



Resolving the ray-finned fish tree of life

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The Challenge of Reconstructing Fish Phylogeny

When it comes to vertebrate evolutionary history, our understanding of lobe-finned fishes—the branch of the vertebrate tree leading to coelacanths plus the tetrapods (amphibians, turtles, birds, crocodiles, lizards, snakes, and mammals)—far outstrips our knowledge of ray-finned fishes (Actinopterygii). Actinopterygians exhibit extraordinary species richness (>33,000 described species) and have evolved a staggering diversity in morphology and ecology over their 400+ million y history. Ray-finned fishes include some of the smallest vertebrates [the adult cyprinid *Paedocypris progenetica* measure just under 8 mm (1)], some of the largest (adult ocean sunfish weigh more than 2,000 kg), some of the longest (oarfishes may reach a length of more than 13 m), some of the longest lived [rougheye rockfishes, *Sebastes aleutianus*, may live for more than 200 y (2)], and some of the shortest lived [the coral reef pygmy goby, *Eviota sigillata*, has a maximum lifespan of 59 d (3)]. In marine waters, ray-finned fishes include the tremendously diverse and ecologically rich coral reef fish families, such as wrasses, angelfishes, butterfly fishes, and damselfishes, and they comprise most important commercial and recreational fishing stocks. Within freshwaters, ray-finned fishes have produced several ecologically dominant radiations, including cyprinids, characiforms, catfishes, and cichlids. Efforts to reconstruct the phylogenetic history of this group have proven extremely challenging (4, 5), especially within acanthomorphs (6, 7), a hyperdiverse subclade comprising almost two-thirds of all ray-finned fish species.

One of the major impediments to understanding higher-level ray-finned fish relationships has been the lack of obvious morphological characters that unite major lineages (reviewed in ref. 8). Within the last 5 y, large-scale multilocus sequencing efforts have produced novel insights in the ray-finned fish backbone phylogeny, including unexpectedly close relationships among morphologically disparate lineages (9–11). Testing these new hypotheses is critical for understanding vertebrate evolutionary history, yet little evidence beyond the original set of nuclear markers

responsible for generating the trees in the first place has emerged. In PNAS, Hughes et al. (12), use over 1,000 new markers using phylogenomic and transcriptomic techniques to reconstruct the evolutionary history of ray-finned fishes. Hughes et al.'s phylogeny reveals many key features of the backbone of the ray-finned fish tree of life and provides the most comprehensive evolutionary framework to date for this major radiation of vertebrates.

Phylogenomics has revolutionized evolutionary reconstruction by vastly expanding both the number of loci that can be interrogated across nonmodel organisms, as well as the efficiency with which the data may be collected (13). The most recent multilocus studies that underlie our current understanding of the ray-finned fish tree of life are based upon 10–20 markers (e.g., refs. 9 and 11). These studies represent the culmination of decades of effort to identify genes with reasonable phylogenetic performance, as well as the laborious effort to amplify and sequence each marker for each species (14). In contrast, modern genome sequencing and transcriptomics enable rapid and cost-effective collection of genome-scale data for any species, shifting the challenge of phylogenetic analysis from intensive laboratory work to identifying the markers that are most appropriate for the questions at hand.

Hughes et al. (12) introduce a pipeline that expands the genomic resources for phylogenetic inference in fishes by identifying and extracting 1,105 protein coding genes from genomic alignments. Inferring the ray-finned fish tree of life using phylogenomic data is potentially confounded by two episodes of genomic expansion in the history of the group (15, 16): one at the base of all vertebrates and the second at the base of all teleost fishes (a group comprising over 99% of ray-finned fish species). Hughes et al. (12) show that naive phylogenomic pipelines will likely recover a significant number of paralogs that in turn may mislead evolutionary inferences at the tree-of-life scale. By filtering out gene trees that fail to recover the monophyly of three major fish groups that emerged following

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genomic expansion events (teleosts, euteleosts, and otophysans), Hughes et al. arrive at a final marker set for their analyses with maximal power to accurately reconstruct actinopterygian evolutionary history.

Can More Markers Help Resolve Controversial Nodes and Questions About the Timing of Diversification?

The growth of molecular phylogenetics has led to conflicting perspectives on the evolutionary relationships among several of the most distinctive lineages on the ray-finned fish tree of life. The branching arrangement of the base of the teleost tree is one such area of conflict, with morphological studies generally supporting the elopomorphs (eels and tarpon) and osteoglossomorphs (bony tongues) as successive sister lineages to remaining teleosts (4). This result is recovered by some multilocus (11, 17) and phylogenomic studies (18), while others support a sister group relationship between elopomorphs and osteoglossomorphs (18). The arrangement of the otophysans (a species-rich clade containing more than two-thirds of the world's freshwater species, including characins, knifefishes, minnows, and catfishes), remains highly controversial, as morphological studies unambiguously recover the monophyly of the characiformes (19), while molecular studies generally render this group paraphyletic with respect to other otophysan lineages (20; but see ref. 21). Hughes et al. (12) investigate the strength of support across the genome for alternative resolutions of contentious nodes through a recently developed statistical pipeline (21). The authors (12) show that for at least some controversial nodes, including teleosts and otophysans, conflict between morphological and molecular results appears to be driven by noise in some gene partitions. When only markers with strong phylogenetic signal are considered, the favored molecular and morphological topologies are the same. Their analysis underscores the need to consider both the strength of support for novel phylogenetic results and the distribution of that support across gene partitions in the analysis of massive next-generation datasets.

Another vexing phylogenetic problem in vertebrate evolutionary biology concerns the percomorphs, the largest acanthomorph subclade, with over 17,000 species. Recent multilocus studies (8, 11) resolve the astonishing richness of this group into nine major lineages, including Gobiaria (gobies), Pelagiaria (tunas), Syngnatharia (seahorses and pipefishes), Carangaria (jacks and flatfishes), Ovalentaria (cichlids, blennies, and clingfishes), and Eupercaria (groupers, sculpins). In PNAS, Hughes et al. (12) show strong genome-wide signal for the monophyly of these groups, as well as for relationships among them. Given the extraordinary diversity of living percomorphs today, a key evolutionary question concerns the origin and rise of this group. Most

prior divergence-time studies show that this massive radiation unfolded largely over the Late Cretaceous and some have dubbed this period the "Second Age of Fishes" (9). However, some fossil and phylogenomic studies implicate the mass extinction and recovery at the K-Pg (Cretaceous–Paleogene) boundary as major forces that restructured marine fish communities (22) and fueled the rise of percomorphs (23, 24). Hughes et al. (12) show that the diversification within major percomorph groups predates the K–Pg boundary.

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The underlying causes for the discrepancy in divergence times estimated by these studies is not immediately clear and could reflect differences in the rates of evolution between coding and noncoding phylogenomic markers, differences in the treatment of fossil constrained nodes, or differences in taxonomic sampling. However, resolution of the timing of percomorph diversification is critical to evaluating explanations for the evolutionary success of the groups. If the modern percomorph fauna can be linked to postextinction recovery, then the mass extinction at the K–Pg was key to shaping diversity across major groups of both terrestrial and aquatic groups today. Alternatively, if diversification happened over a broader window of time that predates the mass extinction, percomorph diversity may be better understood as a consequence of lineage-specific factors relating to the ecological flexibility of the group as a whole (9).

The success of multilocus studies substantially improved the prospects of resolving higher-level relationships among ray-finned fishes. In PNAS, Hughes et al. (12) deliver a significant advance on this front. Their phylogeny, the most robustly supported hypothesis of relationship of ray-finned fishes to date, provides a stable framework for understanding actinopterygian evolutionary history as well as a road-map for fleshing-out relationship among the ~500 families of this spectacular radiation. Perhaps more importantly, by providing this new framework in conjunction with a powerful set of markers that can be readily sequenced across divergent taxa and that are informative across phylogenetic scales, Hughes et al. (12) make a credible case for building a truly comprehensive, species-level phylogeny for all fishes in the coming decade.

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