. Feeding mechanics and bite force modelling of the skull of *Dunkleosteus terrelli*, an ancient apex predator. *Biol Lett*. 2007 Feb 22;3(1):77–80.
20. Bellwood DR, Hoey AS, Bellwood O, Goatley CHR. Evolution of long-toothed fishes and the changing nature of fish–benthos interactions on coral reefs. *Nat Commun*. 2014 Jan 15;5(1):3144.
21. Mihalitsis M, Bellwood DR. Functional groups in piscivorous fishes. *Ecology and Evolution*. 2021 Sep;11(18):12765–78.

22. Muruga P, Bellwood DR, Mihalitsis M. Forensic odontology: Assessing bite wounds to determine the role of teeth in piscivorous fishes. *Integrative Organismal Biology*. 2022 Apr 29;4(1):obac011.
23. Bakaev AS, Bulanov VV, Kogan I, Johanson Z, Minikh AV. Early ray-finned herbivores: the dental system of the Eurynotoidiidae (Actinopterygii; middle-late Permian, European Russia) and implications for palaeobiology and palaeoecology. *Palaeontology*. 2024;67(3):e12700.
24. Longo SJ, McGee MD, Oufiero CE, Waltzek TB, Wainwright PC. Body ram, not suction, is the primary axis of suction feeding diversity in spiny-rayed fishes. *Journal of Experimental Biology*. 2016;219:119–28.
25. Norton SF, Brainerd EL. Convergence in the Feeding Mechanics of Ecomorphologically Similar Species in the Centrarchidae and Cichlidae. *Journal of Experimental Biology*. 1993 Mar 1;176(1):11–29.
26. Bellwood DR, Goatley CHR, Bellwood O, Delbarre DJ, Friedman M. The Rise of Jaw Protrusion in Spiny-Rayed Fishes Closes the Gap on Elusive Prey. *Current Biology*. 2015 Oct;25(20):2696–700.
27. Wainwright PC, McGee MD, Longo SJ, Patricia Hernandez L. Origins, Innovations, and Diversification of Suction Feeding in Vertebrates. *Integrative and Comparative Biology*. 2015 Jul 1;55(1):134–45.
28. Camp AL, Olsen AM, Hernandez LP, Brainerd EL. Fishes can use axial muscles as anchors or motors for powerful suction feeding. *Journal of Experimental Biology*. 2020 Sep 15;223(18):jeb225649.
29. Day SW, Higham TE, Cheer AY, Wainwright PC. Spatial and temporal patterns of water flow generated by suction-feeding bluegill sunfish *Lepomis macrochirus* resolved by Particle Image Velocimetry. *Journal of Experimental Biology*. 2005 Jul 15;208(14):2661–71.
30. Holzman R, Day SW, Mehta RS, Wainwright PC. Jaw protrusion enhances forces exerted on prey by suction feeding fishes. *J R Soc Interface*. 2008 Dec 6;5(29):1445–57.
31. Gibb AC, Ferry-Graham L. Cranial movements during suction feeding in teleost fishes: Are they modified to enhance suction production? *Zoology*. 2005 Jun;108(2):141–53.
32. Staab KL, Holzman R, Hernandez LP, Wainwright PC. Independently evolved upper jaw protrusion mechanisms show convergent hydrodynamic function in teleost fishes. *Journal of Experimental Biology*. 2012 May 1;215(9):1456–63.
33. Evans AJ, Naylor ER, Lujan NK, Kawano SM, Hernandez LP. Deploy the proboscis!: Functional morphology and kinematics of a novel form of extreme jaw protrusion in the hingemouth, *Phractolaemus ansorgii* (Gonorynchiformes). *Journal of Anatomy*. 2024 Jun;244(6):929–42.
34. Schultz LP. *Emmelichthyops atlanticus*, a new genus and species of fish (family Emmelichthyidae) from the Bahamas, with a key to related genera. *Journal of the Washington Academy of Sciences*. 1945;35(4):132–6.
35. Patricia Hernandez L, Bird NC, Staab KL. Using zebrafish to investigate cypriniform evolutionary novelties: functional development and evolutionary diversification of the kinethmoid. *J Exp Zool Pt B*. 2007 Sep 15;308B(5):625–41.
36. Vari RP. A new species of Bivibranchia (Pisces: Characiformes) from Surinam, with comments on the genus. *Proceedings of the Biological Society of Washington*. 1985;98(2):511–22.
37. Gery J. L'appareil protracteur buccal de Bivibranchia (Characoidei) (I) avec une note sur *Phractolaemus* (Chanoidei) (Pisces). *Vie Milieu*. 1962;13:729–40.
38. Douma JC, Weedon JT. Analysing continuous proportions in ecology and evolution: A practical introduction to beta and Dirichlet regression. Warton D, editor. *Methods Ecol Evol*. 2019 Sep;10(9):1412–30.

39. Maier MJ. DirichletReg: Dirichlet Regression for Compositional Data in R. Vienna University of Economics and Business Research Report Series. 2014;125:1–24.
40. Maier MJ. DirichletReg: Dirichlet Regression for Compositional Data in R. 2021.
41. Revell LJ. Ancestral character estimation under the threshold model from quantitative genetics. *Evolution*. 2014 Mar;68(3):743–59.
42. Burns MD, Satterfield DR, Peoples N, Chan H, Barley AJ, Yuan ML, et al. Complexity and weak integration promote the diversity of reef fish oral jaws. *Commun Biol*. 2024 Nov 4;7(1):1433.
43. Corn KA, Martinez CM, Burress ED, Wainwright PC. A Multifunction Trade-Off has Contrasting Effects on the Evolution of Form and Function. Dayrat B, editor. *Systematic Biology*. 2021 Jun 16;70(4):681–93.
44. Martinez CM, McGee MD, Borstein SR, Wainwright PC. Feeding ecology underlies the evolution of cichlid jaw mobility. *Evolution*. 2018 Aug;72(8):1645–55.
45. Kahane-Rapport SR, Savoca MS, Cade DE, Segre PS, Bierlich KC, Calambokidis J, et al. Lunge filter feeding biomechanics constrain rorqual foraging ecology across scale. *Journal of Experimental Biology*. 2020 Jan 1;jeb.224196.
46. Holzman R, Collar DC, Price SA, Hulsey CD, Thomson RC, Wainwright PC. Biomechanical trade-offs bias rates of evolution in the feeding apparatus of fishes. *Proc R Soc B*. 2012 Apr 7;279(1732):1287–92.
47. Cade DE, Kahane-Rapport SR, Gough WT, Bierlich KC, Linsky JMJ, Calambokidis J, et al. Minke whale feeding rate limitations suggest constraints on the minimum body size for engulfment filtration feeding. *Nat Ecol Evol*. 2023 Mar 13;7(4):535–46.
48. Wainwright PC. Biomechanical limits to ecological performance: mollusc-crushing by the Caribbean hogfish, *Lachnolaimus maximus* (Labridae). *Journal of Zoology*. 1987;213:283–97.
49. Trapani J. Position of Developing Replacement Teeth in Teleosts. Schaefer SA, editor. *Copeia*. 2001 Feb;2001(1):35–51.
50. Huysseune A, Witten PE. Continuous tooth replacement: what can teleost fish teach us? *Biological Reviews*. 2024 Jun;99(3):797–819.
51. Huysseune A, Thesleff I. Continuous tooth replacement: the possible involvement of epithelial stem cells. *BioEssays*. 2004 Jun;26(6):665–71.
52. Waltzek TB, Wainwright PC. Functional morphology of extreme jaw protrusion in Neotropical cichlids. *Journal of Morphology*. 2003 Jul;257(1):96–106.
53. Alexander RM. Functional design in fishes. London: Hutchinson Publishing Group; 1967. 160 p.
54. Motta PJ. Dentition patterns among Pacific and Western Atlantic butterflyfishes (Perciformes, Chaetodontidae): relationship to feeding ecology and evolutionary history. *Environmental Biology of Fishes*. 1989;25(1–3):159–70.
55. Bellwood DR. Origins and escalation of herbivory in fishes: a functional perspective. *Paleobiology*. 2003;29(1):71–83.
56. Schaefer SA, Stewart DJ. Systematics of the Panaque dentex species group (Siluriformes: Loricariidae), wood-eating armored catfishes from tropical South America. *Ichthyological Exploration of Freshwaters*. 1993;4(4):309–42.
57. Rosen J, Cohen K, Donatelli CM, Summers AP, Crofts S, Kolmann MA. Something to sink your teeth into: the mechanics of tooth indentation in frugivorous fishes. *Journal of The Royal Society Interface*. 2025;22:20240752.
58. Lucas CM. Within Flood Season Variation in Fruit Consumption and Seed Dispersal by Two Characin Fishes of the Amazon. *Biotropica*. 2008 Sep;40(5):581–9.

59. Heiple Z, Huie JM, Medeiros APM, Hart PB, Goatley CHR, Arcila D, et al. Many ways to build an angler: diversity of feeding morphologies in a deep-sea evolutionary radiation. *Biol Lett*. 2023 Jun;19(6):20230049.
60. Berkovitz B, Shellis P. *The Teeth of Non-Mammalian Vertebrates*. London: Academic Press; 2017. 354 p.
61. Thiery AP, Shono T, Kurokawa D, Britz R, Johanson Z, Fraser GJ. Spatially restricted dental regeneration drives pufferfish beak development. *Proc Natl Acad Sci USA*. 2017 May 30;114(22):E4425–34.
62. Britski HA, Andreucci RD, Menezes NA, Carneiro J. Coalescence of teeth in fishes. *Revta Bras Zool*. 1985;2(8):459–82.
63. Bonaldo RM, Hoey AS, Bellwood DR. The Ecosystem Roles of Parrotfishes on Tropical Reefs. In: Hughes RN, Hughes DJ, Smith IP, editors. *Oceanography and Marine Biology [Internet]*. 0 ed. CRC Press; 2014 [cited 2024 Nov 14]. P. 81–132. Available from: <https://www.taylorfrancis.com/books/9781482220667/chapters/10.1201/b17143-3>
64. Soule JD. Tooth Attachment by Means of a Periodontium in the Trigger-fish (Balistidae). *Journal of Morphology*. 1969;(127):1–6.
65. Geerinckx T, Huysseune A, Boone M, Claeys M, Couvreur M, De Kegel B, et al. Soft Dentin Results in Unique Flexible Teeth in Scraping Catfishes. *Physiological and Biochemical Zoology*. 2012 Sep;85(5):481–90.
66. Streelman JT, Webb JF, Albertson RC, Kocher TD. The cusp of evolution and development: a model of cichlid tooth shape diversity. *Evolution and Development*. 2003 Nov;5(6):600–8.
67. Westneat MW. Evolution of Levers and Linkages in the Feeding Mechanisms of Fishes. *Integrative and Comparative Biology*. 2004 Nov 1;44(5):378–89.
68. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods*. 2012;(9):671–5.
69. Claude J. Log-Shape Ratios, Procrustes Superimposition, Elliptic Fourier Analysis: Three Worked Examples in R. *Hystrix, the Italian Journal of Mammalogy*. 2013 Jul 1;24(1):94–102.
70. Rabosky DL, Chang J, Title PO, Cowman PF, Sallan L, Friedman M, et al. An inverse latitudinal gradient in speciation rate for marine fishes. *Nature*. 2018 Jul;559(7714):392–5.
71. Pinheiro J, Bates D, R Core Team. *Nlme: Linear and Nonlinear Mixed Effects Models [Internet]*. 2025. Available from: <https://CRAN.R-project.org/package=nlme>
72. Paradis E, Schliep K. *ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R*. Schwartz R, editor. *Bioinformatics*. 2019 Feb 1;35(3):526–8.
73. Ives A, Li D. *rr2: An R package to calculate R²s for regression models*. *JOSS*. 2018 Oct 31;3(30):1028.
74. Ives AR. *R²s for Correlated Data: Phylogenetic Models, LMMs, and GLMMs*. *Systematic Biology*. 2019;68(2):234–51.
75. Hahsler M, Grün B, Hornik K. **arules**- A Computational Environment for Mining Association Rules and Frequent Item Sets. *J Stat Soft [Internet]*. 2005 [cited 2025 May 22];14(15). Available from: <http://www.jstatsoft.org/v14/i15/>
76. Beaulieu JM, Jhvueng DC, Boettiger C, O'Meara BC. Modeling Stabilizing Selection: Expanding The Ornstein-Uhlenbeck Model of Adaptive Evolution. *Evolution*. 2012 Aug;66(8):2369–83.
77. Revell LJ. *Phytools: an R package for phylogenetic comparative biology (and other things)*. *Methods Ecol Evol*. 2012 Apr;3(2):217–23.
78. Kassambara R. *rstatix: Pipe-Friendly Framework for Basic Statistical Tests [Internet]*. 2023. Available from: <https://rpkgs.datanovia.com/rstatix/>

Chapter 4

Habitat-specific temporal variation in the pace of fish diversification

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Introduction

Diversity is one of the main currencies of evolutionary biology. Its oscillating dynamics reflect speciation and extinction events concentrated unevenly through space and time^{1–7}, yet debate remains over the underlying causes of this temporal variation. Periods of increased diversification may require environmental upheaval or climatic events that reshape the ecological landscape^{1,8,9}, organismal modifications that increase the adaptive potential of lineages (e.g., whole genome duplications, functional innovations)^{10,11}, or a confluence of the two¹². However, studies more often find that diversification slows through time, an emergent pattern across many groups^{7,13–16}. These temporal slowdowns are frequently attributed to the existence of ecological limits – the accessibility of open niches, resources, or geographic area – which generate diversity-dependent patterns as ecological space becomes saturated^{13,16–19}. Species diversity often scales with these properties (e.g., geographic area)^{20–22}, yet whether and how these ecological factors modulate the rate of species diversification over long timescales is much less studied. Associating these ecological differences with varied diversification rate trends can provide crucial insight into how and why diversification rates change through time. A species' habitat is a multivariate representation of biotic and abiotic characteristics that together provide the

physical substrate for diversification to occur. Habitats vary considerably in their range of microhabitat gradients, trophic niches available, stability, and connectivity, and this heterogeneity has been shown to shape speciation dynamics over short timescales^{23–27}. This raises the possibility that the distribution of extant species diversity has been generated by the accumulated effects of habitat-specific variation in the pace of diversification through time.

Over 35,000 species of ray-finned fishes (Actinopterygii), representing over half of extant vertebrate species, have come to dominate aquatic habitats worldwide. These habitats vary considerably in ecological resources, space, stability and thus, they also vary in the potential for diversification rates to change as opportunity is extinguished or environmental change occurs. The heterogeneity of complex benthic habitats such as shallow coral reefs fosters interactions with multiple, highly diverse resources, promoting niche differentiation and supporting higher species diversity^{25,28–30}. Freshwater systems have continuous habitat gradients and abundant physical barriers to gene flow that stimulate divergence events²⁴. In contrast, environments with comparatively greater homogeneity such as the pelagic zone and deep sea have narrowed ecological opportunities and high connectivity, decreasing the chances of speciation events^{31–34}. These habitats are more stable over geological timescales^{35,36}. Actinopterygian diversity is also characterized by remarkable contrasts; freshwater and marine species richness is similar despite vastly greater habitat area in the oceans^{37,38}. This suggests that temporal trends in the pace of diversification may vary between major aquatic habitats, affording an opportunity to understand how past diversification dynamics have shaped contemporary patterns of biodiversity.

Here, we estimate habitat-specific temporal trends in diversification rates of ray-finned fishes at a global scale. We estimate these rates at an unprecedented resolution to address how major ecological differences (e.g., geographic area, stability, structural complexity) between

habitats influence the rate at which species accumulate through time. We compare diversification rate trends among seven major aquatic habitats, identifying both tree-wide and clade-specific rate shifts across ray-finned fishes and associate these trends with shared habitat traits. By incorporating multiple lines of evidence, we show that accelerating diversification rates are a unique attribute of complex benthic habitats in both marine and freshwater systems, a trend fueled by the emergence of clades that capitalized on the diverse array of benthic resources following major ecological stimulation.

Results and Discussion

Diversification rates through time

To investigate how diversification rates have changed throughout the evolutionary history of ray-finned fishes, we classified 9,560 species into seven discrete categories (reef-associated, marine demersal, marine benthopelagic, marine pelagic, freshwater demersal, freshwater benthopelagic, freshwater pelagic) representing major aquatic habitats that vary in the ecological opportunities they offer lineages^{25,28–30}. We estimated speciation and extinction rates in 1-My intervals for lineages within each habitat using episodic birth-death (EBD) models implemented in RevBayes^{39,40}. Ray-finned fishes show substantial variation in the tempo of diversification through time across habitats (Fig. 1a). Net diversification rates are remarkably constant in marine pelagic (MP), marine demersal (MD), marine benthopelagic (MB), and freshwater pelagic (FP) lineages, which inhabit comparatively homogenous environments that represent the majority of aquatic habitat availability by area and volume (Fig. 1a)⁴¹. This temporal consistency may be a consequence of both the stability of these habitats over long timescales and/or the lack of substrate they provide to stimulate species diversification along multiple ecological axes^{31,35}. In contrast, reef-associated (RA) lineages and those in freshwater demersal (FD) and freshwater

benthopelagic (FB) habitats show a notable increase in net diversification rate beginning in the Eocene (56-33.9 Ma) and continuing until the Middle to Late Miocene (16-5.3 Ma) (Fig. 1a). During this period, tree-wide net diversification increased 1.5-1.7x, after which rates stagnated and gradually decreased towards the present. The rising net diversification of RA, FB, and FD lineages is thus a relatively recent trend in the evolution of ray-finned fishes; median rates vary only slightly (0.025-0.038 lineages/My⁻¹) over the last ~200 My (Fig. 1a). While increasing speciation (λ) explains this trend in RA lineages, declining extinction (μ) underlies net diversification increases in FD and FB lineages (Fig. 1c; *SI Appendix*, Fig. S1). These temporal trends indicate that despite accounting for a minority of total area^{41,42}, these habitats (RA, FD, FB) may have shared properties that promote the continued and accelerated generation of species diversity. These habitats are considered more heterogenous, complex, and vulnerable to change^{24,28,29} indicating that the temporal trends of ray-finned fish diversification (Fig. 1a-c) may reflect key differences in the potential for diversification that these aquatic habitats provide.

Given that speciation and extinction rates estimated under birth-death models are not identifiable⁴³, we verified that these patterns of diversification rate change between time intervals are consistent under alternative diversification scenarios^{44,45}. By jointly sampling alternative λ and μ rate functions to create congruent diversification scenarios, we find consistent patterns of speciation rate change between ~50 and ~10 Ma that closely match those under the observed EBD model (Fig. 1c; *SI Appendix*, Fig. S8). We also investigated whether increasing net diversification rates during this same time period can occur under four specific alternative diversification scenarios: constant λ , constant μ , exponentially decreasing λ , and exponentially increasing μ . We find that the observed net diversification rate trends in RA, FD, and FB lineages are either valid under these four scenarios, or the scenario is not contained within the

congruence class (e.g., λ or $\mu < 0$ at any point in time) (*SI Appendix*, Figs. S4-S6)^{44,45}. In our implementation of the Horseshoe Markov random field (HSMRF) birth-death model, the total rate variability through time is determined in part by the global scale hyperprior (ζ)⁴⁰. We confirmed that the temporal diversification patterns are robust under more “permissive” priors, which effectively allow for more total variation in λ and μ across all intervals (see Materials and Methods; *SI Appendix*, Fig. S2).

While tree-wide shifts in diversification rate may be a consequence of processes affecting clades equally across a broad phylogenetic scale (e.g., climatic events occurring at a global scale), identifying the specific clades that drive these shifts and their commonalities can provide ecologically-centered explanations for why diversification rates change. We used a lineage-specific birth-death-shift (BDS) model⁴⁶ implemented in RevBayes to estimate the timing, magnitude, and phylogenetic placement of speciation rate shifts. We identified 95 rate shifts (posterior probability [PP] of shift > 0.5) which were distributed relatively evenly across habitats when accounting for tree size (RA; 0.0039 shifts per branch, FB; 0.0042, FD; 0.0067, MD; 0.004, MB; 0.005, MP; 0.0071, FP; 0.0104). However, these shifts were distributed unevenly through time and peaked during the Early Miocene (23-16 Ma), a time of considerable climatic and ecosystem reorganization globally (Fig. 1a). The magnitude of rate shifts is higher in RA, FB, and FD lineages (i.e., habitats in which diversification increases through time) (Analysis of Variance [ANOVA]; $F=5.904$, $P<0.05$) (Fig. 1e), indicating that the emergence of some clades within these habitats (e.g., Pomacentridae, Holocentridae, Acanthuridae [RA], Cichlidae, Cyprinidae [FB], Loricariidae, Sicydiinae, Balitoridae [FD]) may have had a disproportionately larger impact on the tempo of tree-wide net diversification. Though recent net diversification rates are highest in reef taxa (0.066 lineages My^{-1}), reef-associated lineages do not have larger

shifts than other habitats (ANOVA; $F=1.935$, $P=0.168$) (Fig. 1f), suggesting an interaction between patterns emerging across the phylogeny (e.g., tree-wide shifts for all lineages within a habitat) and clade-specific rate shifts within these habitats.

Association with the benthos has recently been appreciated as a significant driver of diversification in fishes^{32,47,48}, so we compared the magnitude of rate shifts among clades that do and do not feed on benthic-associated resources. We found that primarily benthic-feeding groups undergo 1.2x larger shifts (ANOVA; $F=19.73$, $P<0.001$) (Fig. 1d) and comprise 40% of all shifts across fishes (Fig. 1a). Our results therefore suggest that the emergence of clades that evolved to successfully exploit benthic resources, coupled with complex habitats and the plethora of ecological niches they offer, are primary contributors to the uneven diversity of extant ray-finned fishes.

Benthic feeding on reef ecosystems

Change in the pace of diversification is a signal of evolutionary radiation⁴⁹. While slowdowns suggest extinguishing ecological opportunity, increasing rates may imply that environmental change or organismal innovation broadened the range of achievable niches. Marine reef ecosystems are one of the most complex aquatic habitats and long-term cradles of biodiversity^{50,51}. Our results show that significant speciation rate shifts in reef lineages most often occur in benthic-feeding groups (60% of shifts within reef-associated lineages), resulting in the largest and fastest increase in net diversification since the Cretaceous-Paleogene (K-Pg) extinction event (Fig. 1a), which raises the possibility that benthic feeding stimulated these changes. While several reef-associated clades have undergone diversification rate shifts, separating the effect of reef habitat itself from other factors such as organismal traits has

remained difficult^{52–54}. Therefore, we further examined how transitions to feeding on benthic-associated resources shaped the tempo of reef fish diversification.

Using a modified classification of reef fish feeding modes⁴⁷ to reflect whether primary diet items either require detachment (e.g., algae) or are firmly associated with the reef benthos (e.g., urchins), as opposed to free-moving (e.g., plankton), we fit a state-dependent speciation and extinction (SSE) model with hidden states⁵⁵ in RevBayes to estimate speciation and extinction rates of lineages feeding on benthic-associated and non-benthic-associated resources while accounting for background rate variation. Net diversification rates are 1.33-1.57x higher ($T_{1.3A}=0.909$; $T_{1.3B}=0.592$; see Materials and Methods) when lineages transition to feeding on benthic-associated resources, such as coral or algae (Fig. 2c). This effect of benthic feeding on net diversification is robust to an alternative prior distribution of extinction rates (*SI Appendix*, Fig. S7). Though rates estimated under SSE models are not time-varying, examining the distribution of hidden states through time can reveal temporal diversification patterns because positive rate shifts can be reflected by transitions to hidden states with a higher background diversification rate. The proportional rise of benthic feeding fishes in hidden state B (in which net diversification rates are 3.35x higher than hidden state A) since the Paleocene-Eocene Thermal Maximum (PETM) mirrors the tree-wide increase in net diversification rate observed under the EBD model (Fig. 1a, 2b). The acceleration of diversification rate in reef-associated lineages is thus for the most part driven by the rise of butterflyfishes (Chaetodontidae), angelfishes (Pomacanthidae), surgeonfishes (Acanthuridae), triggerfishes (Balistidae), rabbitfishes (Siganidae), parrotfishes (*Scarus*, *Chlorurus*), and damselfishes (Pomacentridae) (Fig. 2a, 3), which all together dominate the benthic-feeding fauna on modern reefs. The emergence of specialized benthic feeders that exploited the habitat complexity and abundance of

food resources on reefs is thus a major contributor to the acceleration of species diversification over time in reef associated lineages, which represent a large evolutionary radiation whose diversification has only recently stalled (Fig. 1a).

Evolutionary processes operating across broad spatial and temporal scales could generate consistent diversification trends across phylogenetically-disparate groups⁵⁶. In this case, we would expect to find net diversification rate increases over a similar time period in other dominant reef fauna. Major lineages of reef-building scleractinian corals emerged during the Cretaceous and Paleogene, resulting in a structural overhaul of reef habitat complexity^{57–59}. We therefore tested if reef-associated corals showed a similar temporal trend in diversification rates as reef-associated fishes. Net diversification rates estimated for 842 species of reef-associated scleractinian corals are remarkably constant through time periods during which large changes are seen in reef fish diversification (but see ref. ⁵⁹) (*SI Appendix*, Fig. S3). This contrasts the pattern observed in reef-associated fishes, suggesting that the increased diversification during this same period is thus likely linked to the evolution of fishes themselves and their ecological interactions, rather than reflecting a general pattern of accelerated diversification across reef-associated taxa.

Changing ecological opportunities and innovations

Geological and climatic events occurring globally over evolutionary timescales can stimulate major ecological reorganization, fundamentally changing the nature of how lineages interact with each other and their environment^{60,61}. The abrupt increase in net diversification rate for RA, FD, and FB lineages occurred during a time of substantial change in aquatic systems and their faunal compositions, marked by the diversification of distinct acanthomorph families and shifts towards low trophic level feeding^{62–64}. Intense warming during the PETM ~56 Ma massively reorganized aquatic food webs and habitats^{65–69} by transforming the benthic resources

available to fishes, such as algae and coral^{70,71}. The unequal acceleration of species diversification across habitats following this hyperthermal event (Fig. 1a) suggests the PETM and its residual effects may have disproportionately affected freshwater and shallow marine environments by changing the composition of benthic resources, revitalizing opportunity to diversify along novel ecological axes. Some benthic communities persisted through this hyperthermal event due to thermal tolerance (e.g., molluscs⁷²), providing both stability and turnover of benthic resources. Concordant mass extinction of established lineages during or immediately following the PETM may have promoted further diversification by reducing competitors⁷³, though diverse fish assemblages persisted through this time⁷⁴. In contrast, stable environments with lower ecological opportunity such as the open ocean may have experienced reduced PETM impacts³⁵, leading to the stagnation of species diversification (Fig. 1a) in the absence of major upheaval. The steady pace of diversification through time in these habitats suggests that without input of new ecological substrate to stimulate diversification, dominant lineages likely emerged without widespread diversification.

While extensive restructuring of ecological resources in freshwater and marine reef systems may have opened ecological opportunity, lineages are not equally equipped to exploit these changes. Feeding on benthic resources involves various forms of biting mechanics, which strongly contrasts the ubiquity of prey capture by suction feeding on free-moving prey. Multiple innovations in the feeding system characterize the evolution of benthic-feeding fishes. These include advances in both acquisition (e.g. complex multicuspid teeth, lateral jaw movement, intramandibular joint)^{75–77}, processing (e.g., pharyngeal mill)⁷⁸, and assimilation^{79,80} which together increase performance in these feeding niches. The effect of these clades on diversification is remarkably consistent across models (episodic birth-death [Fig. 1a, b], birth-

death-shift [Fig. 1a, 3], state-dependent speciation and extinction [Fig. 2]), suggesting that benthic-feeding fishes may represent lineages that withstand the loss of speciation potential through time⁷ to generate pulses of increased diversity across the actinopterygian phylogeny. This intertwined interaction between organismal functional innovation and major environmental upheaval most likely caused the recent and revitalized diversification of fishes in complex benthic habitats.

Conclusion

Aquatic habitats differ in their ability to stimulate diversification, leading to major consequences for the distribution of extant biodiversity patterns. Our results add resolution to a striking paradigm. Although the number of freshwater and marine ray-finned fish species is relatively equal³⁷, nearly 70% of species occupy just three complex benthic habitats, a pattern that can be attributed to their accelerated diversification rates. Microhabitat gradients and high structural complexity in freshwater benthic and marine reef habitats provide opportunity in waiting for properly equipped lineages to exploit, whereas vast comparatively homogenous habitats such as the open ocean promote steady diversification through time. Jointly, benthic-feeding lineages that evolved functional feeding innovations capitalized on renewed ecological opportunities following global environmental change. This dynamic interplay between the traits of species and the attributes of benthic habitats prompted the progressive acceleration of species diversification over ~30 My. The resultant tree-wide rate shifts and clade-specific pulses structured the distribution of contemporary fish biodiversity.

Materials and methods

Phylogeny and habitat classification

For all phylogenetic comparative analyses, we used the molecular phylogeny of Rabosky et al.⁸¹. While other phylogenetic hypotheses for subsets of fishes exist, we opted to use a single time calibration to ensure our analyses are commensurate and comparable across habitat subtrees. We used FishBase⁸² to classify species into seven discrete habitats: marine reef-associated (n=1907 species), marine demersal (n=1625 species), marine benthopelagic (n=300 species), marine pelagic (n=635 species), freshwater demersal (n=1647 species), freshwater benthopelagic (n=3107 species), freshwater pelagic (n=339 species). We created seven individual habitat trees by pruning the full phylogeny to match each set of species within each habitat. We excluded species that were found in both freshwater and marine (brackish) habitats, freshwater and brackish, marine and brackish, or all three. Within each salinity environment, we combined the following habitats: (1) bathydemersal, demersal, (2) pelagic, bathypelagic, pelagic-oceanic, pelagic-neritic. We recognize that our method does not fully account for the evolutionary history of transitions into and occupation of these habitats.

Diversification rate through time

To estimate diversification rates across habitats through time, we implemented an episodic birth-death (EBD) model in RevBayes v1.2.1³⁹. This model assumes rates are constant within time intervals but can change between intervals. We used a Horseshoe Markov random field (HSMRF) for the prior distribution of log-transformed rates, which assumes autocorrelation of rates between adjacent time intervals. We estimated speciation (λ) and extinction (μ) rates in 1-My intervals for each of the seven habitat trees separately. We set unique global scale

hyperpriors (ζ) for each tree, which control the overall variability in diversification rates through time, using the ‘*setMRFGlobalScaleHyperpriorNShifts*’ function in the R package ‘*RevGadgets*’⁸³ with a prior number of shifts= $\log(2)$ and a shift size of 2 (2-fold change)⁴⁰ (FD: 368 intervals, $\zeta=0.0003738458$; FB and FP: 187 intervals, $\zeta=0.0009208335$; RA, MP, MD, MB: 193 intervals, $\zeta=0.0008787526$). We tested the sensitivity of our results to the choice of the global scale hyperprior by repeating all analyses with ζ values that produce both 5 and 10 prior shifts, a more “permissive” approach that effectively increases the total rate variability through time compared to ζ for $\log(2)$ shifts, a conservative value suggested by ref.⁴⁰ that follows the framework of ref.⁸⁴. For these sensitivity analyses, we maintained an effective shift size of 2-fold. We report results from $\log(2)$ shifts in the main text because this prior is reasonably conservative but flexible⁴⁰, and results from 5 and 10 shifts in the supplementary information (*SI Appendix*, Fig. S2). We set unique sampling fractions (ρ) for each tree (RA $\rho=0.475$, MP $\rho=0.303$, MD $\rho=0.255$, MB $\rho=0.277$, FP $\rho=0.355$, FD $\rho=0.347$, FB $\rho=0.383$). We used Markov Chain Monte Carlo (MCMC) to estimate model parameters; each MCMC had two independent runs for 500,000 generations each with sampling every 10 generations and a pre-burn-in of 50,000 generations (tuning interval=200). This burn-in period helps ensure acceptance rates remain within an acceptable range and improves MCMC efficiency. We further removed 25% of the combined runs as burn-in. We used the R package *convenience*⁸⁵ to verify convergence of speciation and extinction rates in the combined runs at a precision level (α) of 0.015; the posterior distributions of both rates converged for all habitats. We used *RevGadgets* to visualize diversification rates through time; we report net diversification (r ; $\lambda-\mu$) rates in the main text. For all figures and analyses on the median rates, we retained only rates over the last 193 million

years of the freshwater demersal (FD; root age 368 Ma) tree so that all trees had comparable timescales.

Congruent diversification scenarios

Diversification rates estimated from birth-death models are not identifiable, however shared patterns across congruent birth-death models can be inferred when diversification rates are found to vary through time^{43,44,86}. We explored the validity of alternative hypotheses for diversification rates using the R package *CRABS*⁴⁵ for RA, FD, and FB lineages, which showed rate change through time. We tested hypotheses of constant μ , exponentially increasing μ , constant λ , and exponentially decreasing λ (*SI Appendix*, Figs. S4-S6). We accepted that alternate hypotheses were not contained within the congruence class if μ or λ were negative at any point. We summarized trends in net diversification rate (r) change across reference and congruent models with a threshold of twice the mean change in λ between time intervals, which was unique to each habitat. We also simultaneously generated 20 congruent sets of λ and μ through joint sampling with HSMRF p -trajectories⁸⁶ ($\sigma_{\text{MRF}}=1$, $\beta_1=0.5$, $\beta_2=0.2$) and visualized trends in speciation and extinction rates across models within the congruence class (Fig. 1c; *SI Appendix*, Fig. S8).

Coral diversification

We estimated shifts in diversification rate through time for reef-associated scleractinian corals, the major clade of reef-building invertebrates. We summarized the distribution of trees from Huang and Roy⁸⁷ into a single maximum a posteriori (MAP) tree using the ‘*mapTree*’ function in RevBayes v1.2.1 and pruned this tree to match reef-associated species (n=842 species). We used the EBD model described above to estimate diversification rates through time

in 1-My intervals (351 intervals, $\zeta = 0.0003717236$, $\rho = 0.545$). The MCMC had two independent runs of 1,000,000 generations each with 25% removed as burn-in. We used Kolmogorov-Smirnov (KS) tests to check for convergence of the replicates ($\alpha = 0.015$; *SI Appendix*, Fig. S3) and combined the runs.

Branch-specific diversification rate estimation

We implemented a branch-specific diversification rate model in RevBayes v1.2.1 to estimate the timing, location, and magnitude of clade and branch rate shifts⁴⁶. Under this model, both extant and extinct lineages can undergo shifts in diversification rate. Speciation rates were drawn from a log-normal distribution discretized into 6 quantiles ($k=6$); the mean was drawn from a log-uniform distribution with bounds (1E-6, 1E2), and standard deviation drawn from an exponential distribution ($\lambda = 1.7$). We used the same prior for the distribution of extinction rates, but constrained extinction rates to be equal across all k . The number of rate-shifts was drawn from a uniform distribution with bounds (0, 100). Unique sampling fractions (ρ) were set for each tree; these were equal to those of the EBD analysis and unsampled taxa were assumed to be random. We used MCMC to sample speciation and extinction rates, and stochastic branch rates; two independent chains were run for 500,000 generations each (tuning interval=200), sampling every 10 generations. We combined the runs and removed 50% as burn-in; we used the R package *convenience* to verify convergence ($\alpha = 0.015$). To identify the timing and phylogenetic placement of shifts within each habitat, we first identified all branches where the posterior probability (PP) of a shift was above 0.5. We then quantified the shift magnitude as the ratio between the mean speciation rate on the shift branch and parent branch (multiplier). We compared the magnitude of shifts between reef ($n=15$) and non-reef ($n=80$), between benthic ($n=38$) and non-benthic ($n=57$), and between RA, FD, FB ($n=63$) and MD, MB, MP, FP ($n=32$)

using one-way Analysis of Variance (ANOVA) tests. We log-transformed the shift multiplier to fulfill assumptions of normality (Shapiro-Wilk test $P > 0.05$).

Benthic feeding classification

To categorize reef fish species as feeding on benthic associated resources, we modified the diet classifications of Corn et al.⁴⁷. We defined benthic resources as those that require detachment from the reef surface (e.g. turf algae, sponges, coral polyps, mollusks) or are firmly associated with the reef benthos and resist detachment (e.g., urchins, some crabs). Species feeding on elusive free-swimming resources in the water column were categorized as non-benthic feeding. We classified species that feed on ectoparasites (e.g., *Elacatinus*) as benthic-feeding because these resources are attached to a substrate and require detachment. All ‘ram biters’⁴⁷ were categorized as feeding on non-benthic resources; classifications can be found in the Supplementary Data. We pruned the marine reef-associated tree to match the 1288 species for which we had data on feeding mode. We also classified clades in which a speciation rate shift occurred on the parent branch in this same scheme using diet data available in FishBase⁸²; we made classifications based on which diet items were most prevalent across species in the clade. We did not classify shifts that occurred in deep nodes that included multiple disparate clades (e.g., at the base of Cypriniformes).

State-dependent diversification

To test if reef species feeding on benthic-associated resources have higher diversification rates than those that do not, we fit a two-state hidden-state speciation and extinction (HiSSE) model⁵⁵ in RevBayes v1.2.1. The framework of the Bayesian implementation of HiSSE explicitly tests a null hypothesis of character-independent diversification, reducing the need for

extensive model comparison. If character-independent diversification is supported, the posterior distribution of net diversification rates within a hidden state should largely overlap. We used two hidden states (HiSSE-2) to accommodate rate variation independent of the observed characters. Speciation and extinction rates were drawn from a normal distribution with mean $\ln(\# \text{ taxa}/2)$ divided by the age of the tree ($\rho=0.321$). We set a prior of 50 state transitions across the tree, and an equal-probability of non-benthic feeding across either hidden state as the prior on the root state. To test if our results were robust to assumptions on the extinction rate, we placed a lower bound on the prior distribution of extinction rates^{76,88}. Thus, $\mu = A * \lambda + \delta$ where $A = 0.5$ (μ at least half of λ) and δ is a random variable that allows extinction to be greater than $A*\lambda$. We used MCMC to sample λ , μ , and stochastic character maps; two independent chains were run for 50,000 generations each (tuning interval=100), sampling every generation, with 5,000 generations pre-burn-in. We combined the runs, removed the first 10% as burn-in, and verified convergence using the *convenience* package ($\alpha=0.015$). We processed the posterior distribution of model parameters using *RevGadgets*.

To further evaluate the probability that benthic-feeding species have higher net diversification than non-benthic-feeding species, we used a simple test statistic, T_{ab} , where a is a specified magnitude of difference (e.g. 1.5x) and b specifies the hidden state (e.g. A, B). Thus, $T_{1.5A}$ represents the number of generations for which net diversification for state 1 (r_1) is 1.5x greater than the net diversification for state 0 (r_0), divided by the total number of generations, for hidden state A. This is effectively the posterior probability that $r_1 > r_0$ at a user-specified threshold (e.g., 1.5x greater) within a hidden state under a Bayesian HiSSE model.

Figures and Tables

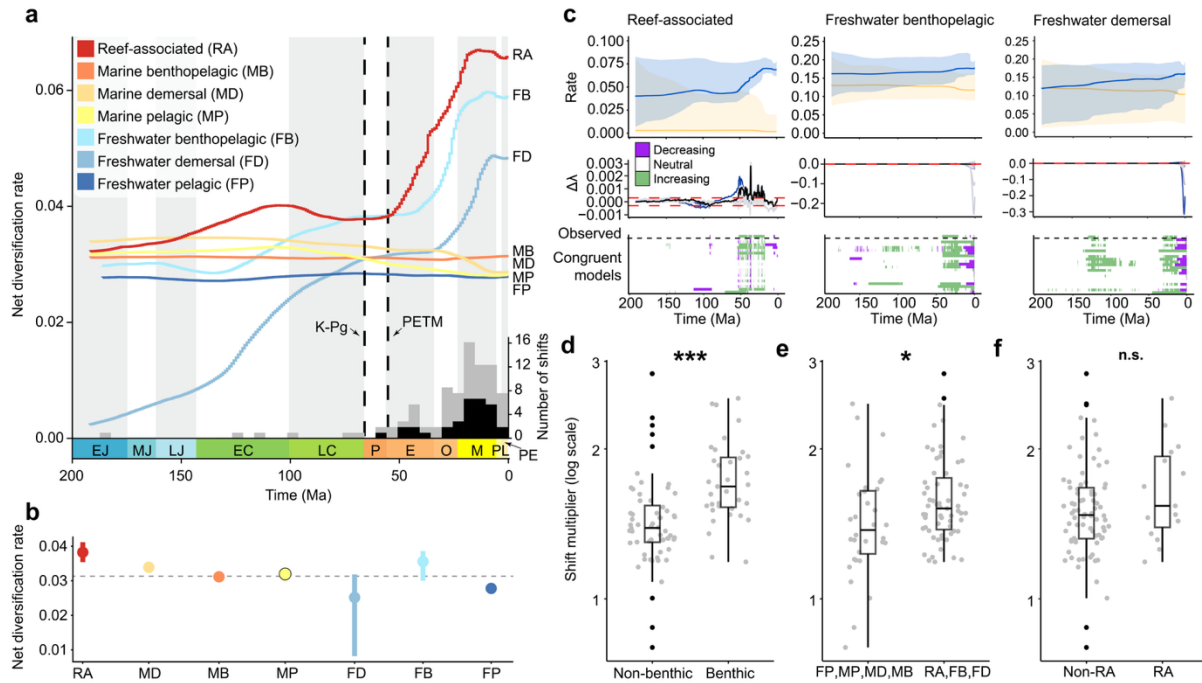


Figure 1 | The tempo of ray-finned fish diversification through time. **a**, Net diversification rates in 1-My intervals for reef-associated (RA; n=1907), freshwater benthopelagic (FB; n=3107), freshwater demersal (FD; n=1647), marine benthopelagic (MB; n=300), marine demersal (MD; n=1625), marine pelagic (MP; n=635) and freshwater pelagic (FP; n=339) lineages. Abbreviations of geological epochs: EJ, Early Jurassic; MJ, Middle Jurassic; LJ, Late Jurassic; EC, Early Cretaceous; LC, Late Cretaceous; P, Paleocene; E, Eocene; O, Oligocene; M, Miocene; PL, Pliocene; PE, Pleistocene. Stacked bar chart reporting the number of branch-specific rate shifts through time; black bars correspond to primarily benthic feeding clades. **b**, Median rates for each habitat; the dashed line represents the global median. Bars represent the interquartile range. **c**, Speciation (blue) and extinction (orange) rates, with shaded areas representing 95% confidence intervals, and the range of speciation rate trends under congruent diversification scenarios for RA, FB, and FD lineages. Qualitative trends are reported relative to

the observed data. The magnitude of branch-specific rate shifts between **d**, benthic (n=38) and non-benthic (n=57) feeding clades ($P<0.001$), **e**, habitats undergoing net diversification rate increases through time (n=63) and those that do not (n=32) ($P<0.05$), and **f**, reef (n=15) and non-reef associated (n=80) taxa ($P=0.168$). Black points represent outliers.

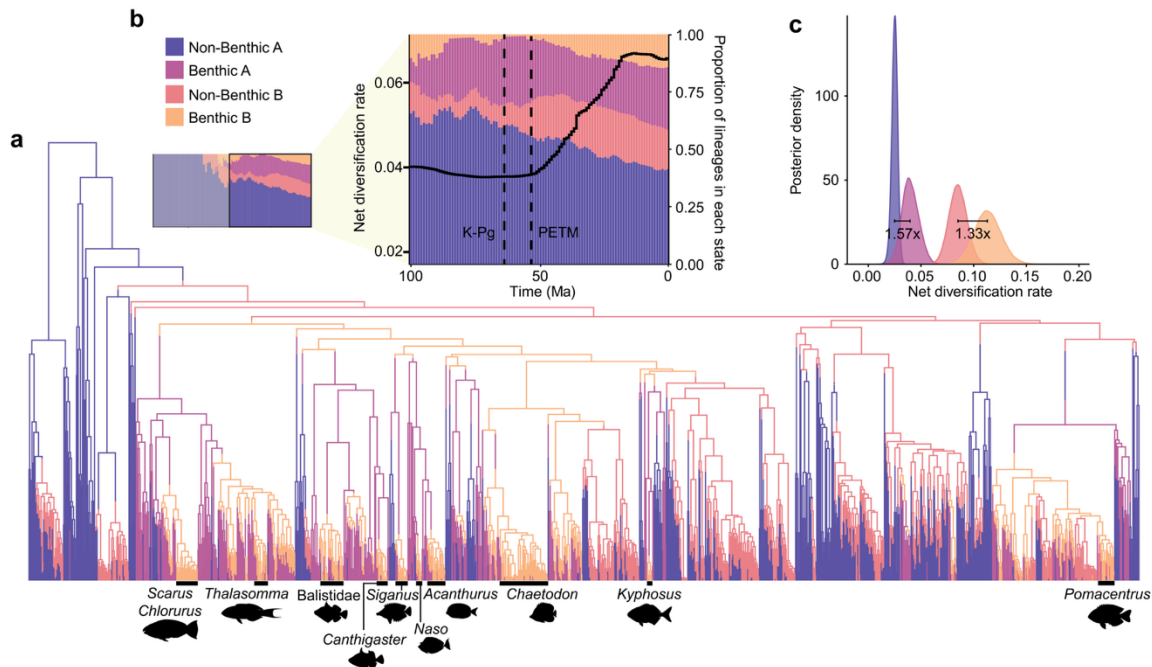


Figure 2 | The evolution of benthic-feeding reef fishes. **a**, Ancestral state reconstruction using the HiSSE-2 model of benthic feeding on a phylogeny of coral reef fishes. **b**, The proportion of benthic and non-benthic feeding across two hidden states (A & B) through time, in 1-My intervals. The trend line corresponds to the net diversification rates for reef-associated lineages estimated over the same interval. **c**, Posterior distribution of net diversification rates for benthic and non-benthic feeding lineages across two hidden states. The difference between median rates of feeding types (benthic vs. non-benthic) within each hidden state is reported.

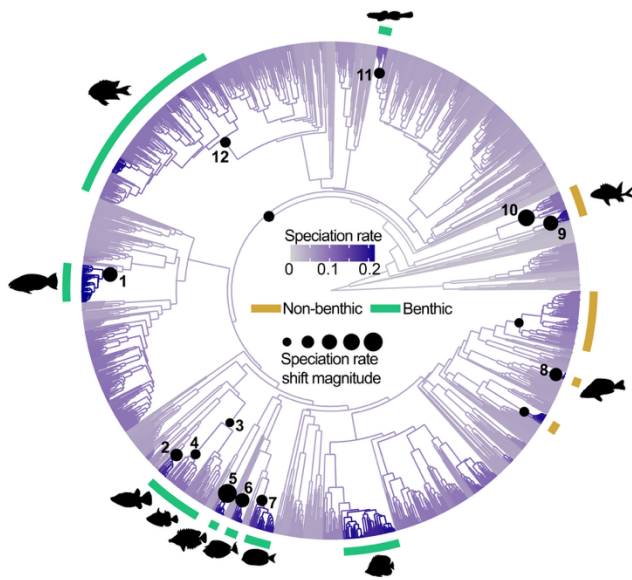


Figure 3 | Shifts in the tempo of reef fish diversification. Phylogeny of reef-associated fishes, with black points indicating nodes where significant rate shifts were detected; points are scaled to the magnitude of speciation rate change between parent and daughter branches. Speciation rate shifts in 1) *Scarus* and *Chlorurus*, 2) *Canthigaster*, 3) Balistidae, 4) *Rhinocanthus*, 5) Siganidae, 6) *Naso*, 7) *Acanthurus*, 8) *Hypoplectrus*, 9) and 10) Holocentridae, 11) *Elacatinus*, 12) Pomacentridae. Green bars represent predominantly benthic-feeding groups. Ectoparasite feeding (*Elacatinus*) requires detachment of resources and thus, functionally resembles a biting-associated feeding mode rather than a suction-based mode.

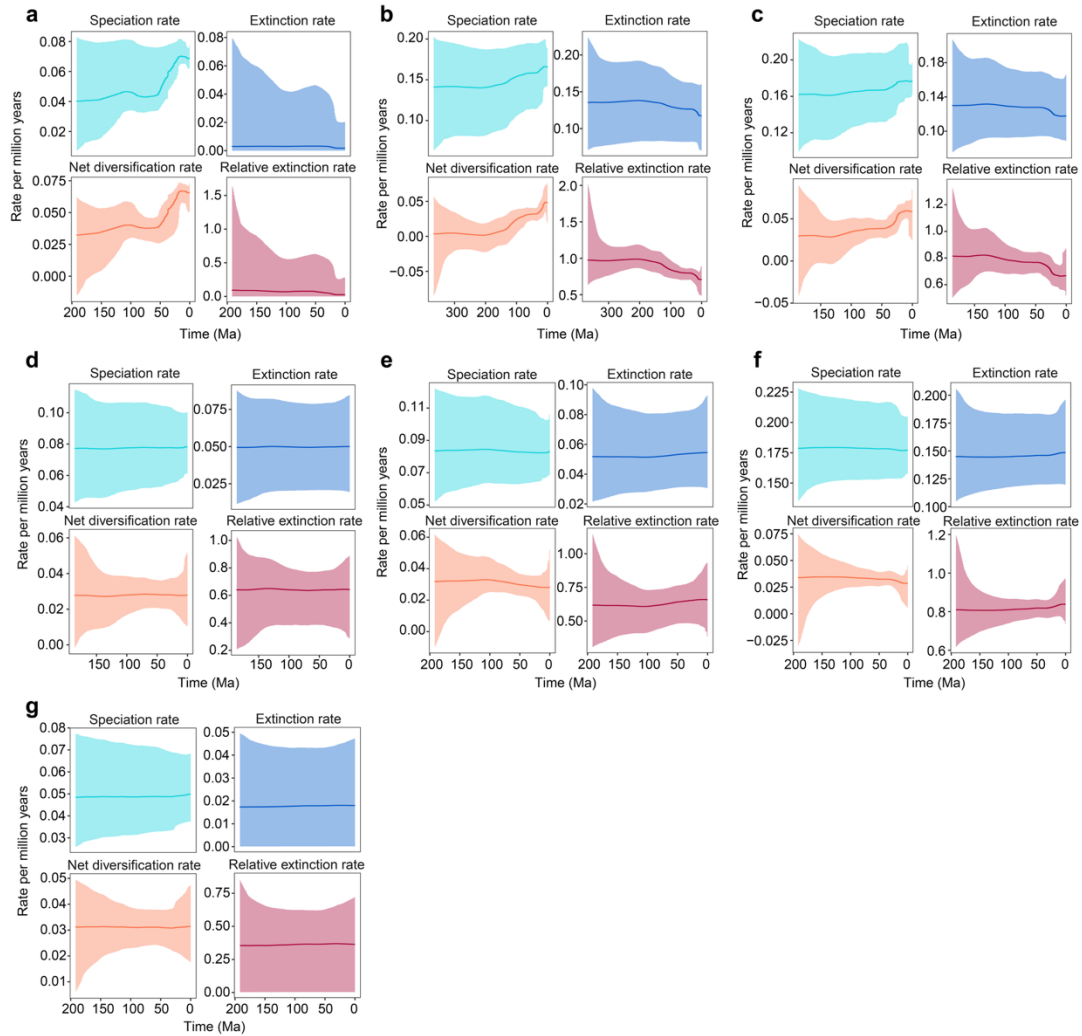


Fig. S1. Speciation, extinction, net diversification, and relative extinction rates through time in 1-My intervals across seven major aquatic habitat types. Shaded areas represent 95% confidence intervals, and relative extinction is reported as the ratio of extinction (μ) to speciation (λ). Rates estimated in 1-My intervals. **a**, reef-associated (RA), **b**, freshwater demersal (FD), **c**, freshwater benthopelagic (FB), **d**, freshwater pelagic (FP), **e**, marine pelagic (MP), **f**, marine demersal (MD), **g**, marine benthopelagic (MB).

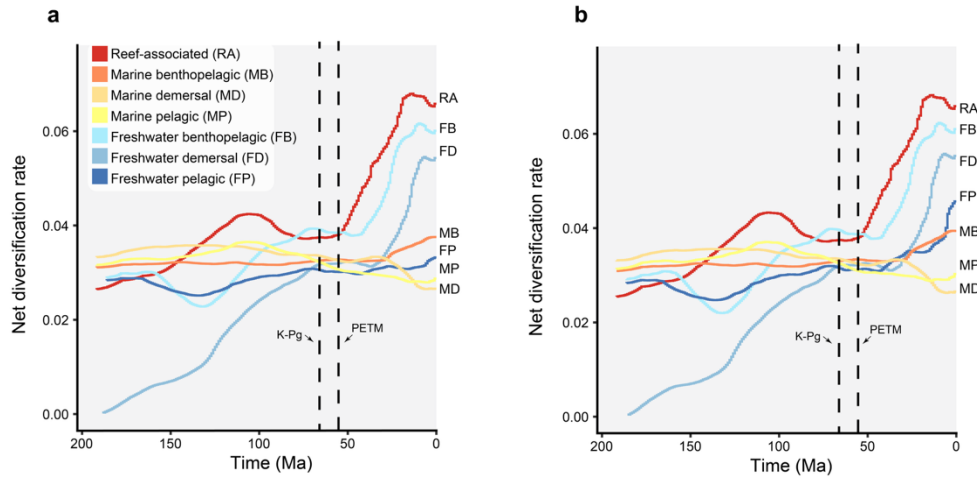


Fig. S2. Temporal trends in net diversification rates across habitats are stable across different global scale hyperpriors. Net diversification rates estimated in 1-My intervals with a prior of **a**, 5 shifts and **b**, 10 shifts between intervals. The dashed lines mark the K-Pg extinction and the Paleocene-Eocene Thermal Maximum (PETM).

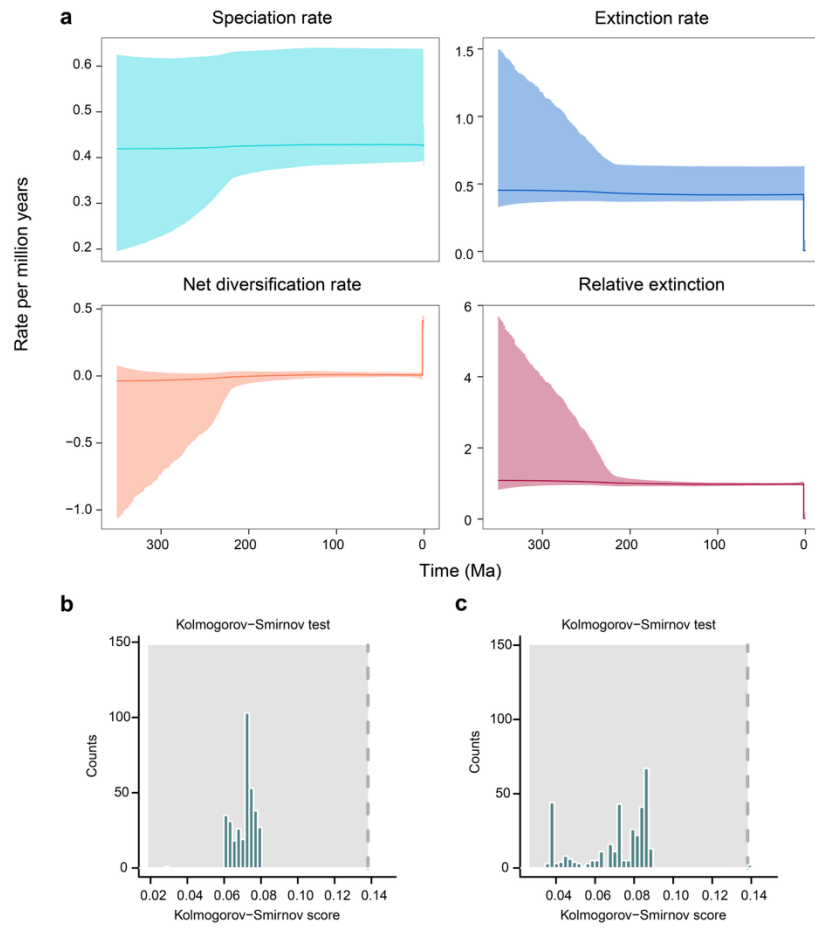


Fig. S3. Temporal trends in scleractinian (stony coral) diversification rates. **a**, Speciation, extinction, net diversification, and relative extinction through time in 1-My intervals for 842 species of reef-associated scleractinian corals. Kolmogorov-Smirnov tests for convergence of **b**, speciation and **c**, extinction rates between runs.

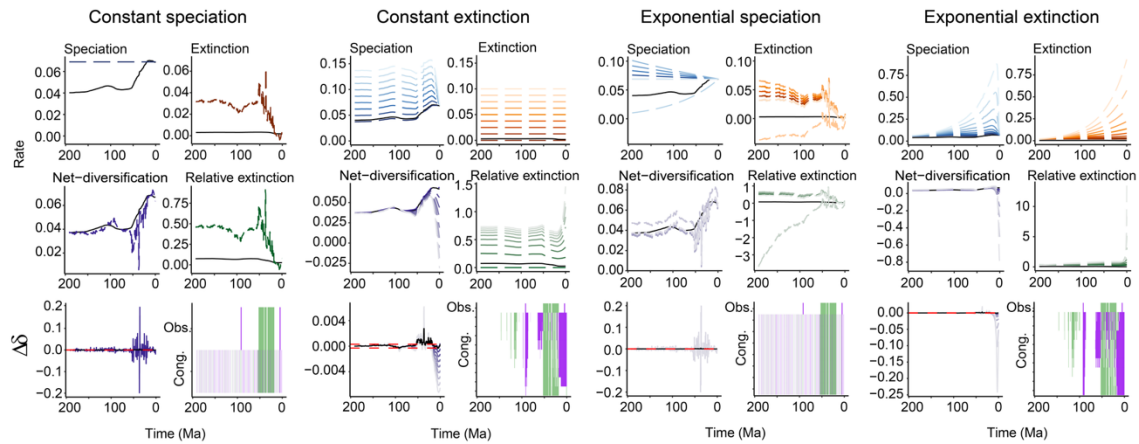


Fig. S4. Congruent diversification scenarios for reef-associated species. The range of congruent diversification patterns. Constant speciation, constant extinction, exponentially decreasing speciation, and exponentially increasing extinction scenarios are reported. For each scenario, congruent trends are reported relative to the observed rate (solid black line); purple represents qualitative patterns of rate decreases, green represents qualitative patterns of rate increases, and white represents no rate change between intervals.

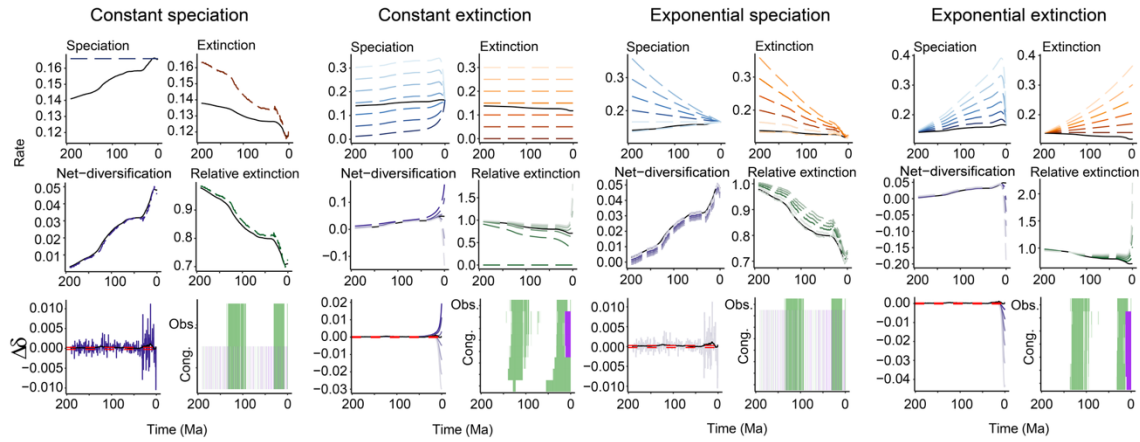


Fig. S5. Congruent diversification scenarios for freshwater demersal species. The range of congruent diversification patterns. Constant speciation, constant extinction, exponentially decreasing speciation, and exponentially increasing extinction scenarios are reported. For each scenario, congruent trends are reported relative to the observed rate (solid blackline); purple represents qualitative patterns of rate decreases, green represents qualitative patterns of rate increases, and white represents no rate change between intervals.

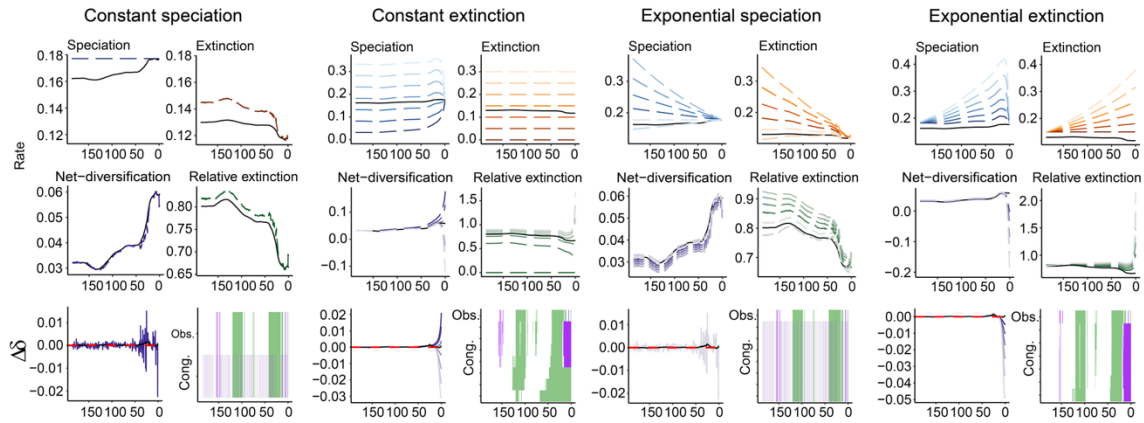


Fig. S6. Congruent diversification scenarios for freshwater benthopelagic species. The range of congruent diversification patterns. Constant speciation, constant extinction, exponentially decreasing speciation, and exponentially increasing extinction scenarios are reported. For each scenario, congruent trends are reported relative to the observed rate (solid black line); purple represents qualitative patterns of rate decreases, green represents qualitative patterns of rate increases, and white represents no rate change between intervals.

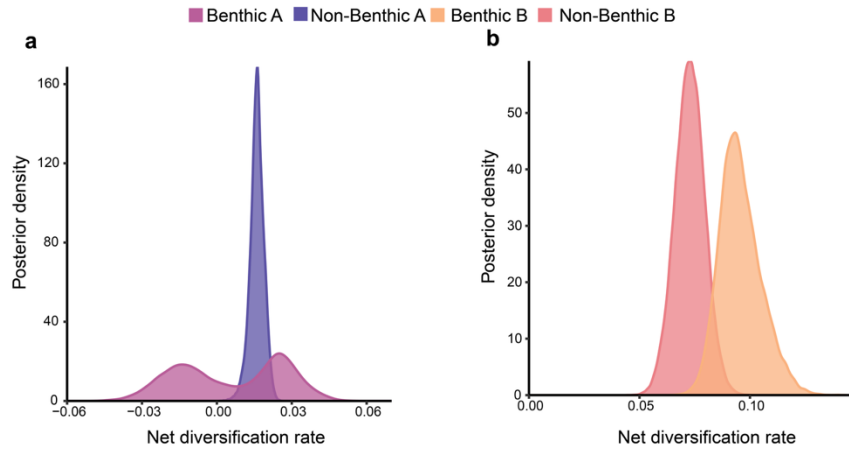


Fig. S7. The effect of benthic feeding is stable under alternate extinction priors. Net diversification rates in **a**, hidden state A and **b**, hidden state B, estimated under a HiSSE-2 model, when the extinction rate is at least half of the speciation rate.

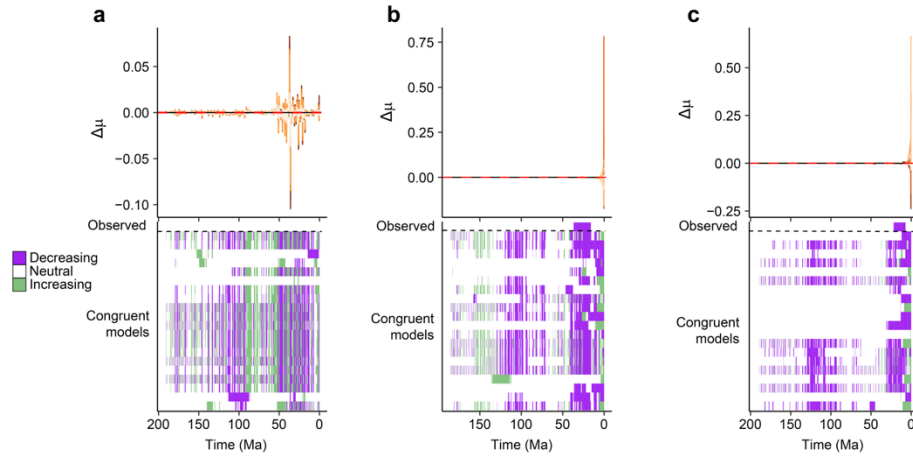


Fig. S8. Extinction rate trends of congruent diversification scenarios. Trends in the extinction rate change between intervals for **a**, reef-associated lineages, **b**, freshwater benthopelagic lineages, and **c**, freshwater demersal lineages under congruent diversification scenarios generated through joint sampling of speciation and extinction rates with horseshoe Markov random field (HSMRF) *p*-trajectories.

References

1. Stadler, T. Mammalian phylogeny reveals recent diversification rate shifts. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 6187–6192 (2011).
2. Quintero, I., Lartillot, N. & Morlon, H. Imbalanced speciation pulses sustain the radiation of mammals. *Science* **384**, 1007–1012 (2024).
3. Alfaro, M. E. *et al.* Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. *Proc. Natl. Acad. Sci. U.S.A.* **106**, 13410–13414 (2009).
4. Sepkoski, J. J. Rates of speciation in the fossil record. *Philosophical Transactions of the Royal Society B: Biological Sciences* **353**, 315–326 (1998).
5. Bininda-Emonds, O. R. P. *et al.* The delayed rise of present-day mammals. *Nature* **446**, 507–512 (2007).
6. Magallón, S. & Castillo, A. Angiosperm diversification through time. *American J of Botany* **96**, 349–365 (2009).
7. Quintero, I., Andréoletti, J., Silvestro, D. & Morlon, H. Loss of macroevolutionary species fitness explains the rise and fall of clades. *Nat Ecol Evol* <https://doi.org/10.1038/s41559-025-02873-7> (2025) doi:10.1038/s41559-025-02873-7.
8. Valente, L. M., Savolainen, V. & Vargas, P. Unparalleled rates of species diversification in Europe. *Proc. R. Soc. B.* **277**, 1489–1496 (2010).
9. Cai, T. *et al.* The role of evolutionary time, diversification rates and dispersal in determining the global diversity of a large radiation of passerine birds. *Journal of Biogeography* **47**, 1612–1625 (2020).
10. Tank, D. C. *et al.* Nested radiations and the pulse of angiosperm diversification: increased diversification rates often follow whole genome duplications. *New Phytologist* **207**, 454–467 (2015).
11. Miller, A. H., Stroud, J. T. & Losos, J. B. The ecology and evolution of key innovations. *Trends in Ecology & Evolution* **38**, 122–131 (2023).
12. Donoghue, M. J. & Sanderson, M. J. Confluence, synnovation, and depauperons in plant diversification. *New Phytologist* **207**, 260–274 (2015).
13. McPeck, M. A. The Ecological Dynamics of Clade Diversification and Community Assembly. *The American Naturalist* **172**, E270–E284 (2008).
14. Moen, D. & Morlon, H. Why does diversification slow down? *Trends in Ecology & Evolution* **29**, 190–197 (2014).
15. Morlon, H., Potts, M. D. & Plotkin, J. B. Inferring the Dynamics of Diversification: A Coalescent Approach. *PLoS Biol* **8**, e1000493 (2010).
16. Phillimore, A. B. & Price, T. D. Density-Dependent Cladogenesis in Birds. *PLoS Biol* **6**, e71 (2008).
17. Toussaint, E. F. A. *et al.* Explosive Cenozoic radiation and diversity-dependent diversification dynamics shaped the evolution of Australian skipper butterflies. *Evolutionary Journal of the Linnean Society* **1**, kzac001 (2022).
18. Rabosky, D. L. Ecological limits and diversification rate: alternative paradigms to explain the variation in species richness among clades and regions. *Ecology Letters* **12**, 735–743 (2009).
19. Rabosky, D. L. & Lovette, I. J. Density-dependent diversification in North American wood warblers. *Proc. R. Soc. B.* **275**, 2363–2371 (2008).
20. Losos, J. B. & Schluter, D. Analysis of an evolutionary species–area relationship. *Nature* **408**, 847–850 (2000).

21. Davies, T. J., Savolainen, V., Chase, M. W., Goldblatt, P. & Barraclough, T. G. Environment, Area, and Diversification in the Species-Rich Flowering Plant Family Iridaceae. *The American Naturalist* **166**, 418–425 (2005).
22. Ricklefs, R. E. Global variation in the diversification rate of passerine birds. *Ecology* **87**, 2468–2478 (2006).
23. Ivory, S. J. *et al.* Environmental change explains cichlid adaptive radiation at Lake Malawi over the past 1.2 million years. *Proc. Natl. Acad. Sci. U.S.A.* **113**, 11895–11900 (2016).
24. Seehausen, O. & Wagner, C. E. Speciation in Freshwater Fishes. *Annu. Rev. Ecol. Evol. Syst.* **45**, 621–651 (2014).
25. Rocha, L. A., Robertson, D. R., Roman, J. & Bowen, B. W. Ecological speciation in tropical reef fishes. *Proc. R. Soc. B.* **272**, 573–579 (2005).
26. Schluter, D. Ecological Speciation in Postglacial Fishes. *Proceedings of the Royal Society B: Biological Sciences* **351**, 807–814 (1996).
27. Connell, J. H. Diversity in Tropical Rain Forests and Coral Reefs. *Science* **199**, 1302–1310 (1978).
28. Gratwicke, B. & Speight, M. R. The relationship between fish species richness, abundance and habitat complexity in a range of shallow tropical marine habitats. *Journal of Fish Biology* **66**, 650–667 (2005).
29. Kovalenko, K. E., Thomaz, S. M. & Warfe, D. M. Habitat complexity: approaches and future directions. *Hydrobiologia* **685**, 1–17 (2012).
30. Graham, N. A. J. & Nash, K. L. The importance of structural complexity in coral reef ecosystems. *Coral Reefs* **32**, 315–326 (2013).
31. Burress, E. D. & Hart, P. B. Pelagic zone is an evolutionary catalyst, but an ecological dead end, for North American minnows. *Evolution* **78**, 1396–1404 (2024).
32. Friedman, S. T. *et al.* Body shape diversification along the benthic–pelagic axis in marine fishes. *Proc. R. Soc. B.* **287**, 20201053 (2020).
33. Palumbi, S. R. Genetic Divergence, Reproductive Isolation, and Marine Speciation. *Annual Review of Ecology and Systematics* **25**, 547–572 (1994).
34. McClain, C. R. & Hardy, S. M. The dynamics of biogeographic ranges in the deep sea. *Proc. R. Soc. B.* **277**, 3533–3546 (2010).
35. Sibert, E., Norris, R., Cuevas, J. & Graves, L. Eighty-five million years of Pacific Ocean gyre ecosystem structure: long-term stability marked by punctuated change. *Proc. R. Soc. B.* **283**, 20160189 (2016).
36. Leckie, R. M. Paleoecology of Mid-Cretaceous Planktonic Foraminifera: A Comparison of Open Ocean and Epicontinental Sea Assemblages. *Micropaleontology* **33**, 164 (1987).
37. Rabosky, D. L. Speciation rate and the diversity of fishes in freshwaters and the oceans. *Journal of Biogeography* **47**, 1207–1217 (2020).
38. Miller, E. C. Comparing diversification rates in lakes, rivers, and the sea. *Evolution* **75**, 2055–2073 (2021).
39. Höhna, S. *et al.* RevBayes: Bayesian Phylogenetic Inference Using Graphical Models and an Interactive Model-Specification Language. *Syst Biol* **65**, 726–736 (2016).
40. Magee, A. F., Höhna, S., Vasylyeva, T. I., Leaché, A. D. & Minin, V. N. Locally adaptive Bayesian birth-death model successfully detects slow and rapid rate shifts. *PLoS Comput Biol* **16**, e1007999 (2020).
41. Horn, M. H. The amount of space available for marine and freshwater fishes. *Fishery Bulletin* **70**, 1295–1297 (1972).
42. Spalding, M. D., Ravilious, C. & Green, E. P. *World Atlas of Coral Reefs*. (University of California Press, Berkeley, CA, 2001).

43. Louca, S. & Pennell, M. W. Extant timetrees are consistent with a myriad of diversification histories. *Nature* **580**, 502–505 (2020).
44. Kopperud, B. T., Magee, A. F. & Höhna, S. Rapidly changing speciation and extinction rates can be inferred in spite of nonidentifiability. *Proc. Natl. Acad. Sci. U.S.A.* **120**, e2208851120 (2023).
45. Höhna, S., Kopperud, B. T. & Magee, A. F. CRABS: Congruent rate analyses in birth–death scenarios. *Methods Ecol Evol* **13**, 2709–2718 (2022).
46. Höhna, S. *et al.* A Bayesian Approach for Estimating Branch-Specific Speciation and Extinction Rates. Preprint at <https://doi.org/10.1101/555805> (2019).
47. Corn, K. A. *et al.* The rise of biting during the Cenozoic fueled reef fish body shape diversification. *Proc. Natl. Acad. Sci. U.S.A.* **119**, e2119828119 (2022).
48. Siqueira, A. C., Morais, R. A., Bellwood, D. R. & Cowman, P. F. Trophic innovations fuel reef fish diversification. *Nat Commun* **11**, 2669 (2020).
49. Harmon, L. J., Schulte, J. A., Larson, A. & Losos, J. B. Tempo and Mode of Evolutionary Radiation in Iguanian Lizards. *Science* **301**, 961–964 (2003).
50. Kiessling, W., Simpson, C. & Foote, M. Reefs as Cradles of Evolution and Sources of Biodiversity in the Phanerozoic. *Science* **327**, 196–198 (2010).
51. Close, R. A., Benson, R. B. J., Kiessling, W. & Saupe, E. E. Reefal regions were biodiversity hotspots throughout the Phanerozoic. *Science Advances* **11**, eadv9793 (2025).
52. Leprieur, F., Pellissier, L., Mouillot, D. & Gaboriau, T. Influence of historical changes in tropical reef habitat on the diversification of coral reef fishes. *Sci Rep* **11**, 20731 (2021).
53. Cowman, P. F. & Bellwood, D. R. Coral reefs as drivers of cladogenesis: expanding coral reefs, cryptic extinction events, and the development of biodiversity hotspots: Cladogenesis on coral reefs. *Journal of Evolutionary Biology* **24**, 2543–2562 (2011).
54. Alfaro, M. E., Santini, F. & Brock, C. D. Do reefs drive diversification in marine teleosts? Evidence from the pufferfish and their allies (order Tetraodontiformes). *Evolution* **61**, 2104–2126 (2007).
55. Beaulieu, J. M. & O’Meara, B. C. Detecting Hidden Diversification Shifts in Models of Trait-Dependent Speciation and Extinction. *Syst Biol* **65**, 583–601 (2016).
56. Alroy, J. Dynamics of origination and extinction in the marine fossil record. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 11536–11542 (2008).
57. Kiessling, W. Sampling-standardized expansion and collapse of reef building in the Phanerozoic. *Foss. Rec.* **11**, 7–18 (2008).
58. Siqueira, A. C., Kiessling, W. & Bellwood, D. R. Fast-growing species shape the evolution of reef corals. *Nat Commun* **13**, (2022).
59. Vaga, C. F. *et al.* A global coral phylogeny reveals resilience and vulnerability through deep time. *Nature* <https://doi.org/10.1038/s41586-025-09615-6> (2025) doi:10.1038/s41586-025-09615-6.
60. Feichtinger, I. *et al.* Ecological restructuring of North Tethyan marine vertebrate communities triggered by the end-Cretaceous extinction. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2409366122 (2025).
61. Edie, S. M., Collins, K. S. & Jablonski, D. The end-Cretaceous mass extinction restructured functional diversity but failed to configure the modern marine biota. *Science Advances* **11**, eadv1171 (2025).
62. Ghezelayagh, A. *et al.* Prolonged morphological expansion of spiny-rayed fishes following the end-Cretaceous. *Nat Ecol Evol* **6**, 1211–1220 (2022).
63. Friedman, M. Explosive morphological diversification of spiny-finned teleost fishes in the aftermath of the end-Cretaceous extinction. *Proc. R. Soc. B.* **277**, 1675–1683 (2010).

64. Bellwood, D. R. Origins and escalation of herbivory in fishes: a functional perspective. *Paleobiology* **29**, 71–83 (2003).
65. Zachos, J. C., Dickens, G. R. & Zeebe, R. E. An early Cenozoic perspective on greenhouse warming and carbon-cycle dynamics. *Nature* **451**, 279–283 (2008).
66. Gibbs, S. J., Bown, P. R., Sessa, J. A., Bralower, T. J. & Wilson, P. A. Nannoplankton Extinction and Origination Across the Paleocene-Eocene Thermal Maximum. *Science* **314**, 1770–1773 (2006).
67. Kennett, J. P. & Stott, L. D. Abrupt deep-sea warming, palaeoceanographic changes and benthic extinctions at the end of the Palaeocene. *Nature* **353**, 225–229 (1991).
68. Sreenivasan, S. P. *et al.* Increasing primary productivity in the oligotrophic Tethyan coastal ocean during the Paleocene-Eocene warming episode. *Global and Planetary Change* **216**, 103898 (2022).
69. Dunne, J. A., Labandeira, C. C. & Williams, R. J. Highly resolved early Eocene food webs show development of modern trophic structure after the end-Cretaceous extinction. *Proc. R. Soc. B* **281**, 20133280 (2014).
70. Afzal, J., Williams, M., Leng, M. J. & Aldridge, R. J. Dynamic response of the shallow marine benthic ecosystem to regional and pan-Tethyan environmental change at the Paleocene–Eocene boundary. *Palaeogeography, Palaeoclimatology, Palaeoecology* **309**, 141–160 (2011).
71. Scheibner, C. & Speijer, R. P. Decline of coral reefs during late Paleocene to early Eocene global warming. *eEarth* **3**, 19–26 (2008).
72. Foster, W. J. *et al.* Resilience of marine invertebrate communities during the early Cenozoic hyperthermals. *Sci Rep* **10**, 2176 (2020).
73. Arcila, D. & Tyler, J. C. Mass extinction in tetraodontiform fishes linked to the Palaeocene–Eocene thermal maximum. *Proc. R. Soc. B* **284**, 20171771 (2017).
74. El-Sayed, S., Friedman, M., Anan, T., Faris, M. A. & Sallam, H. Diverse marine fish assemblages inhabited the paleotropics during the Paleocene-Eocene thermal maximum. *Geology* **49**, 993–998 (2021).
75. Mihalitsis, M. *et al.* Lateral jaw motion in fish expands the functional repertoire of vertebrates and underpins the success of a dominant herbivore lineage. *Proc. Natl. Acad. Sci. U.S.A.* **122**, e2418982122 (2025).
76. Peoples, N., Burns, M. D., Mihalitsis, M. & Wainwright, P. C. Evolutionary lability of a key innovation spurs rapid diversification. *Nature* **639**, 962–967 (2025).
77. Gibb, A. C., Staab, K., Moran, C. & Ferry, L. A. The Teleost Intramandibular Joint: A mechanism That Allows Fish to Obtain Prey Unavailable to Suction Feeders. *Integrative and Comparative Biology* **55**, 85–96 (2015).
78. Wainwright, P. C. & Price, S. A. Innovation and Diversity of the Feeding Mechanism in Parrotfishes. in *Biology of Parrotfishes* (eds Hoey, A. S. & Bonaldo, R. M.) 26–41 (CRC Press, Boca Raton, FL: CRC Press, Taylor & Francis Group, [2018] “A Science Publishers book.” | Include bibliographical references and index., 2018). doi:10.1201/9781315118079-2.
79. Duque-Correa, M. J. *et al.* Diet and habitat as determinants of intestine length in fishes. *Rev Fish Biol Fisheries* **34**, 1017–1034 (2024).
80. Ghilardi, M. *et al.* Phylogeny, body morphology, and trophic level shape intestinal traits in coral reef fishes. *Ecology and Evolution* **11**, 13218–13231 (2021).
81. Rabosky, D. L. *et al.* An inverse latitudinal gradient in speciation rate for marine fishes. *Nature* **559**, 392–395 (2018).
82. Froese, R. & Pauly, D. FishBase. www.fishbase.org.
83. Tribble, C. M. *et al.* RevGadgets: An R package for visualizing Bayesian phylogenetic analyses from RevBayes. *Methods Ecol Evol* **13**, 314–323 (2022).

84. Drummond, A. J. & Suchard, M. A. Bayesian random local clocks, or one rate to rule them all. *BMC Biol* **8**, (2010).
85. Fabreti, L., Höhna, S. & Magee, A. convenience: Convergence assessment for phylogenetic inference. (2024).
86. Andréoletti, J. & Morlon, H. Exploring congruent diversification histories with flexibility and parsimony. *Methods Ecol Evol* **14**, 2931–2941 (2023).
87. Huang, D. & Roy, K. The future of evolutionary diversity in reef corals. *Phil. Trans. R. Soc. B* **370**, 20140010 (2015).
88. Zenil-Ferguson, R., McEntee, J. P., Burleigh, J. G. & Duckworth, R. A. Linking Ecological Specialization to Its Macroevolutionary Consequences: An Example with Passerine Nest Type. *Systematic Biology* **72**, 294–306 (2023).