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BOOM AND BUST: ANCIENT AND RECENT DIVERSIFICATION IN BICHIRS (POLYPTERIDAE: ACTINOPTERYGII), A RELICTUAL LINEAGE OF RAY-FINNED FISHES

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Understanding the history that underlies patterns of species richness across the Tree of Life requires an investigation of the mechanisms that not only generate young species-rich clades, but also those that maintain species-poor lineages over long stretches of evolutionary time. However, diversification dynamics that underlie ancient species-poor lineages are often hidden due to a lack of fossil evidence. Using information from the fossil record and time calibrated molecular phylogenies, we investigate the history of lineage diversification in Polypteridae, which is the sister lineage of all other ray-finned fishes (Actinopterygii). Despite originating at least 390 million years (Myr) ago, molecular timetrees support a Neogene origin for the living polypterid species. Our analyses demonstrate polypterids are exceptionally species depauperate with a stem lineage duration that exceeds 380 million years (Ma) and is significantly longer than the stem lineage durations observed in other ray-finned fish lineages. Analyses of the fossil record show an early Late Cretaceous (100.5–83.6 Ma) peak in polypterid genus richness, followed by 60 Ma of low richness. The Neogene species radiation and evidence for high-diversity intervals in the geological past suggest a "boom and bust" pattern of diversification that contrasts with common perceptions of relative evolutionary stasis in so-called "living fossils."

KEY WORDS: Africa, depauperon, extinction, Hippopotomine Event, living fossil, paleodiversity, species tree.

The uneven distribution of species richness is one of the most striking patterns observed across the vertebrate Tree of Life. The last decade has seen progress in identifying both lineages that exhibit extraordinarily high levels of species richness and factors that may underlie their higher than expected rates of lineage diversification (Alfaro et al. 2009; Santini et al. 2009; Near et al. 2012a; Near et al. 2013), but less attention is devoted to identifying and understanding the evolution of ancient and exceptionally species-poor lineages (Alfaro et al. 2009; Rabosky et al. 2013). As the study of recently diverged and species-rich lineages illuminate the processes that generate biodiversity (Wagner et al. 2012), ancient and species-depauperate clades provide opportunities to investigate how biodiversity is maintained over long periods of evolutionary time (Casane and Laurenti 2013; Mathers et al.

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2013). However, developing an understanding of the evolutionary diversification of species-poor lineages requires assessment of any available paleontological information, as patterns observed solely from living species may fail to capture diversification dynamics evident in the fossil record (Morlon et al. 2011; Slater et al. 2012).

The survival of several early diverging lineages of actinopterygians (ray-finned fishes) is particularly intriguing. Comprising more than half of the approximately 63,000 living vertebrate species, crown lineage actinopterygians originated in the Devonian (Gardiner and Schaeffer 1989), yet more than 99% of their living diversity is contained within the geologically younger (Triassic-Recent) teleost radiation (Friedman and Sallan 2012; Near et al. 2012b; Betancur-R et al. 2013; Broughton et al. 2013). Early diverging nonteleost actinopterygian lineages, such as Holostei (bowfin and gars), gave examples for Darwin's articulation of the concept of living fossils (1859, p. 107), and continued to be viewed as such on the basis of their ancient origins, low morphological diversity, and presence of ancestral character states relative to living teleosts (Stanley 1979, pp. 124–125; Gardiner 1984; Schultze and Wiley 1984; Wiley and Schultze 1984; Rabosky et al. 2013). Conversely, another of Darwin's living fossils, the sturgeons, exhibit high rates of body size evolution despite their ancient origins and relatively low species richness (Rabosky et al. 2013). This suggests that the label of "living fossils" may not adequately capture the patterns that underlie the origin and maintenance of biodiversity across all ancient ray-finned fish lineages, thus warranting more detailed investigations of early diverging and species poor clades of actinopterygians.

Polypteridae (bichirs and reedfish) comprises 18 species of freshwater fishes endemic to Africa (Eschmeyer and Fricke 2012) that are consistently resolved as the sister lineage of all other ray-finned fishes (Patterson 1982; Gardiner and Schaeffer 1989; Coates 1999; Kikugawa et al. 2004; Broughton et al. 2013). Polypterids have been regarded as living fossils, not because of a resemblance of living species with those preserved in the fossil record, but rather due to their early divergence from other ray-finned fishes and the retention of numerous ancestral actinopterygian character states, including ganoid scales, akinetic upper jaws, a fully enclosed aortic canal, functional lungs, a heterocercal tail, and large gular plates (Boulenger 1909, p. 4; Goodrich 1928; Patterson 1982; Greenwood 1984). Despite the presence of these ancestral features, the overall body plan of polypterids diverges strongly from the stratigraphically earliest ray-finned fishes (Fig. 1), exhibiting striking derived traits, including distinctive dorsal finlets, a unique anatomy of the pectoral fin, a slender eel-like trunk, reduced number of gill arches, uniquely derived dorsal ribs, and absence of a spiracular canal in the neurocranium (Jessen 1973; Greenwood 1984; Britz and Bartsch 2003; Britz and Johnson 2003). All but one of the 18 polypterid species is classified in *Polypterus*. The Reedfish, *Erpetoichthys calabaricus*, is similar morphologically to species of *Polypterus*, but is more elongate and lacks pelvic fins (Fig. 1). Overall there is little disparity in morphology among living polypterid species, which are diagnosed from one another on the basis of scale row counts, number of dorsal finlets, subtle morphometric ratios, and coloration (Greenwood 1984; Britz 2004).

Phylogenetic analyses based on morphological characters that include fossils of early ray-finned fishes indicate that the most recent common ancestor (MRCA) of polypterids and all other ray-finned fish lineages dates to approximately 390 million years (Ma) in the Devonian (Gardiner and Schaeffer 1989; Coates 1999; Long and Trinajstic 2010). In contrast to the antiquity of this divergence, the fossil record of polypterids consists primarily of scales and isolated skeletal elements that extends only to the Late Cretaceous of Africa and South America, leaving at least a 300 Ma gap in the fossil record (Gayet et al. 2002). Given the paucity of complete polypterid fossils (Otero et al. 2006), it is not known if the limited morphological variety among living polypterid species is the consequence of being an ancient lineage with unusually high species longevity that exhibits low rates of phenotypic changes, as is expected from a classic definition of many living fossil lineages (e.g., Stanley 1979, p. 123), or recent diversification relative to their deep evolutionary origins.

We adopted a multidisciplinary approach to distinguish between these alternatives. First, we examined the phylogenetic relationships and timing of diversification of Polypteridae using DNA sequences from eight nuclear protein coding genes sampled from 15 polypterid species. Phylogenies inferred using multispecies coalescent species tree analyses were time calibrated using Bayesian relaxed molecular clock methods. Second, because polypterids are often casually referred to as "living fossils," we characterize lineage diversification dynamics to determine if evolutionary patterns shown by this group deviate significantly from other clades of actinopterygians. Third, we surveyed the fossil record of polypterids, and of freshwater fishes in Africa more generally, to test previous hypotheses about patterns of lineage diversity in the clade. Collectively, our results demonstrate that crown-group Polypteridae originated about 20 million years (Myr) ago in the Miocene, and exhibits a pattern of lineage diversification that is unique among ray-finned fishes. Contrary to other lineages of ray-finned fishes previously labeled as living fossils, for example, holosteans and acipenseriforms (Stanley 1979, pp. 124–126), the diversification of polypterids is characterized by a significantly protracted stem lineage duration that separates the evolutionary origin of the clade from the diversification of the living species by hundreds of millions of years. Based on fossil evidence, we resolve a Late Cretaceous peak of polypterid genus-level richness that is independent of the radiation of living species and persists when the effects of uneven paleontological sampling

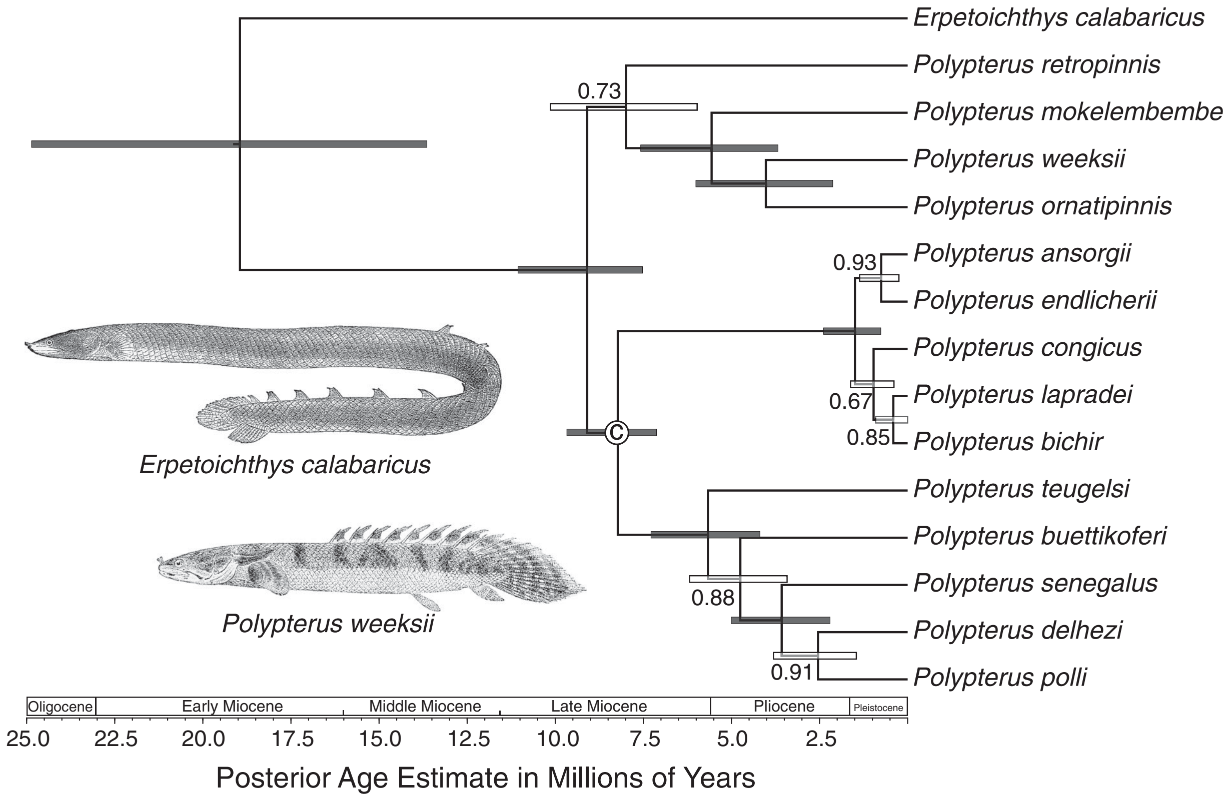

Figure 1. Time-calibrated species tree of Polypteridae that shows the estimated divergence times in millions of years and overlaid onto the geological time scale epochs. Bars at nodes represent the 95% highest posterior density interval of the molecular age estimates, where filled bars highlight nodes strongly supported with (≥0.95) Bayesian posterior probabilities. Posterior values are given for nodes without strong Bayesian support. The node in the phylogeny calibrated with the fossil age prior is marked with a "C" inside of a circle. Drawing of polypterid species modified from Boulenger (1909).

are considered. This interval of elevated polypterid diversity is separated from the onset of diversification of the extant lineages by at least 60 Ma, and implies a volatile "boom and bust" pattern in the history of the polypterid fishes that contrasts with classical notions of evolutionary dynamics in living fossils.

Materials and Methods

GENE SEQUENCING AND PHYLOGENETIC ANALYSES

The taxon sampling was identical to a previous phylogenetic analysis of Polypteridae using mtDNA sequence data (Suzuki et al. 2010b), and included 15 polypterid species sampled from a total of 33 specimens. One to four specimens were sampled for each species, but we were unable to sample *Polypterus lowei* (previously treated as a subspecies of *P. retropinnis*), *P. meridionalis* (previously treated as a subspecies of *P. senegalus*), and *P. palmas*, although *P. polli* and *P. buettikoferi*, which are sampled in this study, were previously treated as subspecies of *P. palmas*. As subspecies within a species exhibit morphological differences, an allopatric geographic distribution, and do not show patterns of intergradation or parapatry, we treated all recognized subspecies of *Polypterus* as distinct species (e.g., Hanssens et al. 1995). Museum voucher information for all specimens sampled for DNA sequencing is presented in Table S1.

We collected nucleotide sequence data from eight protein coding nuclear genes (*Glyt*, *plagl2*, *Ptr*, *rag1*, *rag2*, *ryr3*, *sreb2*, and *zic1*) using previously published primers (Lopez et al. 2004; Sullivan et al. 2006; Li et al. 2007). Individual gene sequences were aligned by eye and guided by the translated amino acid sequences. DNA sequences were submitted to Genbank KF792465-KF792728 and nexus formatted alignments of each gene are available from the Dryad Digital Repository: doi:10.5061/dryad.h6h7c. Partitioning strategies were assessed through comparison of Bayes factor scores with those greater than 10 considered being strong support (Kass and Raftery 1995; Brandley et al. 2005). A two-partition scheme (codons from the first and second combined and third codon positions treated separately) was selected for half of the genes (*Ptr*, *plag12*, *ryr3*, and *sreb2*), each of the three codon positions were partitioned for three genes (*Glyt*, *rag1*, and *rag2*), and the codon positions were not partitioned for the *zic1* locus. Optimal models of nucleotide substitution for each data partition were selected using Akaike information criterion (AIC) as executed in the computer program MrModeltest 2.3 (http://www.abc.se/∼nylander/mrmodeltest2/mrmodeltest2.html).

Gene trees were inferred from each locus using the computer program MrBayes 3.2 (Ronquist et al. 2012) with the optimal data partitioning schemes and nucleotide substitution models. Each MrBayes analysis was run for 3×10^6 generations with two simultaneous runs each with four chains. Monitoring the average SD of the split frequencies between the two runs assessed stationarity of the chains and convergence, and in all of the gene tree analyses this value was less than 0.005 after 1.5×10^6 generations. The first 50% of the sampled generations were discarded as burn-in and the individual gene trees were summarized as a 50% majority-rule consensus tree.

FOSSIL CALIBRATION PRIOR AND RELAXED MOLECULAR CLOCK ANALYSIS

Fossil-calibrated species trees of Polypteridae were generated using multispecies coalescent analyses performed in *BEAST version 1.6.1 (Drummond and Rambaut 2007; Heled and Drummond 2010). A birth-death speciation branching prior was used. To allow for nucleotide substitution rate heterogeneity, we used a model of uncorrelated rates that follow a lognormal distribution for all eight loci (Drummond et al. 2006). Divergence time analyses were calibrated using the Miocene fossil †*Polypterus faraou* from the Toros-Menalla locality, Chad (Vignaud et al. 2002; Otero et al. 2006) as a minimal age prior that was applied to the MRCA of the clade containing *P. bichir*, *P. ansorgii*, *P. endlicheri*, *P. congicus*, and *P. lapradei* and the clade containing *P. teugelsi*, *P. buettikoferi*, *P. senegalus*, *P. delhezi*, and *P. polli*. The justification for this phylogenetic placement is provided by †*P. faraou* exhibiting a protruding lower jaw, a morphological feature that is characteristic of the clade resolved in molecular phylogenies that contains *P. bichir*, *P. ansorgii*, *P. endlicheri*, *P. congicus*, and *P. lapradei* (Suzuki et al. 2010b). A lognormal distribution was used for the calibration prior with 7 Ma as the minimal age offset with a mean of 0.946 and a SD of 0.8 to set 16.6 Ma as the 95% soft upper bound. This upper bound is based on the upper 95% highest posterior density interval of the relaxed molecular clock estimate of the MRCA of *Polypterus* and *Erpetoichthys* using nine nuclear genes and 36 external fossil calibration priors (Near et al. 2012b). The chain lengths in the *BEAST runs were 10^8 generations with parameters sampled every 10^3 generations. Visualization of the chain likelihoods and calculation of the effective sample size (ESS) for each parameter were conducted using the computer program Tracer version 1.5, with ESS values greater than 200 indicating adequate sampling of the posterior distribution of each parameter estimate. The BEAST formatted xml file is available from the Dryad Digital Repository: doi:10.5061/dryad.h6h7c.

LINEAGE DIVERSIFICATION ANALYSIS

To characterize patterns of lineage diversification in polypterids relative to all other ray-finned fishes, we used a step-wise information theoretic approach, MEDUSA, to incrementally fit increasingly complex models of lineage diversification to the time-calibrated phylogeny of ray-finned fishes presented in Near et al. (2012b). This topology was collapsed into a diversity tree that reflected the species richness of stem lineages of missing taxa based on species diversity values presented in the January 2012 Catalog of Fishes (Table 1; Eschmeyer and Fricke 2012). The fit of either birth-death or Yule (pure birth) models of diversification and associated shift points in the time-calibrated diversity tree were calculated using the AIC. More complex parameter-rich models were iteratively added and compared to the previous model until the iterative model building process no longer offered an improvement in AIC score (Alfaro et al. 2009).

To determine if the stem lineage age interval of polypterids is outside of the range expected in other ancient ray-finned fish lineages, we simulated 10,000 birth death trees over the range of inferred speciation and extinction rate estimates obtained from the MEDUSA analyses using the TreeSim package in R (Cavin 2008; Stadler 2011). Simulated trees were conditioned on both the observed species-richness of polypterids and the age of the MRCA of polypterids and all other ray-finned fishes. The observed stem lineage duration was compared to the resulting distribution of expected stem lineage durations. We accounted for the possibility of the end-Cretaceous mass extinction event contributing to the observed disparity between the stem and crown age intervals of polypterids by repeating the birth–death simulations, this time conditioning on the observed number of living species and a single lineage surviving through the Cretaceous–Paleogene boundary 66 Myr ago.

PALEOBIODIVERSITY ANALYSIS

Previous accounts of patterns of taxonomic diversity of Polypteridae have relied upon raw counts of genera and species in the fossil record (Werner and Gayet 1997; Gayet et al. 2002). These studies have reported major peaks and troughs in the counts of species diversity over polypterid history, but it is unclear whether these represent genuine biological patterns or instead reflect patchiness of the fossil record. It has long been appreciated that paleobiodiversity patterns are biased (Raup 1972), and a series of approaches have emerged to account for documented variation in the sedimentary record that might lead to uneven sampling (e.g., Peters and Foote 2001; Smith and McGowan 2007). We explored the relationship between polypterid paleobiodiversity and a series of geological proxies to determine the degree to which the fossil

Table 1. Species Richness Assigned to Lineages in Collapsed Phylogeny of Actinopterygii for MEDUSA Diversification Rate Analyses.

Lineage	Species Diversity
Erpetoichthys	1
Polypterus	17
Acipenseridae	26
Polyodontidae	2
Amia	1
Lepisosteidae	7
Elopiformes	9
Anguilliformes	943
Albulidae	13
Notacanthoidei	27
Hiodon	2
Osteoglossiformes	216
Denticeps	1
Clupeoidei	394
Alepocephaliformes	140
Gonorynchus	5
Chanos	1
Kneriidae	31
Cypriniformes	4039
Siluriformes	3540
Characiformes	1998
Gymnotiformes	200
Lepidogalaxias	1
Argentiniformes	89
Salmonidae	214
Esociformes	13
Stomiiformes	424
Retropinnidae	6
Osmeridae	14
Salangidae	20
Plecoglossus	1
Galaxiidae	50
Ateleopodidae	13
Aulopiformes	260
Myctophidae	260
Neoscopelidae	6
Percopsiformes	9
Polymixia	10
Zeiformes	33
Gadiformes	609
Stylephorus	1
Lampriformes	25
Beryciformes	262
Ophidiiformes	516
Batrachoididae	82
Unnamed clade of Percomorpha	16,574

record of the group might be driven by variation in sampling or the rock record rather than genuine changes in richness.

Although fossils of Polypteridae are known from South America, they occur at too few horizons to make meaningful statements about diversity patterns in the fossil record of this continent (Gayet et al. 2002). We focused exclusively on the African record of Polypteridae, which is richer and more extensive than its South American counterpart and has a direct bearing on our understanding of extant polypterid species, which are all African in origin. A list of known occurrences of African fossil Polypteridae was assembled from the primary literature (Werner and Gayet 1997; Gayet et al. 2002) and the Paleobiology Database (PaleoDB; paleodb.org). Because many fossil polypterid remains are highly fragmentary and can only be identified to genus, this was selected as the level of taxonomic analysis.

Following Lloyd and Friedman (2013), the number of fossil localities was applied as a sampling proxy and the number of named formations was used as an estimate of available rock volume. Those authors found a highly significant ($P < 0.01$) correlation between these geological quantities and genus-level diversity of fishes in the Phanerozoic record of Great Britain. We made two separate estimates of a locality-based sampling proxy, limiting our survey to Late Cretaceous or younger sites from Africa with the potential to yield polypterids on the basis of depositional environment and the presence of taphonomic analogues (i.e., fossil fishes including but not restricted to polypterids). First, we assembled a list of major African freshwater fish fossil localities assembled through a survey of review papers on the record of the continent (e.g., Murray 2000; Friedman et al. 2013). Second, we calculated the number of collections in PaleoDB bearing nontetrapod Osteichthyes (ray-finned and lobe-finned fishes) that conformed to specific environmental profiles (lacustrine, fluvial, karst, other terrestrial, marginal marine). We also recorded the number of named osteichthyan-bearing formations of Late Cretaceous and younger age from Africa in the PaleoDB as an estimate of sedimentary rock volume available for paleontological sampling. The number of formations is an imperfect proxy for rock volume (Benton et al. 2011), but this measure represents a reasonable first approximation in the absence of more detailed information on the African sedimentary record.

Given the considerable uncertainty in the dating of many of these continental deposits, we have generally binned our taxonomic and locality data by series-level divisions of the geological record. There are two important exceptions. First, we combined the Holocene record with that of the Pleistocene to make a composite Quaternary bin. Second, we split the Late Cretaceous, which is substantially longer than any Cenozoic epoch, into two divisions comprising the Cenomanian–Santonian and the Campanian–Maastrichtian. Collections accessed using the PaleoDB were assigned to bins based on their reported age midpoints.

We tested the correlation between these three geological proxies and observed polypterid richness. Because time series will often show temporal autocorrelation, we gauged the strength of relationship using Pearson rank-order correlation of generalized differences (McKinney 1990). We also applied the modeling approach developed by Lloyd (2012) to determine whether major features of polypterid paleobiodiversity patterns cannot be ascribed to variation in sampling, as gauged by a geological proxy, and might instead represent genuine biodiversity signals. Deviations from the null are expressed as residuals from predicted richness, with relative significance gauged through approximate confidence intervals generated from SEs and SDs of the model. These analyses were completed in the statistical programming language R (www.R-project.org) using functions hosted at: http://www.graemetlloyd.com/meth.html. Data files and R scripts are available from the Dryad Digital Repository: doi:10.5061/dryad.h6h7c.

Results

A MOLECULAR TIMESCALE FOR THE DIVERSIFICATION OF MODERN POLYPTERIDS

Gene trees inferred from each locus using a Bayesian method exhibited varying degrees of phylogenetic resolution that was correlated with the estimated mean rate of molecular evolution in the *BEAST multispecies coalescent analysis (Figs. S1 and S2). The time-calibrated species tree was well resolved with more than half of the nodes supported with strong ($\geq$0.95) Bayesian posteriors (Fig. 1). *Polypterus* was monophyletic in all of the gene trees and the *BEAST species tree. Within *Polypterus*, there were three strongly supported clades including (1) *P. ornatipinnis*, *P. mokelembembe*, and *P. weeksii*, (2) *P. endlicheri*, *P. ansorgii*, *P. congicus*, *P. lapradei*, and *P. bichir*, and (3) *P. teugelsi*, *P. buettikoferi*, *P. senegalus*, *P. delhezi*, and *P. polli* (Fig. 1). The phylogeny is very similar to results using mtDNA genes (Suzuki et al. 2010b), including the monophyly of *Polypterus* and the resolution of three clades of *Polypterus* species. However, the mtDNA phylogeny and nuclear gene inferred species tree differed in the resolution of *P. retropinnis*, which was not strongly supported in either set of analyses, and also differed with regard to the phylogenetic placement of the *P. ansorgii* clade within *Polypterus*, which was not supported with a strong Bayesian posterior in the mtDNA phylogeny (Suzuki et al. 2010a,b).

The mean posterior age estimate from the fossil-calibrated species tree analysis for the MRCA of extant Polypteridae was 19.1 Ma with a 95% highest posterior density interval (HPD: [13.6, 25.4 Ma]), overlapping with age estimates from a separate study using nine nuclear genes and 36 external fossil calibration priors (95% HPD: [5.7, 16.4 Ma]; Near et al. 2012b). The estimated age of the *Polypterus* MRCA was 9.1 Ma (95% HPD: [7.5, 11.1 Ma]). Most interspecific divergences within *Polypterus* were young, with all but one dating to the Pliocene and Pleistocene (Fig. 1).

PATTERNS OF LINEAGE DIVERSIFICATION

The MEDUSA analysis identified the 10 shifts in lineage diversification among the major living lineages of ray-finned fishes (Fig. 2). With an estimated speciation rate (r) of 4.6×10^{-6} lineages/Ma and an extinction fraction (ε) of 0.99, polypterids significantly deviate from the background rate of lineage diversification ($r = 1.8 \times 10^{-2}$ lineages/Ma, $\varepsilon = 0.81$) of all other ray-finned fishes (Actinopteri). Within Actinopteri there is no detected shift in lineage diversification in sturgeons and paddlefishes (Acipenseriformes); however, there is a significant decrease in the rate of lineage accumulation in Holostei (Bowfin and gars; $r = 5.0 \times 10^{-3}$ lineages/Ma). The best-fitting MEDUSA model identified significant decreases in the rate of lineage diversification for three deep-branching single species teleost lineages: *Denticeps clupeoides* (Denticle Herring), *Lepidogalaxias salamandroides* (Salamanderfish), and *Stylephorus chordatus* (Tube-eye), fitting a pure-birth model with a speciation rate of 1.0×10^{-6} lineages/Ma to each of these lineages (Fig. 2). Significant increases in diversification rate were detected for the Otophysi (minnows, catfishes, and their allies; $r = 5.7 \times 10^{-2}$ lineages/Ma), Myctophidae (lanternfishes; $r = 9.0 \times 10^{-2}$ lineages/Ma), an unnamed clade of acanthomorph teleosts ($r = 5.1 \times 10^{-2}$ lineages/Ma), and an unnamed clade of percomorph teleosts ($r = 8.0 \times 10^{-2}$ lineages/Ma; Fig. 2).

Tree simulations that conditioned on the age and diversity of Polypteridae using either the range of credible diversification rate estimates of the Holostei ($1.0 \times 10^{-6} < r > 1.1 \times 10^{-2}$ lineages/Ma) or Actinopteri, which includes the sturgeons and paddlefishes ($1.6 \times 10^{-2} < r > 2.1 \times 10^{-2}$ lineages/Ma; $0.72 < \varepsilon < 1.00$), resulted in expected stem age intervals that were significantly smaller than the observed steam age interval of living Polypteridae (385.9 Ma; $P < 0.0001$; Fig. 3A). Repeating this simulation with modeling, the end Cretaceous mass extinction event by culling polypterid diversity to a single lineage also produced stem lineage durations significantly smaller than polypterids ($P < 0.001$; Fig. 3B).

PATTERNS OF TAXONOMIC DIVERSITY IN THE POLYPTERID FOSSIL RECORD

Consistent with previous reviews of the polypterid fossil record (Gayet et al. 2002), we find a peak in genus-level diversity of the clade in the early Late Cretaceous (Fig. 4A). This is based on interpretations drawn from isolated finlets (Werner and Gayet 1997), as well as more complete skeletal remains including articulated material (Dutheil 1999; Grandstaff et al. 2012). These materials derive from multiple localities and geological horizons,

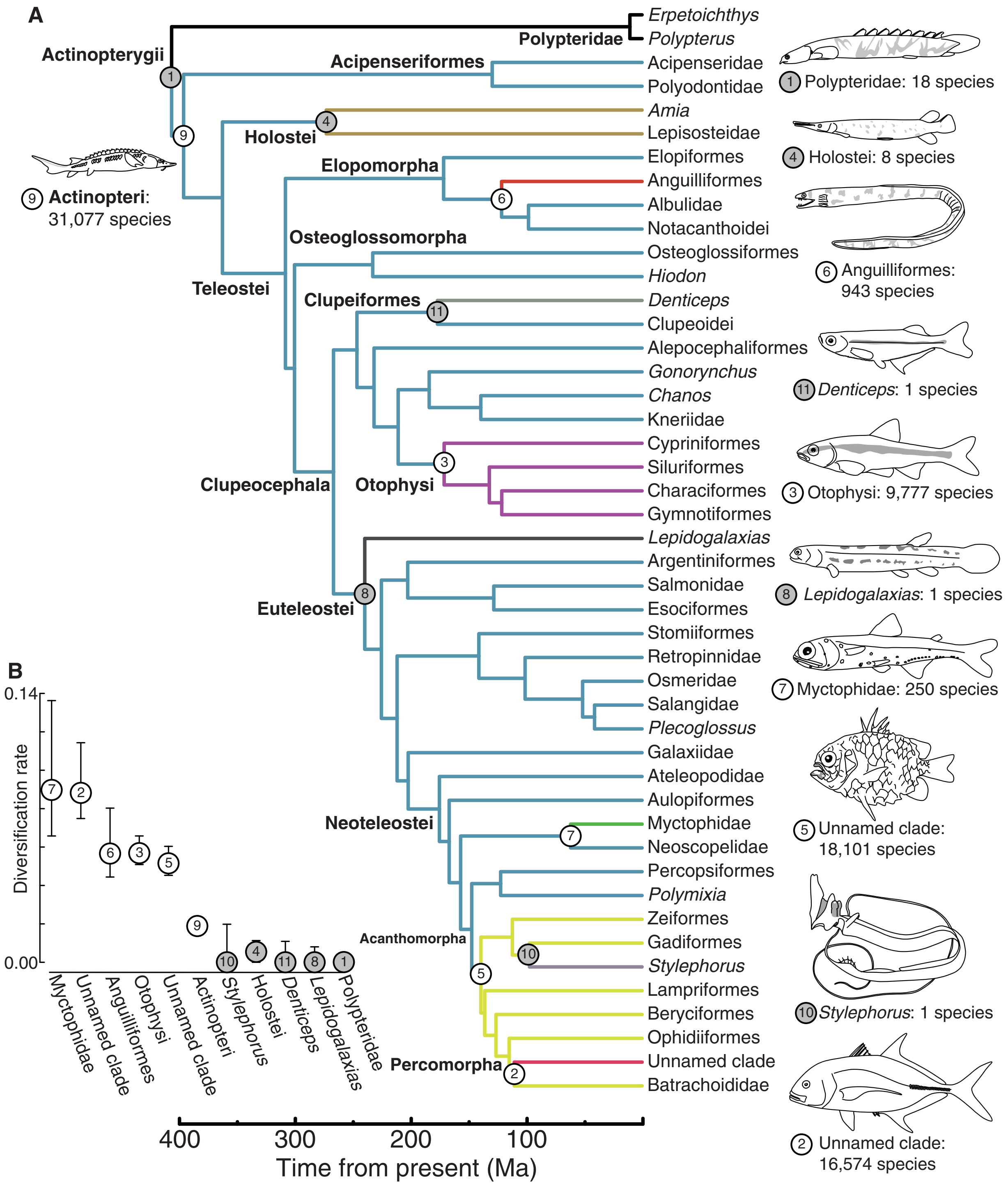

Figure 2. (A) Bayesian inferred time-calibrated phylogeny of major lineages of Actinopterygii taken from Near et al. (2012b). Circled numbers, line drawings, and branch colors identify the 11 lineage diversification regimes identified using MEDUSA with the clade diversity values presented in Table 1. White circles indicate increases in lineage diversification rates, gray circles indicate decreases in lineage diversification rates. Species-richness values of lineages with identified shifts in diversification rate are highlighted under a representative drawing of a species from each clade. (B) Estimates of net diversification rates (birth rate minus death rate) with 95% confidence interval of the 11 identified lineage-diversification regimes. Circled numbers correspond to clades identified in (A). Fish line drawings are by A. Dornburg.

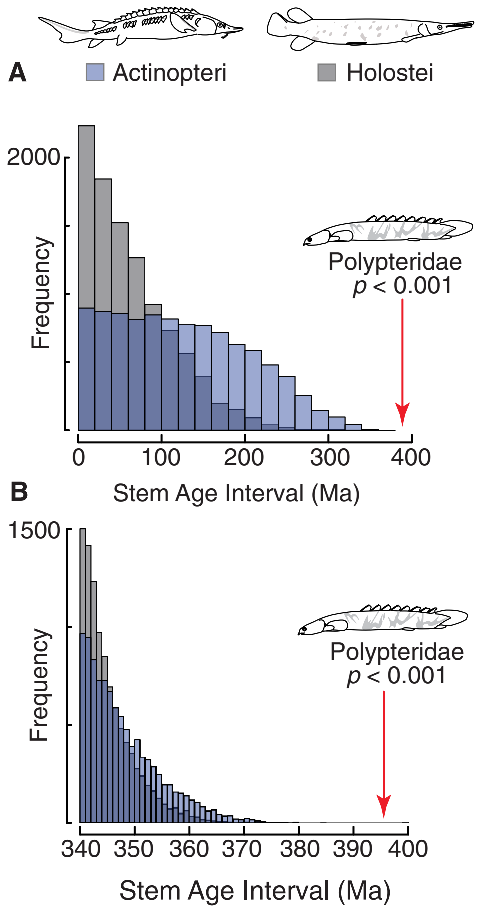

Figure 3. **Expected stem age intervals based on simulations conditioning on the stem age and extant diversity of Polypteridae. Blue and gray histograms correspond to results conditioned on the 95% CI of background lineage diversification rates of Actinopteri ($1.6 \times 10^{-2} < r > 2.1 \times 10^{-2}$ lineages/Ma; $0.72 < \varepsilon < 1.00$) and Holostei ($1.0 \times 10^{-6} < r > 1.1 \times 10^{-2}$ lineages/Ma), respectively. A red arrow indicates the observed stem age interval of Polypteridae. (A) Simulation results based on 10,000 trees with a stem age reflecting the stem age of Polypteridae. (B) Simulation results based on 10,000 trees allowing only a single lineage surviving the end-Cretaceous mass extinction event at 66 Myr ago. Fish line drawings are by A. Dornburg.**

so this pattern is not attributable to a single fossil Lagerstätte. In contrast, the Cenozoic record of polypterids in Africa appears to be restricted to a single genus-level lineage, notionally identified as *Polypterus*. Our molecular results imply that this identification may need revision, as some of these materials likely pertain to stem polypterids that branch outside the divergence between *Polypterus* and *Erpetoichthys* (Fig. 1).

An important question is whether this early peak in polypterid genus diversity, followed by a long period of low richness, reflects genuine patterns of diversity over time or is instead an artifact of sampling. The relationships between our geological proxies (Fig. 4B and 4C) and observed polypterid diversity in the Late Cretaceous and Cenozoic record of Africa are positive but weak (well-studied faunas: $\rho = 0.6$, $P = 0.350$; osteichthyan-bearing formations: $\rho = 0.3$, $P = 0.683$; osteichthyan-bearing collections: $\rho = 0.7$, $P = 0.233$; Spearman rank-order correlation of generalized differences). None of these correlations is significant, although our time series are short due to low stratigraphic resolution and a gap in the record of freshwater fishes in the African Paleocene (Fig. 4B–4D).

Because all of our proxies show positive, albeit nonsignificant, relationships with sampled genus richness of polypterids, we applied an approach that tests for deviations from a simple null model that posits that all variation in apparent diversity is attributable to sampling (Lloyd 2012; Fig. 5A–5C). For each of our proxies, we find genus diversity of polypterids significantly exceeds model predictions in the early Late Cretaceous, coincident with the peak in observed taxonomic diversity in the fossil record. Throughout the Paleogene and Neogene, levels of polypterid diversity are consistent with predictions of the sampling model. For our two sampling proxies (well-studied faunas and osteichthyan-bearing collections), we find that the fewer polypterid genera are known from the Quaternary than predicted by our model (Fig. 5A and 5B). However, fossils from this interval derive from numerous sites in a handful of formations where research emphasis is overwhelmingly focused on fossil hominins and associated mammals rather than fishes, the fragmentary remains of which are treated largely as paleoenvironmental indicators rather than the principal focus of anatomical or taxonomic study (e.g., Fernández-Javlo et al. 1998; Pobiner et al. 2008).

Discussion

Our analyses reveal that polypterids exhibit a pattern of diversification that is different from all other living lineages of ray-finned fishes (Figs. 2 and 3A). Despite the stem lineage divergence from all other ray-finned fishes at least 390 Myr (Gardiner and Schaeffer 1989; Long and Trinajstic 2010), our molecular age estimates indicate that the radiation of living polypterids initiated between the Oligocene and Middle Miocene (~19 Ma; Fig. 1), a result congruent with previous fossil-calibrated relaxed molecular clock analyses (Near et al. 2012b; Betancur-R et al. 2013). At least four other species-depauperate lineages of ray-finned fishes exhibit low rates of lineage diversification (Fig. 2); however, the stem lineage duration of polypterids is exceptional and significantly different from all other lineages of ray-finned fishes (Fig. 3A). Although there is often an expectation of constant richness through time in ancient species poor lineages (Stanley 1979, p. 123; Schopf 1984), we find polypterid diversity in the Late Cretaceous to be exceptionally high, followed by a marked decline in diversity that continues throughout the Cenozoic. The combination of paleontological and neontological approaches

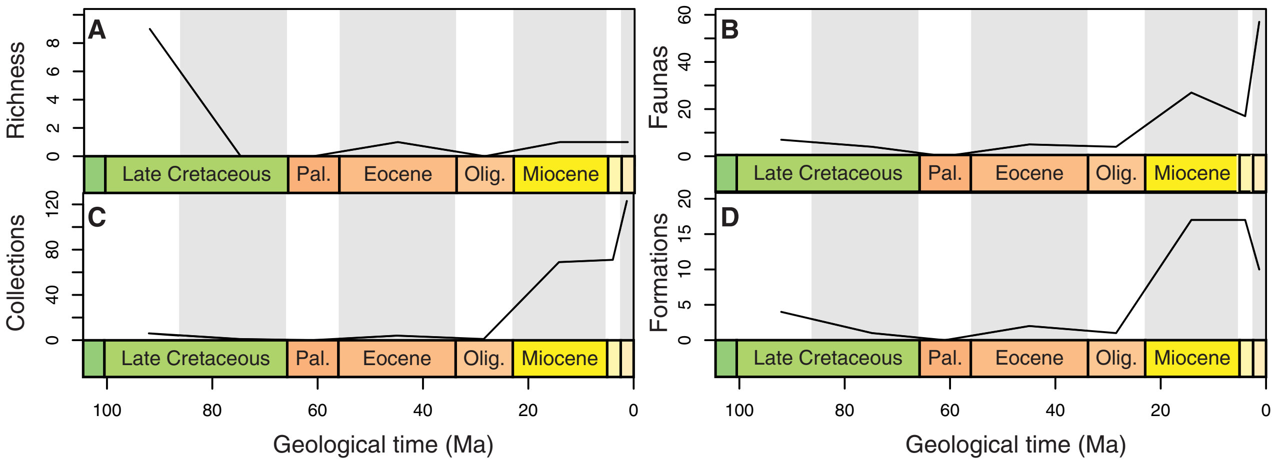

Figure 4. Patterns of genus richness in African fossil Polypteridae. (A) Uncorrected counts of genus-level lineages of Polypteridae known from fossils. (B) Counts of well-studied freshwater fossil fish faunas, assembled from a review of the literature. (C) Counts of osteichthyan-bearing fossil collections with a paleoenvironmental profile consistent with the ecology of fossil and modern polypterids, as recorded in the Paleobiology Database. (D) Counts of osteichthyan-bearing sedimentary formations with a paleoenvironmental profile consistent with the ecology of fossil and modern polypterids, as recorded in the Paleobiology Database. Note that the peak of polypterid lineage diversity shown in (A) does not coincide with peaks in any of the sampling and geological proxies (B, C, and D).

indicate that the persistence of polypterid diversity through time is characteristic as a "boom and bust" economy of species richness, with the majority of living species diversifying in the Pliocene and Pleistocene (Fig. 1).

DIVERSITY OF POLYPTERIDS THROUGH TIME

The polypterid fossil record of Africa exhibits an apparent peak of genus richness in the early Late Cretaceous that cannot be predicted from living species alone (Gayet et al. 2002). Quantitative modeling indicates that high diversity during this interval cannot be explained by sampling or rock volume, and instead might represent a genuine biological signal. It is possible that this peak is even more pronounced than these results suggest because our count of genus diversity excludes four as yet undescribed species of polypterids represented by articulated fossils from the Cenomanian Kem Kem beds of Morocco (Cavin et al. 2010). If these materials represent new genera, then our measure of sampled richness represents a substantial underestimate. Furthermore, this peak in taxonomic diversity corresponds to an interval of high morphological disparity, greatly exceeding that found in living species. For example, the early Late Cretaceous polypterids of Africa span nearly two orders of magnitude in body size, ranging from the 3 m *Bawitius* to the 60 mm *Serenoichthys* (Grandstaff et al. 2012).

Correlates of periods of high polypterid diversity remain unexplored, but the timing of diversification of living polypterids appears coincident with substantial paleoclimatic and tectonic events during the Neogene in Africa. The estimated age of the origin of the living diversity of *Polypterus* in the late Miocene correlates with the onset of the extensive aridification and the disruption of several major watersheds, including the Trans-Saharan and Trans-African river drainage networks (Sepulchre et al. 2006; Otero et al. 2009). These major environmental alterations to aquatic habitats in Africa are hypothesized to have driven species diversification in hippopotomines and ariid catfishes (Otero et al. 2009; Boisserie et al. 2011). The diversity of hippotomines in the fossil record increases dramatically in the late Miocene, and this "Hippopotomine Event" is followed by the rapid expansion of *Hippopotomus* species in the Pliocene/Pleistocene (Boisserie et al. 2011). The proposed mechanisms of this period of lineage diversification, in part, is allopatric diversification resulting from the fragmentation of historically contiguous drainage systems. The radiation of *Polypterus* is coincident with the timescale following the "Hippopotomine Event" and may reflect a pattern eventually discovered in a greater range of organisms, suggesting that similar geomorphic and paleoclimatic events have influenced the diversification of several lineages of aquatic animals in the Neogene of Africa.

LIVING FOSSILS OR ANCIENT SPECIES-DEPAUPERATE LINEAGES?

Since Darwin's (1859, p. 107) articulation of ancient relict lineages as living fossils, there have been changing perspectives on how ancient and species-depauperate lineages persist through time (Simpson 1953, pp. 319–335; Stanley 1975; Gould 2002, pp. 815–817; Rabosky et al. 2013). Lineages classically defined

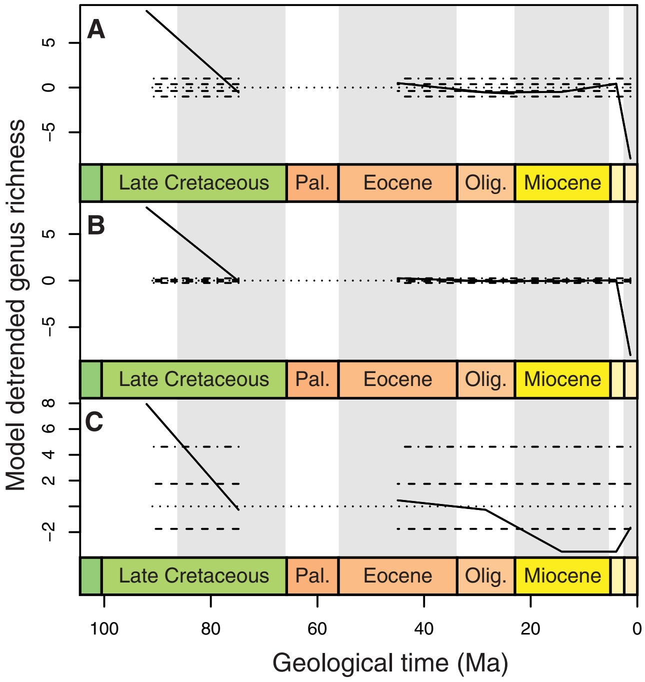

Figure 5. **Deviations in observed polypterid genus-level lineage richness from a null model that posits observed diversity patterns are controlled by sampling or the geological record. Solid line, deviations in observed richness from model predictions; horizontal dotted line, perfect agreement with model; dashed line, 1.96 SEs; dot-dash line: 1.96 SDs of the model. Residuals that extend beyond this error envelope represent substantial excursions from model predictions. Gaps in the model represent intervals where no suitable sedimentary deposits are available, and observed zero richness is uninformative. (A) Model residual estimates using the number of faunas (see Fig. 4B) as a sampling proxy. (B) Model residual estimates using the number of collections (see Fig. 4C) as a sampling proxy. (C) Model residual estimates using the number of formations (see Fig. 4D) as a rock-volume proxy. In all cases, observed genus-level richness of polypterids is significantly higher than model predictions in the early Late Cretaceous.**

as "living fossils" are generally expected to survive over long periods of evolutionary time with low rates of phenotypic evolution that results in morphological similarity of living species with those preserved in the fossil record (Stanley 1975; 1979, pp. 122–132; Fisher 1990; Mathers et al. 2013). The presence of living fossils was cited as "a corollary of the punctuational model" of evolution (Stanley 1979, pp. 122–123), as punctuated equilibrium predicts that the magnitude of phenotypic evolution is associated with the frequency of speciation (Eldredge and Gould 1972; Gould and Eldredge 1977). Alternatively, the persistence of ancient species-depauperate lineages of ray-finned fishes may point to a lack of evolvability (Rabosky et al. 2013), which is the inherent ability of lineages to produce morphological and ecological novelties (Wagner and Altenberg 1996).

Polypterids do exhibit a number of morphological features that are considered ancestral character states in ray-finned fishes (Patterson 1982; Greenwood 1984; Gardiner and Schaeffer 1989) and the morphology of extant species is fairly conserved (Britz 2004; Suzuki et al. 2010b). However, the fossil record of polypterids is insufficient to substantiate inferences about rates of morphological change throughout their history. Available material from Cretaceous deposits provides evidence that polypterid genera vary in body size by almost two orders of magnitude (Grandstaff et al. 2012), suggesting the potential for high rates of body size evolution similar to those observed in extant sturgeons (Rabosky et al. 2013). Coupling this with the observed Neogene age of the living species suggests that the low morphological disparity among living polypterid species may be a consequence of their recent diversification and not reflective of any constraints on phenotypic evolution.

Polypterids are among the clades of ray-finned fishes that exhibit a significantly low rate of lineage diversification (Fig. 2). However, the Neogene diversification of the living lineages and a history of high-diversity intervals in the geological past both indicate polypterids do not fit the characteristics of the classic definition of "living fossils." This is in line with evidence from recent molecular phylogenetic studies that demonstrate that the diversification dynamics of other classic textbook examples of living fossils show patterns of recent lineage diversification (Nagalingum et al. 2011; Mathers et al. 2013). For example, both cycads and tadpole shrimps (Notostraca) exhibit a signature of recent lineage diversification, but there are several reconstructed instances of diversification that date to the Mesozoic and Paleozoic (Nagalingum et al. 2011; Mathers et al. 2013), whereas diversification of living polypterids is entirely in the Neogene (Fig. 1). The pattern of recent speciation after a substantially long-stem lineage duration observed in polypterids is unique among ray-finned fishes, as the other exceptionally species-depauperate lineages are characterized by either species with long intervals between diversification events (e.g., Holostei; Boreske 1974; Grande and Bemis 1998; Grande 2010; Wright et al. 2012) or the persistence of deeply branching single species lineages (e.g., *Denticeps*, *Lepidogalaxias*, and *Stylephorus*).

Our analyses illustrate that polypterids represent anachronistic actinopterygians that do not exhibit the evolutionary patterns classically associated with living fossils (Greenwood 1984), despite their retention of ancestral character states and position as the sister lineage of all other ray-finned fishes. Instead, we provide evidence of at least one earlier pulse of lineage diversification in the Cretaceous, suggesting that the continued integration of paleontological and neontological approaches provides an exciting framework from which to begin generalizing the evolutionary dynamics that underlie the persistence of ancient lineages across the Tree of Life.

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DATA ARCHIVING

DNA sequences are on Genbank, KF792465-KF792728. Data is archived on Dryad, doi:10.5061/dryad.h6h7c.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Gene trees inferred from each sampled locus using MrBayes 3.2.
Figure S2. Gene trees inferred from each sampled locus using MrBayes 3.2.
Table S1. Museum Voucher Information for Specimens Housed at the Kyoto University Museum (FAKU) Used in DNA Sequencing.