Syst. Biol. 62(3):351–365, 2013

DOI:10.1093/sysbio/syt001
Advance Access publication January 9, 2013

Continental Diversification of an African Catfish Radiation (Mochokidae: *Synodontis*)

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Received 6 August 2012; reviews returned 30 October 2012; accepted 3 January 2013
Associate Editor: Thomas Near

Abstract.—Despite African rivers containing high species diversity, continental-scale studies investigating the mechanisms generating biological diversity of African riverine faunas are limited compared with lacustrine systems. To investigate the build-up of diversity in a tropical aquatic continental radiation, we test different models of lineage diversification and reconstruct the biogeographic history in a species-rich siluriform genus, *Synodontis* (~130 species), with a broad distribution across all major tropical African drainage basins. The resulting robust species-level phylogeny (~60% complete, based on a multigene data set) exhibits a near constant rate of lineage accumulation throughout the mid-Cenozoic to recent, irrespective of missing species and despite the changing environmental conditions that were prevalent during this time period. This pattern contrasts with the findings for species-level diversification of large clades that commonly show an early burst of cladogenesis followed by declining rates through time. The identification of distinct biogeographic clades demonstrates a correlation between river hydrology and cladogenesis, although there is evidence of recent repeat dispersal into the southern range of the focal group. We conclude that diverse freshwater fish radiations with tropical continental distributions represent important organisms to test hypotheses of diversification and investigate the effects of palaeo-landscapes and climates on present day biodiversity. [Biogeography; Cenozoic; diversification rates; molecular dating; phylogeny; Siluriformes; tropical rivers.]

The majority of biological diversity resides on continents (May 1994), yet understanding the processes driving species build-up has largely centered around studies focused on insular systems, such as islands and lakes. In tropical freshwater systems, there is a disparity in the current understanding of processes generating biodiversity in lake radiations, compared with continental radiations composed of riverine taxa. This is largely attributable to the spectacular adaptive radiations of African cichlid fishes, for which numerous hypotheses as to the mechanisms of speciation have been offered (e.g., Schliewen et al. 1994; Albertson et al. 1999; Seehausen 2004; Seehausen et al. 2008; Genner et al. 2010; Wagner et al. 2012), but which may not exemplify the typical evolution of other fish groups. In contrast to lacustrine settings, the patterns and processes shaping the biodiversity in Africa's rivers are considerably less well documented. Because continental radiations are less spatially limited and occur over longer time scales, their evolutionary history is likely to be more complex, so that processes generating biological diversity may not follow the same trend as island radiations (Derryberry et al. 2011).

An established pattern inferred from empirical studies investigating diversification in both insular and continental lineages is early, rapid cladogenesis followed by declining diversification rates (e.g., Harmon et al. 2003; Rüber and Zardoya 2005; Seehausen 2006; Day et al. 2008; McPeek 2008; Phillimore and Price 2008; Rabosky and Lovette 2008a; Burbrink and Pyron 2009; Gavrilets and Losos 2009). This departure from the predictions of constant rate models is mostly based on adaptive radiation theory (Schluter 2000), in which ecological opportunity facilitates an early burst of speciation into new adaptive zones that become saturated over time causing diversification rates to decline (Gavrilets and Vose 2005; Rabosky and Lovette 2008a). This may not be the only explanation, as more recently a similar pattern has been identified considering geographic speciation in the absence of ecology (Pigot et al. 2010). However, it has recently been shown that unusually large clades, which constitute the majority of diversification studies, are more likely to show an "early burst" pattern even when speciation and extinction rates are constant through time (Pennell et al. 2012).

In contrast, a near constant rate of diversification has recently been reported in a highly diverse continental family of Neotropical birds (Furnariidae), which is coupled with evidence of constrained morphological evolution (Derryberry et al. 2011). These authors suggest that diversification in tropical continental radiations may not be as limited by ecological opportunities as in island radiations. However, the commonality of this trend among tropical continental faunas is not established due to the paucity of studies at a similar scale and density.

To provide insights into the processes generating biological diversity, species-rich and spatially extensive clades offer useful systems (Barraclough and Vogler 2002; Kozak and Wiens 2010; Derryberry et al. 2011; Near et al. 2011). Sampling should preferably include 80% of species in a clade (Cusimano and Renner 2010), because the sensitivity of diversification rate models to missing species can lead to inaccurate estimates of diversification rate and rate downturns (Cusimano and Renner 2010);

the latter pattern having been taken as evidence of adaptive radiation (e.g., Harmon et al. 2003; Day et al. 2008) or density-dependent diversification (Phillimore and Price 2008).

Near-complete sampling is a particular challenge for continental-scale species-rich clades, which is compounded in Africa because the rich biodiversity of this continent is poorly known (Schwarzer et al. 2009). This is a result of a combination of scale (Africa is the world's second largest continent, occupying ~20% of total land area), socioeconomic factors, for example, lack of local taxonomists (Swartz et al. 2008; Skelton and Swartz 2011) and continuing political instability (Williams and Kniveton 2011). Continental-scale studies investigating diversification of African freshwater taxa are therefore rare.

The limited dispersal capabilities of most freshwater fishes make them excellent subjects for investigating the effects of palaeo-landscapes on present day biodiversity. This is because physical features, for example, rapids (Markert et al. 2010), changes in substrate (Markert et al. 1999), or changes in water chemistry or physical properties (Val et al. 2006) can act as barriers to fishes leading to isolation of populations. Freshwater fish dispersal has, therefore, been linked to climatic or geological episodes, which affect direct connections or headwaters captures between adjacent drainage basins (Bermingham and Martin 1998; Martin and Bermingham 2000; Montoya-Burgos 2003). The hydrogeological hypothesis (Montoya-Burgos 2003) is based on the idea that such palaeohydrological changes promoting dispersal followed by vicariant events (Lundberg et al. 1998) are important in shaping current biological diversity in Neotropical freshwater faunas.

To date, African freshwater fish systematic studies tend to be restricted within regions or drainage basins, for example, Southern Africa (serranochromin cichlids, Katongo et al. 2007; *Pseudobarbus*, Swartz et al. 2007), West Africa (aplocheiloid killifishes, Collier et al. 2009), Central Africa (mormyrid electric fish, Sullivan et al. 2004), or focus on lacustrine diversification including some broader sampling (e.g., de Graaf et al. 2009; Day et al. 2009; Brown et al. 2010). Alternatively, studies focus at the intercontinental scale and thus include only limited African sampling, for example, notopterid knifefishes (Inoue et al. 2009), aplocheiloid killifishes (Murphy and Collier 1997), characiform fishes (Calcagnotto et al. 2005), cichlid fishes (Genner et al. 2007), and siluriforms (Sullivan et al. 2006). The few reasonably well sampled (e.g., Koblmüller et al. 2008; Schwarzer et al. 2009) or densely sampled (e.g., Wagner et al. 2012) African continental studies available have focused on cichlids, but these studies have not tested models of diversification or quantitatively reconstructed their biogeographic history.

To investigate tropical continental diversification in the freshwater environment, we test different models of lineage diversification and biogeographic scenarios in endemic African *Synodontis* catfish (squeaker catfish) with a truly continental distribution. These catfish present a useful system due to the combination of their broad distribution across all major drainages of the Afrotropical ecoregion and as one of the most species rich African fish genera (129 species, Vreven and Zamba 2010). The genus *Synodontis* is 1 of 9 genera within the endemic African family Mochokidae (Vigliotta 2008) containing ~70% of familial diversity (Ferraris 2007). The distribution of *Synodontis* is similar to African cichlid fishes, although in contrast to cichlids they are principally riverine (~90%), with peak diversity occurring within the Congo drainage basin (~30%). Unlike the mochokid genus *Chiloglanis* that inhabit fast flowing streams, *Synodontis* occur in mature rivers and are also found in the East African rift lakes (Poll 1971). A radiation of these catfishes in Lake Tanganyika (LT) has been the focus of recent molecular phylogenetic studies (Day and Wilkinson 2006; Koblmüller et al. 2006; Day et al. 2009). These data combined with behavioral data indicate the species flock may represent Müllerian mimics (Wright 2011) with some lake species also exhibiting brood parasitism (e.g., Sato 1986). As with many continental lineages, the patterns and processes of their broader diversification have not been addressed to-date, which is particularly pertinent in aquatic settings.

Aims of Investigating a Tropical Aquatic Continental Radiation

To test hypotheses regarding the tempo and pattern of cladogenesis in *Synodontis* catfish, we present a novel multigene species-level phylogeny that includes up to 81 species representing ~60% of known diversity from across all major river basins within their range. We combine a time-calibrated phylogeny with biogeographical data and apply diversification rate analyses including missing species at random to avoid potential inaccuracies caused by incomplete taxonomic sampling (e.g., Cusimano and Renner 2010). To ensure a robust species-level phylogeny we assembled a molecular data set that includes mitochondrial genes: Cytochrome *b* (Cyt *b* + tRNA-pro), Cytochrome oxidase I (COI), and nuclear genes; first and second intron of the S7 ribosomal protein (S7) and the second subunit of the recombination activating gene-2 (Rag 2). These genes were selected based on their performance to infer phylogenetic resolution at different depths of the phylogeny because they cover a range of fast (e.g., Cyt *b*), moderate (S7), and slow-evolving (Rag 2) genes. COI was also sequenced due to its utility for DNA taxonomy and verifying molecular operational taxonomic units (Hebert et al. 2003).

Continental diversification is likely to be highly complex, because many extrinsic and intrinsic processes may affect patterns of lineage accumulation. This is particularly acute in a radiation spanning sub-Saharan Africa that diversified across a time period (mid-Cenozoic to recent) affected by major climatic and geological events (e.g., Sepulchre et al. 2006; Zachos et al. 2001). The evolutionary history of *Synodontis* covers

most of the tectonic events (landscape uplift and volcanic eruptions) associated with East African rifting that occurred between 10 and 25 Ma (Partridge et al. 1995). There have also been major climatic events, for example, the Middle Miocene Climatic Optimum (MMCO) (15–17 Ma) was the warmest period in the last 35 Ma of Earth history when precipitation would have been higher (Zachos et al. 2001), causing rivers to have greater discharge and thus more connectivity. Specifically, we address the following: (i) Is there a signal of an early-burst of cladogenesis that is a common pattern observed in large empirical phylogenies? Alternatively, has lineage accumulation been constant? Or are there any rate shifts associated with major geological/climatic events? (ii) Is there a correlation between river palaeohydrology and cladogenesis? (the hydrogeological hypothesis; Montoya-Burgos 2003). The alternative hypothesis is that dispersal has been an active mechanism in their diversification, or a combination of both processes and (iii) Did *Synodontis* originate in the Congo Basin (CB)? Previous authors (e.g., Livingstone et al. 1982 and references therein) identify the Congo River (formerly Zaire River) as the source for fish diversity of less ichthyologically diverse rivers. To our knowledge, this hypothesis has not been explicitly tested.

Methods

Taxon Sampling

The broad distribution of *Synodontis* across sub-Saharan Africa presents logistical problems regarding sampling particularly as peak diversity (Poll 1971) occurs in the politically unstable region of the Democratic Republic of Congo. Such factors have meant that the samples used here are the result of collecting over a 9-year period. A total of 146 samples representing 72 described species and 9 possible new species giving a putative total of 81 species are included within this study (Supplementary Table S1, doi: 10.5061/dryad.b6225). Species are included from all main river basins with multiple sampling incorporated where possible for those taxa with broad geographic ranges to test species validity. Based on the current taxonomy of 129 species (116 species, Ferraris 2007) that includes a further 13 species described since the publication of this work (Friel and Vigliotta 2006; Wright and Page 2006; De Weirdt et al. 2008; Friel and Sullivan 2008; Wright and Page 2008; Vreven and Milondo 2009; Vreven and Zamba 2010), plus undescribed taxa included in this study, our sampling is ~60% complete. This is approximately double the coverage of a previous study (Day et al. 2009).

To test the monophyly of *Synodontis* and relationships within the Mochokidae, a family within the "Big Africa" clade (Sullivan et al. 2006), 8 outgroups comprising representatives of all but 2 (*Acanthocleithron* and *Atopodontus*) mochokid genera are included (Supplementary Table S1). We include the genera *Atopochilus*, *Chiloglanis*, *Euchilichthys*, *Microsynodontis*, *Mochokiella*, *Mochokus* and selected a more distant outgroup, the Malapteruridae (*Malapterurus*), also a member of the "Big Africa" clade.

Specimen Collection, DNA Extraction, PCR, and Sequencing

Fishes were collected using a variety of techniques (e.g., electrofishing, seining, hook, and line) and preserved as voucher specimens. White muscle tissue and/or fin clips were taken before preservation and stored in ethanol for genomic use with DNA subsequently extracted using a DNeasy Blood and Tissue kit (Qiagen Ltd., UK). Polymerase chain reactions were used to amplify selected genes using standard conditions. A total of ~3586 base pairs (bp) were sequenced, including the mitochondrial genes: Cyt *b* (1138 bp) + tRNA-pro (117 bp), the "bar-coding" gene COI (676 bp), and 2 nuclear genes: ribosomal protein-coding gene S7 (S7) intron 1 (527–545 bp), intron 2 (145 bp), and the recombination activating gene-2 (Rag 2) (921 bp). Genes were selected based on their performance from previous studies, Cyt *b* + tRNA-pro (Day and Wilkinson 2006; Day et al. 2009), S7 (Day et al. 2009), and Rag 2 (Sullivan et al. 2006). We use published primers for Cyt *b* including tRNA-pro, Rag 2 (Hardman and Page 2003; Hardman 2004, 2005), S7 (Chow and Hazama 1998), and CO1 (Ward et al. 2005). Annealing temperatures for the selected genes were as follows: Cyt *b* + tRNA-pro (49–55 °C), COI, S7 and Rag 2 (55 °C). Samples were analyzed using an ABI 3730xl sequencer. Published sequence data from *Synodontis* species (Day and Wilkinson 2006; Day et al. 2009) and several outgroup species (Sullivan et al. 2006) were also used. Voucher information and GenBank accession numbers for new and previously published sequences are provided in Supplementary Table S1.

Sequence Alignment, Model Selection, and Phylogenetic Inference

Presumed orthologous DNA sequences were aligned in Clustal X (Thompson et al. 1997) using default parameters and checked manually in Se-Al (Rambaut 2002). The importance of selecting an appropriate partitioning scheme has been highlighted in phylogenetic studies, as different schemes can affect the accuracy of phylogenetic reconstruction, for example, Brandley et al. (2005). We therefore employed the program PartitionFinder (Lanfear et al. 2012) that statistically selects best-fit partitioning schemes as opposed to ad hoc selection based on potential partitions that are predefined by the user, which here correspond to by gene and codon position. We selected the Akaike Information Criterion (AIC) with branch lengths unlinked and use the greedy search algorithm. Analyses were performed on: (i) individual genes Cyt *b* + tRNA-pro ($n = 149$, where n includes ingroup and outgroup taxa), COI ($n = 149$), S7 ($n = 107$),

Rag 2 ($n=105$); (ii) mtDNA ($n=149$) and ncDNA data sets ($n=142$); and (iii) the concatenated (mt and ncDNA) matrix ($n=155$) in a total evidence approach (Huelsenbeck et al. 1996) to ascertain the behavior of the independent data sets on inferring a robust tree topology. The complete matrix is available at TreeBASE.

Data were analyzed using Bayesian Markov Chain Monte-Carlo, implemented using the parallel version of the program MrBayes v3.1.2 (Ronquist and Huelsenbeck 2003) on a Linux cluster (Legion, UCL). Three separate analyses for each data set were run for 5 000 000 generations, sampling every 100 generations, with an initial burn-in set to 5000 (chain temperature 0.2, 4 chains). Convergence of individual runs was assessed using TRACER version 1.5 (Rambaut and Drummond 2009) and any remaining burn-in discarded prior to tree construction. Branch support was determined by Bayesian Posterior Probabilities (BPPs) and was further evaluated by Maximum Likelihood (ML) bootstrap (BS) support. ML analyses were implemented using the parallel version of the program GARLI 2.0 (Zwickl 2006) on the Linux cluster applying the data partitions from PartitionFinder and running 1000 BS replicates.

To determine if areas of weak support in the phylogenetic hypothesis are the result of unstable taxa, we employed the method of leaf stability (Thorley and Wilkinson 1999) as implemented in the program RadCon (Thorley and Page 2000). Alternative tree topologies based on the concatenated, mtDNA, and ncDNA data were evaluated using the approximately unbiased (AU) test (Shimodaira 2002) in the program CONSEL (Shimodaira and Hasegawa 2001) using site likelihood scores generated from PAUP* (Swofford 2003). We compared trees based on total mtDNA versus ncDNA as opposed to individual gene trees, to include as many taxa as possible common to these data sets.

Node Calibrations and Estimation of Divergence Times

The mochokid fossil record is represented largely by *Synodontis*, predominately isolated robust fin spines (Pinton et al. 2006). There are good diagnostic characters for fin spines of African catfish genera developed through extensive comparative material (Gayet and Van Neer 1990; Pinton et al. 2006), although extant *Synodontis* are currently defined by 2 synapomorphies based only on soft tissue characters (see Vigliotta 2008).

The oldest reported fossil *Synodontis* comes from the earliest Lower Oligocene, Sultanate of Oman at Thaytiniti dated to 34 Ma (Roger et al. 1993; Otero and Gayet 2001) and is represented by fin spines. These fossils possess a number of characters that in combination are used to diagnose *Synodontis* from other African catfish (Gayet and Van Neer 1990). These include: strong denticulation on the posterior border, round tubercles on the anterior border, a cleithral surface developed ventrally, a dorso-lateral process developed both anteriorly and laterally, and a well-developed auxiliary process (Gayet and Van Neer 1990; Otero and Gayet 2001).

Previous phylogenetic studies (e.g., Day and Wilkinson 2006; Day et al. 2009) calibrated their tree based on a fragment of *Synodontis* crania from the Early Miocene (Burdigalian) of Egypt (Priem 1920) dated at ~17–18 Ma (Miller 1999) which has been erroneously reported in the literature as the oldest *Synodontis*. A similarly aged fossil from Kenya (Greenwood 1951) was used to date a further study (Koblmüller et al. 2006). A more recent discovery based on isolated teeth from the Birket Qarrun Formation (BQF), Egypt claim to represent the oldest record of a mochokid catfish (Murray et al. 2010) as this formation is slightly older than the Oman site, dated at 37 Ma, Late Eocene (Priabonian) (Seiffert et al. 2008). However, although the BQF teeth are a distinctive S-shape that is characteristic of some mochokid premaxillary teeth (Vigliotta 2008), this character has yet to be shown as diagnostic for this family. Therefore, until these fossil teeth receive more clarity regarding their assignment we therefore discount them as a calibration for the Mochokidae.

To estimate divergence times the Oman fossil is applied to the *Synodontis* crown group. As fossils provide only minimal soft bounds, we also apply a maximum hard bound to the root of our tree. We use a conservative age of 65.8 Ma based on the absence of catfish fossils during the majority of the Paleocene, as the oldest African catfish *Nigerium* (Longbottom 2010), a member of the family Claroteidae (because it possesses 3 of the 8 derived features used by Mo (1991) to diagnose this family), occurs in the Landenian (uppermost Late Paleocene–Lower Eocene).

To ascertain the appropriate method of molecular dating we tested the assumption of a molecular clock. This was rejected after comparison of likelihood scores for clock and nonclock BI trees (concatenated data set) using a likelihood ratio test implemented in PAUP* ($\ln L$ clock-enforced tree = 38 969.49, $\ln L$ unconstrained tree = 38 689.10, d.f. = 153, $P = < 0.05$). Bayesian relaxed-clock analyses were implemented in BEAST version 1.7.4 (Drummond et al. 2012) using a pruned version of the concatenated data set containing only one specimen per sampled species and implementing a partitioning scheme based on these data using PartitionFinder. We selected an uncorrelated lognormal relaxed clock rate variation model (Drummond et al. 2006) with a speciation model implementing a Yule process assuming a constant rate of speciation per lineage. A lognormal prior distribution was selected for the *Synodontis* fossil calibration: absolute age estimate of 34 Ma, with a 0.8% tail probability (based on the occurrence of possible older isolated mochokid teeth). A uniform prior was applied to the root incorporating a conservative upper bound of 65.8 Ma and a lower bound of 34 Ma. Two independent MCMC analyses were run for 50 million generations and combined using LogCombiner 1.7.4, with 25% of chains discarded as burn-in. Convergence of model parameter values (effective sample size [ESS]) were subsequently checked in Tracer (ESS values >200),

with the posterior probability density of the combined tree and log files summarized using TreeAnnotator version 1.7.4 and results of divergence times visualized on the chronograms using FigTree version 1.3.1. To assess the influence of calibration priors on the posterior divergence time estimates a data matrix containing no sequence data was run in BEAST, which resulted in no priors needing to be updated.

Ancestral Range Reconstruction

Synodontis have a broadly sub-Saharan distribution covering all but 2 (Maghreb and Cape) of the 9 African ichthyo-provinces (after Roberts 1975). These include: Nilo-Sudan (N-S); Upper Guinea forest (UGF), for example, Guinea, Sierra Leone; Lower Guinea forest (LGF), for example, Cameroon, Gabon, Equatorial Guinea; Congo Basin (CB); East Africa (EA); Quanza (Q), that is, Angola; Zambezi (Z), that is, Southern Africa, excluding the Cape. Roberts (1975) includes LT and Lake Malawi (LM) as part of the CB and Z ichthyo-provinces, respectively, but due to the high-endemic biodiversity of these lakes we prefer to place them in separate geographic categories. We use the defined areas as discrete biogeographic units to infer ancestral range patterns. In addition, we also combine the N-S ichthyo-province with the UGF and LGF ichthyo-provinces to form a broad "West Africa" (WA) region, thus reducing the complexity of analyses and providing clarity when describing biogeographic regions. Distributional data for *Synodontis* (Supplementary Table S2, doi: 10.5061/dryad.b6225) were largely taken from Poll (1971), Paugy et al. (2003), and museum databases.

Geographic range evolution was estimated using a likelihood-based Dispersal-Extinction-cladogenesis (DEC) model (Ree et al. 2005) implemented in LAGRANGE (www.reelab.net/lagrange) that takes into account divergence time estimates (Ree and Smith 2008). The ultrametric tree generated in BEAST was used to infer ancestral distributions using a uniform dispersal matrix of dispersal across the 9 or simplified 7 geographic regions.

Diversification Rates

The species-level phylogeny ($n = 89$) was used to estimate a net diversification rate for *Synodontis* (Magallón and Sanderson 2001) implemented in GEIGER (Harmon et al. 2008). Using this method we calculated net diversification rates in the absence of extinction ($\varepsilon = 0$) and under a high relative extinction rate ($\varepsilon = 0.9$). To test the null hypothesis that diversification rates have been constant over time, we compared the likelihood of these data under constant-rate models (pure birth, birth–death) to the likelihood under variable-rate models (two-rate Yule model, exponential and linear density dependent models) using LASER (Rabosky 2006a). The resulting test statistic (ΔAIC) is the difference in AIC scores between best-fit rate constant and rate variable models. A positive ΔAIC value indicates these data are best explained by a rate-variable model (Rabosky 2006b). Due to type 1 errors, the significance of ΔAIC was addressed through simulations (Rabosky 2006b) implemented in LASER. To investigate if our data are better supported by models of declining speciation and increasing extinction scenarios, we also fitted models employing time-varying speciation and constant extinction (SPVAR), time-varying extinction and constant speciation (EXVAR), or both speciation and extinction varying through time (BOTHVAR) to our data (Rabosky and Lovette 2008b). To further test whether rates have decreased through time we computed the gamma (γ) statistic (Pybus and Harvey 2000). As our data are not completely sampled, we also investigated the effects of missing species using the Monte Carlo Constant Rate (MCCR) test (Pybus and Harvey 2000). To avoid any bias caused by incomplete sampling, we also added known missing taxa (48 species) randomly to the tree, apart from the LT clade (which to our knowledge is comprehensively sampled, e.g., Day and Wilkinson 2006). The LT clade aside, taxonomic sampling of *Synodontis* species is random as opposed to overdispersed. We subsampled 100 Bayesian chains and used a R script (Barraclough T.G., unpublished data) modified from the PERL script used in Day et al. (2008), in which each missing species was added randomly to the tree with equal probability along the branches. Lineage-through-time (LTT) plots were generated in APE (Paradis et al. 2004). All packages are implemented in the R programming language (R Development Core Team 2008).

Table 1. Substitution models for nucleotide data partitions selected using the AIC in PartitionFinder

PartitionFinder	Model
Cyt b first codon *tRNA* *CO1* first codon	K81+I+G
Cyt b second codon *CO1* second codon	HKY+I+G
Cyt b third codon	TrN+G
S7 all codon positions	HKY+G
RAG2 first+second codons	TrNef+I+G
RAG2 third codon	TrNef+G
CO1 third codon	GTR+G

Results

Phylogenetic Analyses

For the concatenated data set, a total of 7 partitions were identified using PartitionFinder (Table 1). Based on the concatenated data set and individual gene trees, the genus *Synodontis* is monophyletic (Fig. 1; Supplementary Fig. S1a–d, doi: 10.5061/dryad.b6225). *Synodontis* forms a clade with its successive sister taxa *Microsynodontis*, *Mochokus*, and *Mochokiella*, which itself is sister to a clade, including *Chiloglanis*, *Atopochilus*,

FIGURE 1. Bayesian phylogenetic hypothesis of *Synodontis* catfish reconstructed from the concatenated data set. Outgroups (gray branches), BPPs are given in full below nodes, BS values are given above nodes: black square > 95%, gray square 94–90%, and gray star 89–85%.

and *Euchilichthys*. *Brachysynodontis batensoda* and *Hemisynodontis membranaceus*, 2 previously monotypic genera (Poll 1971), nest within *Synodontis* supporting morphological data (Vigliotta 2008). Based on our results and previous morphological findings, we therefore place these genera in the synonymy of *Synodontis*.

Within *Synodontis* 2 principal clades are identified (A and B), which are further subdivided into the 4 main subclades (A1, A2 and B1, B2, Fig. 1). Support for a monophyletic *Synodontis* and main subclades based on BPPs is excellent, with all major nodes except clade A (0.55 BPP) receiving >98% support, with BS values slightly lower (Fig. 1). Support for internal nodes within the main clades is generally good, although some relationships forming the base of clade A1 are less well supported by BPP and receive no BS support. Support for clade A is considerably improved (> 0.95 BPP) by the removal of the unstable sister taxa *Synodontis albolineatus* and *Synodontis batesii* that are sister to clade A2 (0.53 BPP). The position of these taxa is contentious, as while they are also placed as sister to clade A2 in the Cyt *b* gene tree (Supplementary Fig. S1b) and mtDNA tree, they are alternately sister to all other *Synodontis* taxa in the Rag2 gene tree (Supplementary Fig. S1d). Their placement

in the CO1 gene tree is unresolved (Supplementary Fig. S1a), but these taxa are not resolved as sister to clade A2 as found in the Cyt *b* tree. Unfortunately, these taxa did not amplify for S7 (Supplementary Fig. S1c). The disparity in placement of these taxa may represent insufficient data or real conflict, for example, homoplasy or cyto-nuclear discordance. However, to test between these hypotheses additional nuclear genes and samples of these taxa would need to be sequenced. Support for the position of *Synodontis ocellifer* as sister to clade B2 is weak (0.70 BPP, 57% BS) with this taxon also indicated as unstable using leaf stability metrics. Individual gene trees for Cyt *b* + tRNA-pro, COI, and S7 reveal reasonably good support for the majority of relationships identified when these data are concatenated and although there is no support for clades A1, B1 in the CO1 or S7 gene trees, there is no support for alternative hypotheses (Supplementary Fig. S1b and c). Conversely, Rag 2 (Supplementary Fig. S1d) does not perform as well and yields only partial support for these clades. Comparison of the concatenated tree ($P = 0.577$) to the mtDNA ($P = 0.414$) and ncDNA ($P = 0.000$) trees based on AU tests rejects the latter tree as a significantly worse fit to the data.

Comparison of the results presented here with a previous phylogeny (Day et al. 2009) based on a smaller data set both in terms of taxa (half the present taxon sampling) and characters (Cyt *b* + tRNA-pro and S7 intron 1), reveals remarkable congruency regarding the 4 subclades although the current data set provides greater support for these relationships. Sequence divergence for the genus *Synodontis* using kimura 2 parameter (K2P) distances for mtDNA genes Cyt *b* and COI is 17.4% and 14.76%, respectively. The COI distances are similar to those observed in North American freshwater fish, for example, *Etheostoma and Notropis* (13.5%, April et al. 2011).

Timing and Biogeography

LAGRANGE recovered both dispersal and extinction rates of *Synodontis* to be 0.003 per million years, respectively. Figure 2 shows the most likely scenario of ancestral area reconstructions for nodes of interest summarized on the dated species-tree of the study group. Three distinct biogeographical groupings are identified for *Synodontis* based on the analysis combining WA regions. These comprise WA (that includes the combined ichthyo-provinces: N-S, LGF, UGF) EA and the CB, with WA rendered polyphyletic. In this scenario, the genus *Synodontis* is inferred to have originated in the broad region of WA with subsequent dispersal within this region (relative probability [RP] 0.99). The picture is, however, less clear when these regions are coded separately, with the best likelihood reconstruction inferred for the origin of this genus as N-S with subsequent dispersal within this region, although this finding is equivocal (likelihood scores were not significantly different between multiple reconstructions, RP 0.44).

The principal *Synodontis* clades (A and B) diverged at a similar time although the former is possibly older (95% highest posterior density [HPD]: A, 26.2–34.1 Ma; B, 20.1–28.7 Ma). Clade B is also inferred to have originated in WA (more specifically the N-S region), diverging more or less simultaneously within the Late Oligocene–Early Miocene (95% HPD: clade B1, 18.1–26.7 Ma and clade B2 + *S. ocellifer*, 16.3–25.7 Ma). These subclades are inferred to have had very different biogeographic histories, with the former clade composed completely of WA taxa that are predominately from the N-S region, but also include several UGF taxa (referred to as clade WA N-S). Conversely the latter clade, apart from the 2 early branching taxa (*S. ocellifer* and *Synodontis gobroni*) that are from the N-S region, consists entirely of EA taxa (clade B3) and also includes independent dispersal into LT and LM (referred to as clade EA). Clade EA diversified much later than clade WA N-S and is suggested to be Late Miocene (95% HPD: 8.6–14.3 Ma), which is consistent with the possibility that diversification occurred toward the end of, or just after EA rifting events (10–25 Ma; Partridge et al. 1995). Despite using an older calibration in this study, the age of colonization of LT is estimated to be 7.9 Ma (95% HPD: 5.7–10 Ma), which is only slightly earlier than previous estimates (e.g., Day and Wilkinson 2006; Day et al. 2009).

The other principle clade (A) also reveals disparate biogeographic histories regarding its 2 subclades irrespective of analysis. The biogeographic scenario is better supported based on the simplified analysis indicating the inferred ancestral ranges of WA and the CB are respectively inherited by each of its daughter lineages (clades A2 + *S. albolineatus* and *S. batesii* and A1) arising from this node (RP 0.99). The analysis including 9 regions identifies clade A as having originated in N-S and having subsequently dispersed to the LGF and the CB regions, although this result is highly equivocal (likelihood scores were not significantly different between multiple reconstructions, RP 0.23). Under this scenario, clade A1 subsequently dispersed into the CB (with local extinction in N-S and LGF regions), while conversely for clade A2 + *S. albolineatus* and *S. batesii* there has been subsequent dispersal into N-S and LGF and local extinction within CB (Fig. 2). The divergence of these biogeographic clades, unlike clades EA and WA N-S, occurred contemporaneously as the species rich CB clade is suggested to have diverged some time between 21.9–30.5 (95% HPD) Ma, with its sister clade composed of a mixture of N-S and LGF taxa (referred to as WA LGF) diverging at 19.5–30.3 (95% HPD) Ma. Irrespective of absolute dates, our analyses identifies the biogeographic clades WA N-S, WA LGF, and CB diverging in a similar time window earlier in the history of the group, compared with the EA clade that diversified more recently.

In contrast to the identification of distinct CB, EA, and WA biogeographic clades, taxa from the Zambezi region reveal multiple colonisations (dispersals) into the former 2 regions. Two independent lineages have descended from EA members, with a third lineage originating

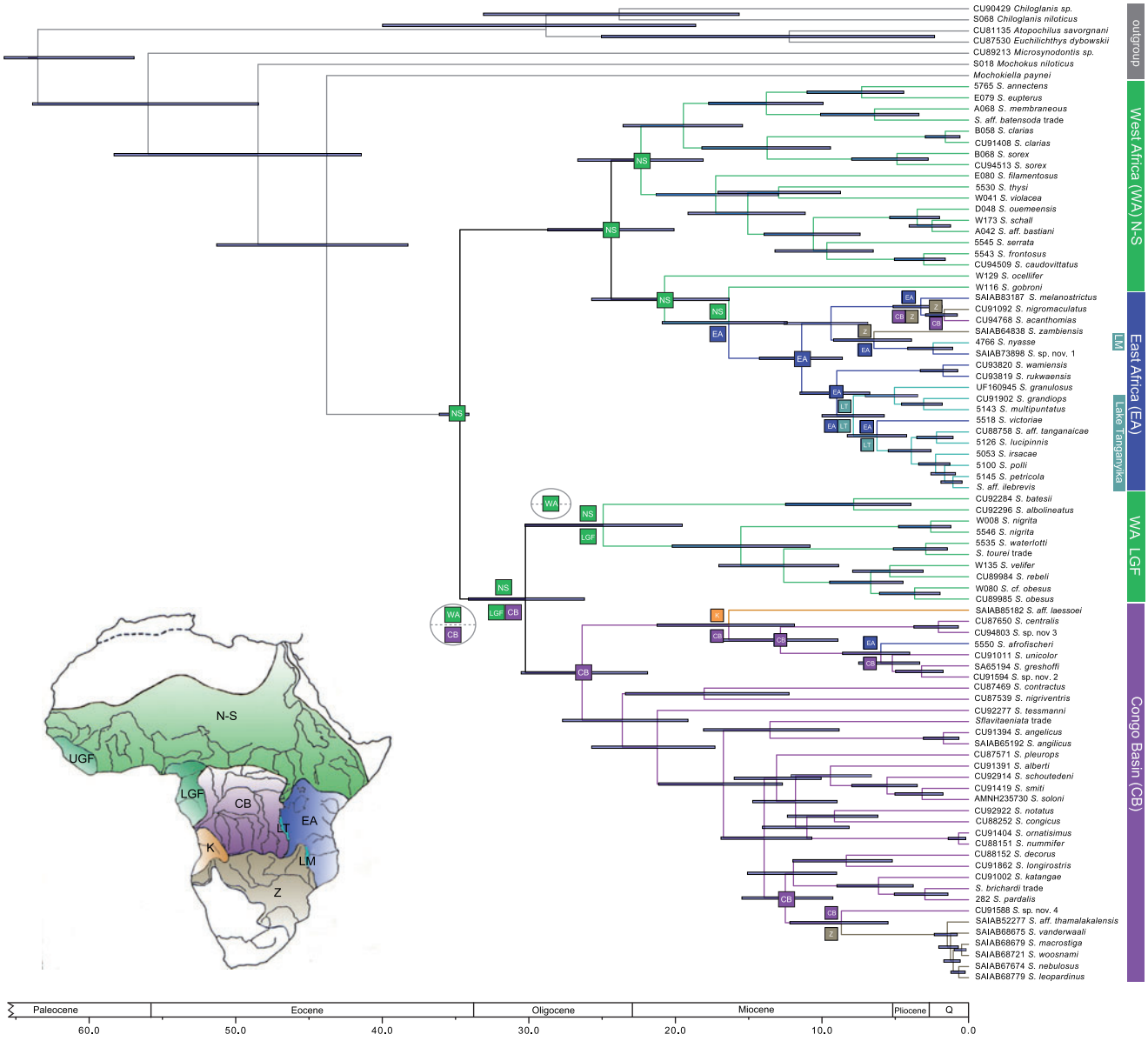

FIGURE 2. Chronogram of *Synodontis* including outgroups inferred from Bayesian relaxed clock dating methods (BEAST software) and calibrated from the mochokid fossil record. Biogeographic range inheritance is modeled using LAGRANGE based on 9 areas: N-S; UGF; LGF; CB; EA; K; Z; LT; and LM. Results from a simplified analysis combining the regions N-S, UGF, and LGF as WA are indicated in the gray circles for those nodes that are otherwise equivocal. LT and LM are analyzed as separate regions despite being highlighted by the same color. Colored boxes at nodes indicate ancestral area reconstructions with the highest likelihood. Individual boxes indicate an ancestor limited to a single area, whereas combined boxes indicate an ancestor with a distribution including more than one area. Boxes either side of a node indicate ancestral ranges inherited by each of the daughter lineages arising from that node. Inset, map of Africa indicating areas (modified after Roberts [1975]).

from an ancestor in the CB and subsequently dispersing into the Zambezi region. The latter lineage forms a very recent radiation that could have diversified 0.8–2.3 (95% HPD) Ma (see Day et al. 2009). The repeated colonisation of Southern Africa is estimated to have occurred relatively recently during the Late Miocene–Pliocene.

There is also evidence of other recent dispersal events between the biogeographic clades. This is apparent within the EA clade, with dispersal into the CB (95% HPD: 0.8–2.9 Ma) as the distinctive Congolese taxon *Synodontis acanthomias* is sister to the Southern African *Synodontis nigromaculatus*. In the reverse direction, the EA taxon *Synodontis afrofischeri* (Lakes Victoria/Albert) nests within the CB clade (95% HPD: 4.0–8.6 Ma). The single taxon (*Synodontis aff. laessoei*) described from the Cuanza (Angola) ichthyo-province is also identified as having originated within the CB, but diverged much earlier from its Congolese ancestor 11.9–21.3 (95% HPD) Ma.

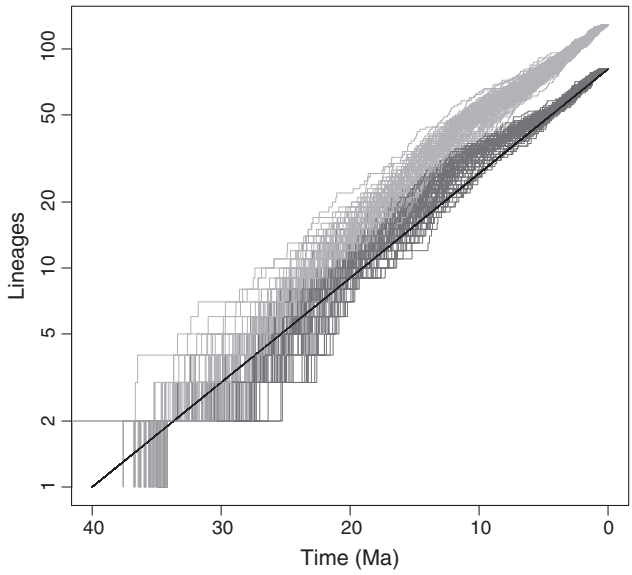

FIGURE 3. LTT plots for the original *Synodontis* data set (dark gray) and including missing species added at random (light gray), estimated from sampling 100 Bayesian trees generated from the concatenated data set. The solid line indicates the expected number of lineages under a constant rate model of diversification with no extinction.

Pattern and Tempo of Diversification

Visual inspection of the LTT plot for *Synodontis* indicates a near constant rate of diversification with no strong indication of a slowing down, irrespective of missing species added (Fig. 3). Net diversification rate for *Synodontis* is 0.12 per Ma assuming no extinction ($\varepsilon = 0$), decreasing to 0.07 per Ma when assuming a high relative rate of extinction ($\varepsilon = 0.9$). Our data, however, are better explained by low-extinction rates, that is, a pure-birth model ($\varepsilon = 0$), as opposed to a birth–death model ($\varepsilon = 0.9$), based on likelihood ratio tests generated in LASER (Rabosky 2006a).

This pattern is supported by comparisons of diversification models using a ML approach for which a constant rate pure birth model is preferred (Table 2). Although a potential decline in diversification rate is identified when missing species are randomly added to the tree, the preferred rate-variable (Yule 2-rate) model does not provide a better fit than the preferred rate-constant (Pure Birth) model, albeit this result is marginally insignificant ($\Delta AIC_{rc} = 4.44$, $P = 0.071$). Irrespective of adding missing taxa the AIC score of the density-dependent linear (DDL) model is very similar to the Yule-2 rate model, implying that there is little power to distinguish between these models. The best estimate of models incorporating speciation and extinction rates that vary through time is the variable speciation rate (SPVAR) model, however, this model is not preferred based on delta AIC scores (Table 2).

A more detailed examination of the *Synodontis* radiation reveals that a DDL model is preferred for the EA ($\Delta AIC_{rc} = 0.280$, $P = 0.232$) and WA LGF ($\Delta AIC_{rc} = -1.723$, $P = 0.667$) clades, although we cannot reject the null hypothesis that these clades have diversified at a constant rate (Supplementary Table S3, doi: 10.5061/dryad.b6225). While this result is not significant, it highlights the biogeographic regions in which diversification rates may have changed.

The gamma (γ) statistic (Pybus and Harvey 2000) computed for the *Synodontis* radiation supports our results being slightly negative (−1.17), but nonsignificant ($P = 0.24$, two-tailed test) indicating no evidence for rapid initial diversification. To determine if this result is affected by missing species, we employed the MCCRs test. As sampling in this study was largely random (as opposed to overdispersed), the MCCR test is appropriate (but see criticisms of this test for overdispersed sampling, Brock et al., 2011). We simulated 5000 trees of 129 taxa (the total number of described and possible new *Syndontis* taxa reported in this study) and compared the empirical value (−1.17) to this distribution (LASER Rabosky 2006a). Although the critical value from the simulations is negative (−2.68) this is again nonsignificant ($P = 0.48$). Notably, inclusion of missing species does not alter model selection or greatly alter diversification rates (Supplementary Table S3).

DISCUSSION

Previous evolutionary investigations of African freshwater fishes have primarily focused on discrete regions (Sullivan et al. 2004; Katongo et al. 2007; Swartz et al. 2007; Collier et al. 2009) or insular environments (e.g., Day and Wilkinson 2006; de Graaf et al. 2009; Brown et al. 2010), with the majority of studies focused on the hyperdiverse cichlid radiations (e.g., Schliewen et al. 1994; Joyce et al. 2005, 2011; Day et al. 2008; Seehausen et al. 2008; Wagner et al. 2012). With the exclusion of African cichlid studies, no previous studies have included dense taxonomic sampling of a species-rich clade or applied quantitative methods to investigate diversification. Our study demonstrates the utility of phylogenetic data in understanding temporal and spatial patterns of diversification during the mid- to late-Cenozoic period that experienced extreme environmental changes with respect to climate and geological tectonic events. By studying a species-rich group with a broad scale continental distribution, we shed light on the generation of biological diversity in the understudied Afrotropical riverine environment.

Lineage Diversification

Despite fluctuating environmental conditions, which include major climatic shifts during the late Cenozoic, our results suggest that lineage accumulation occurred at a near constant rate for much of the history of *Synodontis*. These findings differ from the majority of empirical phylogenetic studies that recover a pattern of initial, rapid cladogenesis followed by a decline in diversification rate (e.g., Nee 2001; Rüber and Zardoya 2005; Weir 2006; Day et al. 2008; McPeek

TABLE 2. Testing for rate variation in the original data set using the ML ΔAIC test statistic

Model	R^1	R^2	Model parameters	st	Log L	AIC	ΔAIC
Rate constant and variable rate models							
Pure birth	0.1005786				13.22471	−24.44942	0
Birth death	0.1005786		0[a]		13.22471	−22.44942	2
Yule 2-rate	0.106833	0.0405053		0.9264017	15.08104	−24.16208	0.29
DDX	0.1811997		0.1670749[x]		14.0049	−24.0098	0.44
DDL	0.138667		161.9996[k]		14.09183	−24.18366	0.27
$\Delta AIC_{rc} = -0.2657625$, $n = 81$, $P = 0.502$							
Variable speciation/extinction models							
SPVAR					13.73027	−21.46055	2.99
EXVAR					13.19705	−20.39409	4.06
BOTHVAR					13.73602	−19.47204	4.98

2008; Phillimore and Price 2008; Rabosky and Lovette 2008a, b; Burbrink and Pyron 2009; Gavrilets and Losos 2009). Such departures from the constant rate model are mostly taken as evidence of saturation of niche space (e.g., Phillimore and Price 2008), although a recent study (Pennell et al. 2012) suggests that analysing "usually large" clades in isolation may result in misleading inferences of diversification rates since early bursts may not be attributable to any biological phenomena.

If we assume that density dependence is common to all lineages interpretation of our results suggest that this catfish clade may be too young to have reached its ecological limit. Alternatively, tropical continental diversification may not be as limited by ecological opportunities. Compared with islands, continental areas have less restriction of movement of species ranges and greater fluctuations in species abundances that may lead to much weaker interactions within clades (Barraclough et al. 1999). Notably, a constant rate of lineage accumulation cannot be rejected for several Corvoidea bird families that originated in Indo-Pacific archipelagos, but which have large geographical distributions as a consequence of their high dispersal and colonizing abilities (Fritz et al. 2011). In a recent study, Pigot et al. (2010) simulated ecologically neutral cladogenesis under geographic speciation and found that a pattern of equal and constant diversification is only expected under a peripatric model of speciation with high rates of range expansion and range volatility over time. This scenario maybe reasonable for *Synodontis* given that this radiation is spatially extensive occurring across the Afrotropics and spans a climatically and geologically highly dynamic time period, although the extent of peripatric speciation in the group has not been investigated.

While models that explicitly incorporate speciation and extinction rates that vary through time were not preferred for our data, comparison of our "best" model assuming these variables (SPVAR) to previous studies reveals a slow decrease in the rate of speciation (λ) (Fig. 4), rather than marked decreases identified in a number of continental radiations, for example,

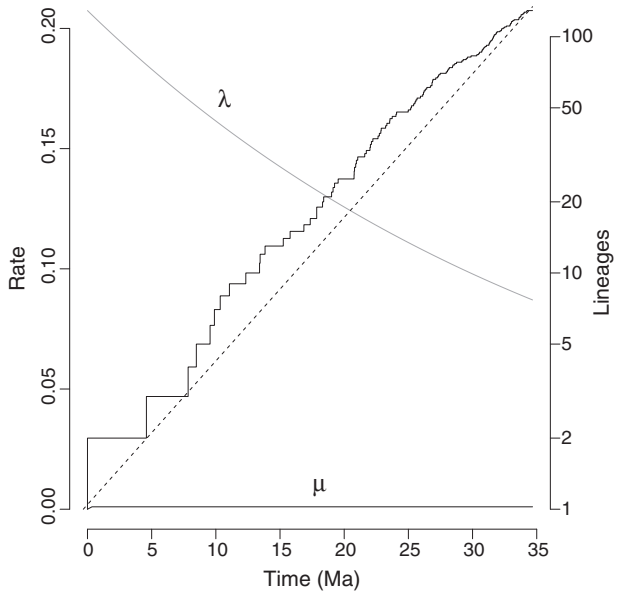

FIGURE 4. ML estimate of speciation rates (λ) through time with a constant extinction rate (μ), under the variable speciation rate (SPVAR) model. The LTT plot is also included.

Australian agamid lizards, North American wood warblers (*Dendroica*), and Australo-Papuan python radiations (Rabosky and Lovette 2008b). Interpretation of the SPVAR model suggests that the *Synodontis* clade could still be growing and that this radiation has not fully exploited niche space. Simple rearrangement of the fitted SPVAR model (equation 9, Rabosky and Lovette 2008b) predicts speciation rate will not decline to zero until ~92.5 million years (including missing species), suggesting the clade is still in the early stages of radiation.

Our study parallels recent findings of a near constant diversification rate reported in a neotropical avian family (Derryberry et al. 2011) with a broad continental distribution and high diversity also spanning a time period of major climatic shifts and geological events, highlighting that lineage diversification in continental freshwater environments maybe responding in a similar

fashion to terrestrial settings. However, more studies at a similar scale and density are needed across a range of different taxonomic lineages and environments to determine if this is a common trend among tropical continental faunas.

Historical Biogeography

The identification of 3 distinct biogeographical groupings (WA, EA, and the CB) indicates that river palaeohydrology has been important in shaping the evolution of these catfish. The inclusion of additional taxa compared with previous studies (e.g., Day et al. 2009) demonstrates the necessity of more complete taxon sampling in deducing biogeographic scenarios. Although the phylogenetic hypothesis of this previous study is congruent to ours (including only taxa common to both studies), the biased sampling of dispersers, for example, Southern African taxa, in Day et al. (2009) implies spurious nonmonophyly of many geographic regions. That *Synodontis* is reconstructed to have originated and diversified within the broad region of WA, is contrary to a previous hypothesis in which the Congo River is assumed to have acted as the source for fishes of less ichthyologically diverse rivers (Livingstone et al. 1982). Results using a likelihood-based DEC model of biogeography imply dispersal followed by range expansion has been important in shaping the evolution of these catfish, although vicariance attributed to EA rifting may have been responsible for the isolation and subsequent radiation within the EA region. Our results (Fig. 2) indicate contemporaneous divergence of the 3 biogeographic clades CB, WA N-S, and WA-LGF (95% HPD: 18.1–30.5 Ma), which although precedes the MMCO that occurred between 15 and 17 Ma (Flower and Kennett 1994; Zachos et al. 2001), this event may have facilitated subsequent early diversification within these clades. The MMCO was a period that experienced considerable warming in mid-latitudes, with an increase in temperature of 6 °C and a precipitation maximum (Flower and Kennett 1994) that would have led to greater river discharge. Increased discharge volume is correlated with higher fish diversity in African rivers (Livingstone et al. 1982) and therefore may contribute to the diversification of lineages. Notably, the EA biogeographic clade did not diverge until much latter (95% HPD: 8.6–14.3 Ma), towards the end of major uplifting phases (10–25 Ma) of the EA rift system (Sepulchre et al. 2006). The younger age of the EA radiation may explain the lower diversity in this region compared with the N-S and the CB. Furthermore, during the Late Neogene (6–8 Ma), EA also experienced a trend toward drier conditions with savannah habitats replacing rain forests (Sepulchre et al. 2006). These conditions may have led to a reduction of river discharge that may also have limited diversity in this region.

Comparison of net diversification rates across biogeographic clades indicates that despite the high species richness of the Congo clade, diversification rates remain unspectacular (0.12 per Ma), supporting the finding that rates and species richness are not correlated (McPeek and Brown 2007). The majority of lineage divergences within the Congo radiation suggest that these took place during the Mid-Late Miocene. If correct, then diversification within this clade predates any existence of a Congo palaeo-lake (2–5 Ma) that is proposed based on biogeographic patterns (Beadle 1981), not geological evidence. Radiations within palaeo-lakes (e.g., Lake palaeo-Makgadikgadi) are hypothesized for serrachromin cichlids (Joyce et al. 2005) and possibly the Southern African *Synodontis* (Day et al. 2009). Closer inspection of the CB biogeographic clade identifies a decrease in diversification rate at a minimum age of ca. 11.7 Ma, although this rate change is not statistically significant (Supplementary Table S3). However, a major event that would have altered available habitats during this timeframe is the formerly landlocked CB system becoming captured in the Miocene (5–15 Ma) by short rivers draining into the Atlantic Ocean (Stankiewicz and Wit, 2006). Rates estimated for the less species rich EA clade are marginally more rapid (0.22 per Ma), consistent with the possibility of a Late Miocene timeframe. The faster rates may also be attributed to the inclusion of the Lake Tanganyikan *Synodontis* radiation within the EA lineage. Conversely, the lower rates estimated for the two broadly sympatric WA lineages (0.08 per Ma WA LGF and 0.12 per Ma WA N-S) maybe because sympatric clades constrain each other's diversification and local richness (Wiens et al. 2011).

Our results also indicate evidence of multiple relatively recent dispersal events due to the polyphyletic nature of the Zambezi ichthyo-province taxa (< 8.5 Ma). Our findings indicate that the single taxon from the Cuanza (Kuwanza) River (*S. aff. laessoei*) originated from Congo ancestors early on in the history of the CB biogeographic clade, which is contrary to previous opinion that postulated that the origin of the Cuanza ichthyological fauna is thought to be distinct from that of the Zambezi and Congo Rivers (Darwall et al. 2009). It has also been previously hypothesized that LT fish faunas have a strong affinity with the CB (e.g., Roberts 1975; de Vos and Snoeks 1994), although we find no support for this hypothesis. Not only has LT been colonized by Tanzanian riverine species but also the EA clade originated from N-S ancestors. However, there is evidence of several recent colonization events, suggested to have occurred during the Late Miocene–Pliocene between EA and the CB regions. Thus any affinity between LT and the CB appears to have occurred relatively recently in the group's history. A much younger affinity between these regions is also reported for lamprologine cichlids (e.g., Day et al. 2007). While we highlight the impact of the geographic isolation of river basin and drainages on modern biodiversity patterns, processes driving diversification within biogeographic clades undoubtedly involve the interplay of several different mechanisms.

Phylogeny and Diversity

Our broad sampling and sequencing of several mitochondrial and nuclear genetic markers supports a largely robust topology. The inclusion of greater outgroup sampling support the monophyly of *Synodontis* as reported in previous molecular (Day and Wilkinson 2006; Koblmüller et al. 2006; Day et al. 2009) and morphological studies (Vigliotta 2008). However, we find no support for mochokid intrarelationships based on morphological data (Vigliotta 2008). Our analyses instead support 2 major clades that may represent distinct subfamilies; these include the clades (*Synodontis*, *Mochokiella*, *Mochokus*, and *Microsynodontis*) and (*Chiloglanis*, *Euchilichthys*, and *Atopochilus*), with the monotypic taxon *Mochokiela paynei* known only from its type locality (Kassawe Forste Reserve, Sierra Leone, Gosse 1986) being sister to *Synodontis*. Further sampling including the genera *Atopodontus* and *Acanthocleithron* is required to fully investigate relationships within this family. Analyses of individual genes revealed good levels of performance of the mitochondrial genes Cyt *b* + tRNA-pro, COI, and the nuclear intron S7. The nuclear gene Rag 2 performed less well in resolving relationships, but this maybe a consequence of the relatively young age of this catfish radiation. Additional rapidly evolving nuclear genes are needed to further explore areas of instability regarding the taxa *S.ocellifer*, *S. albolineatus*, and *S. batesii.*

Since the publication of the most recent taxonomic review of *Synodontis* (Poll 1971), a further 13 species have been described. The inclusion of multiple samples for species that have broad distributions within our study reveal reasonably distinct genetic divergences within taxa with large ranges, particularly across the N-S biogeographic region. K2P distances based on Cyt *b* and COI range between 0.7% and 2.1% and may imply at least some cryptic speciation within the following taxa (K2P distances for Cyt *b* and COI given in parenthesis): *Synodontis sorex* (2%, 1.6%), *Synodontis schall* (2%, 2.1%), *Synodontis nigrita* (1.6%, 1.2%), *Synodontis obesus* (1.8%, 1%), *Synodontis clarias* (1%, 0.8%), and the Congo species *Synodontis angelica* (1.2%, 0.7%). Although not all these distances are >2%, often indicative of distinct species (see April et al. 2011), they are much greater than mean within species divergence (0.39% based on COI) reported in various marine fish species, for example (Ward et al. 2005). The minimum timing of divergences within those taxa whose distances are <2% is 0.7–5 Ma based on our multi gene data set. This Pliocene–Pleistocene timeframe was when the subtropical African climate periodically fluctuated between distinctly wetter and drier conditions, with evidence for increases in aridity at 2.8, 1.7, and 1.0 Ma (deMenocal 2004). These oscillations would have led to expansion and contractions of waterways potentially leading to isolation of populations. However, more extensive sampling of populations is needed to fully test the hypothesis for cryptic speciation in these taxa. Additional new species from the CB that are highly cryptic in nature are also identified in this study (Fig. 1, *S*. sp. nov. 2–4). As such, our study highlights the need for further biodiversity surveys of African rivers, particularly the comparatively unexplored Congo Basin that will undoubtedly yield further species new to science and enhance the opportunity to test hypotheses of diversification scenarios.

Supplementary Material

Data files and/or other supplementary information related to this paper have been deposited at http://datadryad.org/ under doi: 10.5061/dryad.b6225.

Funding

This work was supported by the Natural Environment Research Council (NERC) [NE/F000782/1]. Many of the CU and SAIAB tissues samples used in this study were collected with support from the All Catfish Species Inventory, National Science Foundation (NSF) [DEB-0315963].

Acknowledgments

We thank Thomas Near, Ron Debry, Mark Wilkinson, and two anonymous reviewers for their highly constructive and helpful comments. We thank Tim Barraclough (Imperial College) for an unpublished R script, David Murrell (UCL) for help with R and Richard Ree (Field Museum of Natural History) for advice on LAGRANGE software. The authors additionally acknowledge the support of UCL's Research Computing team and the use of the Legion facility. Ernst Swartz (SAIAB) is thanked for providing samples from Angola.

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