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Multiple independent colonizations into the Congo Basin during the continental radiation of African *Mastacembelus* spiny eels

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Abstract

Aim: There has been recent interest in the origin and assembly of continental biotas based on densely sampled species-level clades, however, studies from African freshwaters are few so that the commonality of macroevolutionary patterns and processes among continental clades remain to be tested. Within the Afrotropics, the Congo Basin contains the highest diversity of riverine fishes, yet it is unclear how this fauna was assembled. To address this, and the diversification dynamics of a continental radiation, we focus on African *Mastacembelus* spiny eels.

Location: Afrotropical freshwaters.

Methods: The most complete molecular phylogeny to date was reconstructed for African spiny eels. Divergence times were estimated applying a Bayesian relaxed clock comparing fossil and geological calibrations across nuclear and mitochondrial trees. Biogeographic reconstructions, applying a dispersal–extinction–cladogenesis model and lineage diversification dynamics were examined.

Results: Spiny eels originated in Asia and colonized Africa *c*. 15.4 Ma (95% HPD: 23.9–8.8 Ma) from which their subsequent radiation across the Afrotropics was best fitted by a constant rate model. Ancestral state estimation identified multiple colonization events into the Congo Basin, whereas all other regions were likely to have been colonized once indicating considerable geographic constraints. Application of the fossil calibration gave similar age estimates across datasets, whereas a geological calibration estimated considerably older nuclear divergences.

Main conclusions: Despite profound environmental events during the evolutionary history of the group, there is no evidence for rapid lineage diversification. This finding supports several recent studies on tropical continental radiations that contrast to the common pattern of density-dependent diversification. We further show that dispersal has occurred into, as well as out of the Congo Basin, indicating the importance of this region in the generation of biodiversity.

KEYWORDS
biogeography, Congo Basin, continental radiation, dispersal, diversification dynamics, Lake Tanganyika, Mastacembelidae, molecular dating

1 | INTRODUCTION

Continental scale species radiations represent excellent opportunities to elucidate patterns and mechanisms responsible for the generation of diversity across heterogeneous landscapes and over geologically complex time periods (e.g. Daniels, Phiri, Klaus, Albrecht, & Cumberlidge, 2015; Day et al., 2013; Derryberry et al., 2011; De-Silva, Elias, Willmott, Mallet, & Day, 2016; Liedtke et al., 2016; Schenk, Rowe, & Steppan, 2013). Speciation and the persistence of diversity through time may be particularly evident in freshwater systems given landscape heterogeneity through fragmentation of aquatic networks as well as ephemerality (Seehausen & Wagner, 2014). Despite river drainage evolution having been shown to shape species distributions and phylogeographical relationships of fish clades (e.g. Goodier, Cotterill, O'Ryan, Skelton, & de Wit, 2011; Montoya-Burgos, 2003; Morris et al., 2016; Near & Keck, 2005), densely sampled species-level studies at the continental scale are lacking. This is particularly pertinent for the Afrotropics, where the processes generating riverine ichthyofauna diversity are largely untested.

The spatial and temporal complexity of continental systems contrast to insular systems where radiations are often described as adaptive, and ecological processes ultimately limit the build up of diversity (e.g. Harmon, Melville, Larson, & Losos, 2008; Reddy, Driskell, Rabosky, Hackett, & Schulenberg, 2012). While such a scenario is not always identified in insular systems (e.g. Harmon et al., 2010), it remains unclear to what degree ecological processes limit the generation of diversity in radiations that span continents (Harmon & Harrison, 2015; but see Rabosky & Hurlbert, 2015). Several recent individual taxon studies of tropical species radiations spanning continents (Day et al., 2013; Derryberry et al., 2011; Liedtke et al., 2016) highlight a near constant rate of diversification. Explanations offered for an absence of density-dependent diversification in these truly continental radiations maybe because widely distributed clades are not limited by ecological opportunities (see Kisel, McInnes, Toomey, & Orme, 2011) or are too young to have reached their ecological limit (Derryberry et al., 2011).

Within the Afrotropics, the dense and extensive hydrological network of the Congo Basin, covering 4 million km^2 and only second in size to the Amazon Basin, contains the highest diversity of riverine fishes in Africa (Stiassny, Brummett, Harrison, Monsembula, & Mamonekene, 2011). Despite such elevated levels of species richness (*c.* 1200 species) and endemism (>80%) (Harrison, Brummett, & Stiassny, 2016), it is unclear how this diversity arose and is maintained. Evolutionary studies focused at the population-level have shown the importance of hydrographic barriers that prevent mixing between freshwater populations, even on a microscale, facilitating diversification (Alter, Munshi-South, & Stiassny, 2017; Markert, Schelly, & Stiassny, 2010). However, the role of the Congo Basin in generating and shaping present day Afrotropical riverine faunas, specifically its importance as a source (i.e. allowing only dispersal out of the Congo) or sink (i.e. allowing only dispersal into the Congo Basin) region, or whether a combination of these processes has led to the build up of its diversity, has to our knowledge not been specifically tested.

Here we combine historical biogeography and diversification analyses, focusing on Afrotropical mastacembelid spiny eels that differ in their ecology to other African freshwater fishes investigated from a continental perspective (Day et al., 2013; Schwarzer et al., 2009), to offer further insights into the macroevolutionary processes that shaped freshwater biodiversity of the Afrotropics. We address how the fauna of the Congo Basin has been assembled, and test: Has the Congo Basin acted as a source or a sink? Diversification dynamics are also investigated in which we test the null model: Have rates remained constant during a continental-wide radiation?

The anguilliform Mastacembelidae (Teleostei: Synbranchiformes) currently contains 85 valid species (Eschmeyer, Fricke, & van der Laan, 2016) and has an intercontinental distribution throughout the Afrotropics (41 valid *Mastacembelus* species; Vreven, 2001; Vreven & Stiassny, 2009; Brown, Britz, Bills, Rüber, & Day, 2011), southeast Asia (includes *Mastacembelus*, *Macrognathus* and *Sinobdella*) and a single species (*Mastacembelus mastacembelus*) from the Middle East. Previous molecular studies have focused on regions with high levels of sympatric diversity (Alter, Brown, & Stiassny, 2015; Brown, Rüber, Bills, & Day, 2010), however, continental scale biogeographical and diversification patterns and processes within the group remain to be investigated.

By reconstructing the most comprehensive molecular phylogeny of African mastacembelid spiny eels to date, we show repeated colonization of the Congo Basin, whereas conversely all other biogeographic areas are remarkably conserved phylogenetically indicating considerable geographic constraint. Furthermore, irrespective of the geological and climatic perturbations that occurred during the evolutionary history of this continental radiation, there is a lack of strong evidence for declining (density-dependent) diversification.

2 | MATERIALS AND METHODS

2.1 | Taxonomic sampling

A total of 255 mastacembelids representing 36 out of 41 valid African species (*c.* 88% completeness) and additional undescribed taxa are included (see Appendix S1 in Supporting Information), expanding sampling from previous studies (Brown et al., 2010; Alter et al., 2015 [49 and 157 samples respectively]). Sampling encompassed all seven Afrotropical ecoregions (Roberts, 1975), plus Lakes Tanganyika and Malawi (see Appendix S2), and included *M. mastacembelus* and five Asian mastacembelid species. The inclusion of *M. mastacembelus* from the Tigris and Euphrates basin is of particular significance regarding resolution of intrafamilial continental relationships. For molecular dating analyses the purported related family Channidae (Betancur_R et al., 2013) was selected, represented by two Asian (*Channa*) and one African (*Parachanna*) species. The inclusion of non-African mastacembelids and channids as out-groups allowed us to test the monophyly of African *Mastacembelus*.

2.2 | Data collection

Five molecular markers were sequenced (5,297 bp) that included those selected in Brown et al. (2010): cytochrome *b* (Cytb), cytochrome *c* oxidase sub-unit (CO1), nuclear S7 introns 1 and 2, and two additional nuclear loci: recombination activating gene 1 (RAG1) and ectodermal neural cortex 1 (ENC1). DNA was extracted from white tissue/fin clip samples using DNeasy Blood and Tissue kit (Qiagen, UK). For PCR primers and amplification conditions, see Appendix S3. Cleaned PCR products were sequenced on an ABI 3730 sequencer (Applied Biosystems, UK). Molecular sequence data were aligned in GENEIOUS 5.6 (Kearse et al., 2012) using default settings and subsequently checked by eye for stop codons and reading frame shifts.

2.3 | Phylogenetic inference

A detailed version of the phylogenetic methods is available within Appendix S3. Analyses were performed on (1) single markers, (2) mitochondrial data, (3) nuclear data, (4) combined mitochondrial and nuclear data.

2.4 | Estimation of divergence times

In the absence of fossil Synbranchiformes, our phylogeny was dated using fossil Channidae (snakeheads), a related family (Betancur_R et al., 2013). We followed Brown et al. (2010) and selected the oldest African Channidae fossil (Murray, 2006) from the latest Eocene to earliest Oligocene (35–33 Ma, Kappleman, 1992) as a minimum age constraint on the Channidae crown group. Older fossils from the Middle Eocene that have been placed within this family (Roe, 1991) were applied to a recent study of the Channoidei (Adamson, Hurwood, & Mather, 2010). However, these fossils were not utilized here as a minimum age constraint since their taxonomic placement is ambiguous. Nevertheless we acknowledge that the channid fossil record could be older and therefore placed a lognormal prior (initial value = 33 Ma, mean = 1.8, *SD* = 0.5; 95% CPD = 46.77) on the calibration node assuming that the most recent common ancestor (MRCA) of the Channidae may be as old as 48 Ma (Roe, 1991).

We also performed further analyses applying a geological calibration based on the age of Lake Tanganyika (LT, 9–12 Ma, Cohen, Soreghan, & Scholz, 1993) as a hard maximum for the node pertaining to the endemic radiation using a normal prior (mean = 10.5 Ma, standard deviation = 0.7; 95% CPD = 11.65). While geological calibrations can lead to wide variability in their precision and reliability (Ho et al., 2015), it is plausible to assume these endemic taxa evolved in situ. Although calibrations based on lake age does not allow for independent assessment of colonization and diversification scenarios of lake endemics, the use of the LT calibration allows us to examine the degree of congruence between fossil and geological calibrations.

As the utility of the mitochondrial genome for accurately estimating divergence dates has been questioned (e.g. Lukoschek, Scott Keogh, & Avise, 2012; Zheng, Peng, Kuro-o, & Zeng, 2011), we analysed mitochondrial and nuclear data separately. Divergence time estimates were implemented in BEAST 1.8.2 (Drummond, Suchard, Xie, & Rambaut, 2012) for mitochondrial and nuclear data (datasets 2–3). A single representative per species (including undescribed taxa) was selected based on having all loci sequenced (see Appendix S1). Several additional taxa were included where they had shown to be non-monophyletic (i.e. *M. congicus*, *M. frenatus*, see Appendix S3). Separate analyses were run implementing linked and unlinked (nuDNA data only) uncorrelated lognormal relaxed molecular clocks (i.e. allowing different distributions of rates among branches depending on gene) selecting a Yule speciation prior. Optimal models and partitioning schemes for these datasets were selected using PARTITIONFINDER 1.1 (Lanfear, Calcott, Ho, & Guindon, 2012; see Appendix S3) in which one partition per gene was defined in order to account for over-parameterization. Each analysis was conducted three times (using the CIPRES Science Gateway server; Miller, Pfeiffer, & Schwartz, 2010) and Markov chain Monte Carlo (MCMC) chains were run for 50,000,000 generations. Burnin run convergence and tree visualization were assessed using the same methods as outlined in Appendix S3.

2.5 | Ancestral range estimation

Geographic range evolution was estimated using dispersal–extinction–cladogenesis (DEC; Ree, Moore, Webb, & Donoghue, 2005; Ree, Smith, & Baker, 2008) implemented in the R package BIOGEOBEARS (Matzke, 2013). To test between alternative biogeographic scenarios regarding the build up of fauna within the Congo Basin the following models were implemented: M0, (unconstrained) allowing dispersal to and from the Congo Basin; M1, allowing only dispersal out of the Congo Basin (i.e. Congo Basin acting as a source); M2, allowing only dispersal into the Congo Basin (i.e. Congo Basin acting as a sink). Models were evaluated based on Akaike's information criterion (AIC) scores and weights. We did not test founder–event speciation (DEC+J) as this model is preferred for island clades (Matzke, 2014).

Eight geographic areas were included in our analysis (see Appendix S2) based on the Afrotropical ichthyo-provinces (after Roberts, 1975): Congo Basin (CB), East Africa (EA), Quanza (Q), Zambezi (Z), Nilo-Sudan (N-S), Upper Guinea Forest (UGF), Lower Guinea Forest (LGF), in which N-S, UGF and LGF were combined as the broad region West Africa (WA) to simplify analyses. Lake Tanganyika (LT) and Lake Malawi (LM) were also included as separate regions (following Day et al., 2013) based on the high levels of lake endemicity. All non-African mastacembelids are placed into a single Asian (A) grouping. Distributional data (see Appendix S1) was based on museum databases and FISHBASE (http://www.fishbase.org). Models allowed for geographic ranges to include any combination of ichthyo-provinces.

2.6 | Lineage diversification

To test whether diversification rates had changed over time, the gamma (γ) statistic was calculated (Pybus & Harvey, 2000) using the

R package APE 3.4 (Paradis, Claude, & Strimmer, 2004), and the effects of missing species examined using the Monte Carlo constant rates test (Pybus & Harvey, 2000). The tempo of diversification was visualized by generating a lineage-through-time (LTT) plot from 100 Bayesian chains sampled randomly from the posterior distribution of the nuDNA dated tree using PHYTOOLS 0.5-10 (Revell, 2012) and APE 3.4 (Paradis et al., 2004). This tree included $n = 41$ taxa (36 valid species, and five possible undescribed species). A total of 20 missing species (five valid and five undescribed species [Vreven, 2001] and 10 cryptic species [we included a very liberal estimate based on results from the mtDNA tree]) were included at random, with equal probability along the branches, using code adapted from Day, Cotton, and Barraclough (2008).

As a likelihood approach is considered more powerful for testing between diversification scenarios (Morlon, 2014), we tested the null hypothesis that diversification rates remained temporally constant across the African radiation, and constituent subclade comprising Lake Tanganyika taxa, by comparing constant rate (pure birth [B] and birth–death [BD]) to variable rate (i.e. varying speciation and extinction) models (B variable; B constant, D variable; B variable, D constant; BD variable) using RPANDA 1.1 (Morlon et al., 2016). Models were compared using the second order AIC scores (AICc and ΔAICc) and Akaike weights.

To test if the African radiation diversified under a distinct rate regime, we used BAMM 2.5 (Bayesian Analysis of Macroevolutionary Mixtures, Rabosky, 2014) and BAMMTOOLS 2.0 (Rabosky et al., 2014). The following priors were generated and applied to downstream analysis (expected number of shifts = 1; λ Init.Prior = 1.02; λShiftPrior = 0.07; μ Init.Prior = 1.02; λIsTimeVariablePrior = 1). Three independent MCMC runs were each run for five million generations sampling every 1,000th and assessed using TRACER 1.6 to confirm convergence of replicates. The global sampling fraction was set to 0.88 (to account for missing species). Bayes factors (BF) were calculated for model (i.e. rate regime) comparison. Rate dynamics were further investigated by a rate-through-time (RTT) analysis. All analyses were carried out in R 3.3.3 (R Core Team 2015).

3 | RESULTS

3.1 | Phylogenetic inference

Inclusion of channid samples suggested the paraphyly of Asian mastacembelids (Figure 1, see Appendix S3), although increased sampling is needed to robustly test these relationships. Phylogenetic trees strongly supported the monophyly of an African *Mastacembelus* radiation plus the Middle Eastern species, *M. mastacembelus* (Figure 1, see Appendix S3). However, the position of this taxon is uncertain, as it is alternatively resolved as sister to the African radiation (nuDNA: 70% BS; 0.79 BPP), or is nested within it (mtDNA: 0.60 BPP/<50% BS; concatenated <50% BS, BPP), in which support for the former scenario is stronger (see Appendix S3). A species tree (generated using *BEAST from a minimum of two individuals across all loci) supported the nuDNA hypothesis regarding the position of this taxon (data not shown).

The African radiation formed five reasonably well-supported clades (A–E) plus a single lineage *M. ansorgii* from the Quanza (Q). The relationships between these groups are largely congruent with the exception of clade B (Figure 1, see Appendix S3). Individual gene trees generally yielded well-resolved topologies with high support (see Appendix S3) indicating the overall utility of selected markers, although resolution at the base of the African radiation was weaker (CO1, and particularly ENC1 trees). A high level of genetic divergence within several species with broad distributions (e.g. *M. congicus*, *M. frenatus*, *M. niger*) was identified, as well as non-monophyly for the former two species (see Appendix S3), indicating the need for future taxonomic revision.

Within the African radiation endemic species from the Lower Congo River are resolved as non-monophyletic (see also Alter et al., 2015; Brown, 2012). *M. crassus*, *M. simbi* and *M. aviceps* form a subclade within clade D and form a sister group to a subclade composed of Southern and East African species including the LM endemic *M. shiranus* (Figure 1, see Appendix S3). A further clade of Lower Congo River endemics (*M. brichardi*, *M. brachyrhinus*) is conversely supported as nested within the otherwise endemic LT radiation (see also Alter et al., 2015). The mtDNA tree resolved these taxa as a clade (100% BS/BPP) indicating a single colonization from LT to the Lower Congo River, although their relationships with respect to one another are not resolved in the nuDNA tree. In both the mtDNA and nuDNA trees, these Lower Congo River endemics form a subclade with the LT endemics *M. tanganicae*, *M. albomaculatus* and *M. flavidus* (100% BS/BPP). All five taxa have exceptionally short branch lengths suggestive of rapid diversification, and some taxa are not always resolved as reciprocally monophyletic (see Appendix S3).

3.2 | Molecular dating

3.2.1 | Effects of genomes and calibrations on divergence estimates

BEAST analyses of mtDNA data gave similar estimates for key events in the evolutionary history of Mastacembelidae regardless of calibration implemented (Table 1). Analyses of nuDNA data implementing the fossil calibration revealed greater variation of divergence estimation, although confidence intervals broadly overlapped. While unlinking nuDNA clocks gave similar estimates to the mtDNA data for older divergences, this analysis yielded the youngest estimates for more recent divergences, for example, the LT radiation (see Table 1). Generally linking nuDNA clocks gave more similar dates to the mtDNA data, with the exception of older divergences, for example, MRCA of the Mastacembelidae (Table 1). The linked clock is however preferred as it reduces over-parameterization of the data by decreasing the number of partitions and parameters. In contrast, application of the geologic calibration to nuDNA data generated much older dates (and broader confidence intervals) to all other analyses. As such (and on the basis of the position of *M. mastacembelus*), the dated nuclear tree (fossil calibration, linked clocks; Figure 1) was selected for downstream biogeographic and diversification analyses.

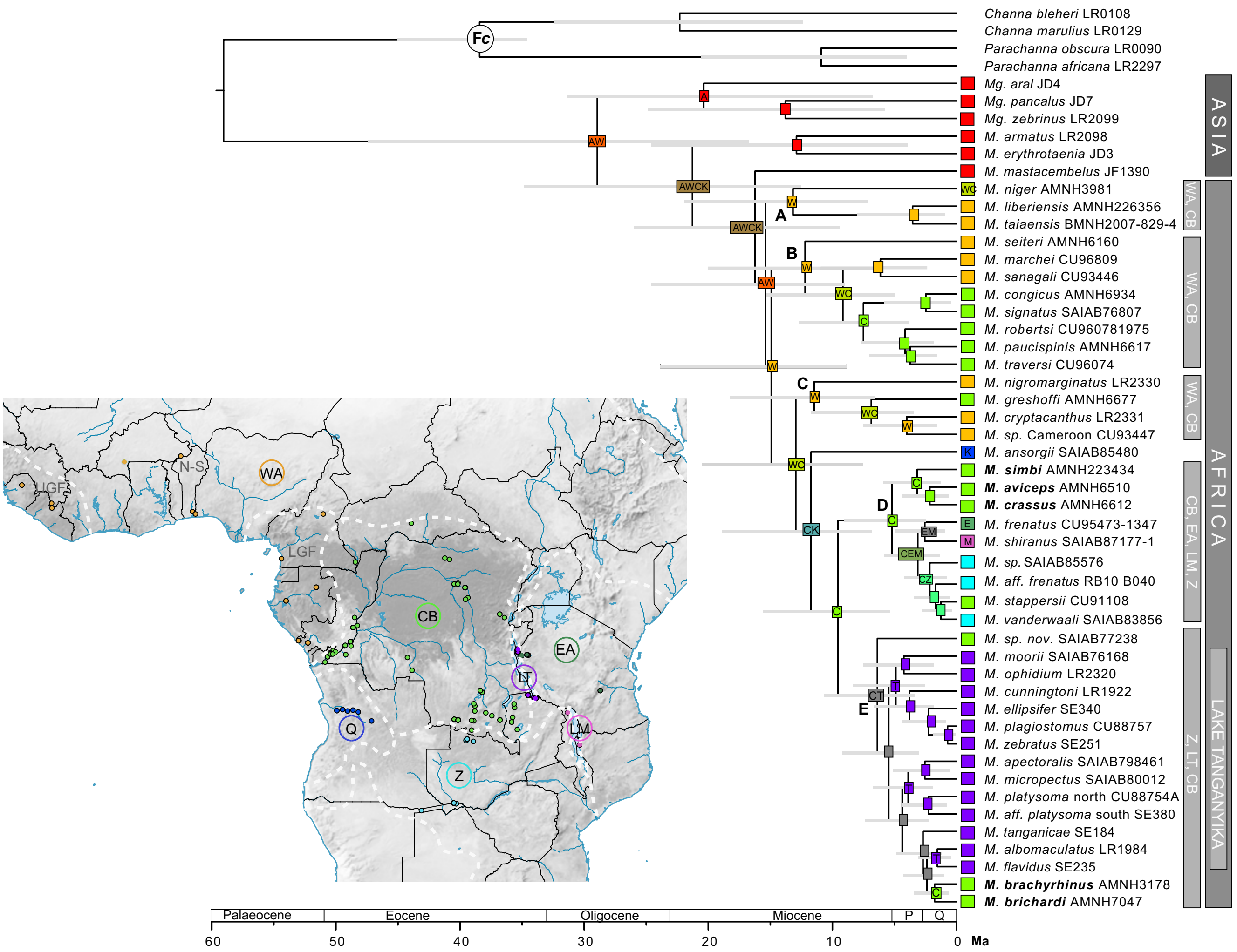

FIGURE 1 Spatiotemporal reconstruction of African *Mastacembelus* spiny eels. Dated nuclear tree inferred using a Bayesian relaxed clock (BEAST) including 95% confidence intervals (HPD). Biogeographic range inheritance based on the DEC model (M0) implementing BioGeoBEARS. Colours at species tips reflect geographic designations (see inset map for African regions [ichthyo-provinces] and Appendix S2); Asia (A, red); West Africa (W, light orange, including N-S, LGF, UGF regions); Congo Basin (C, light green), Zambezi (Z, aqua), Quanza (Q, dark blue), East Africa (E, dark green), Lake Tanganyika (T, purple), Lake Malawi (M, pink); C+W (olive). States at nodes represent the most probable ancestral area before an instantaneous speciation event; stem labels (state of the descendant lineages immediately after speciation) are omitted on this figure to help with clarity, but see these reconstructions in Appendix S3. AR, African radiation; A–E, main clades; Fc, fossil calibration node; Lower Congo River endemics in bold

3.2.2 | Divergence estimates of the *Mastacembelus* radiation

The African radiation diverged 15.4 Ma (95% highest posterior density [HPD]: 23.9–8.8) based on the dated nuclear tree (Figure 1). This is sometime after the estimated age of the family at 28.9 Ma (95% HPD: 47.4–16.7), indicating that the colonization and subsequent diversification within Africa is relatively chronologically recent, but we note confidence intervals are broad. Divergence of the LT radiation at 5.5 Ma (95% HPD: 9.2–3.0) is younger than previous estimates generated from combined mt- and nuDNA data: 7.9 Ma (95% HPD: 10.6–5.5, Brown et al., 2010); 7.0 Ma (95% HPD: 9.9–4.7, Alter et al., 2015) although confidence intervals overlap with these studies.

3.3 | Historical biogeography

Overall our results show strong geographic signal. Model comparison using AIC favoured the unconstrained model (M0)—allowing dispersal to and from the Congo Basin, over either of the constrained models that the Congo Basin is acting solely as a sink (M2) or source (M1) region (Table 2, see Appendix S3). Our analyses revealed multiple independent colonization of the Congo Basin with five to six possible colonization events identified (Figure 1). These events are seeded from several adjacent regions including West Africa, Zambezi and Lake Tanganyika.

Estimation of geographic ranges, using the DEC model, indicated West Africa as the ancestral range for the African radiation, although some nodes in the tree (particularly deep nodes) are ambiguous

TABLE 1 Divergence time estimates implementing a Bayesian uncorrelated lognormal relaxed clock model (BEAST) of key events in the evolutionary history of the Mastacembelidae. Divergence times are indicated as a mean value with 95% confidence intervals [Highest Probability Density (HPD)] in parenthesis.

Dataset	nuDNA			mtDNA			
Calibration	Fossil		Geological	Fossil		Geological	
Prior	Lognormal	Lognormal	Normal	Lognormal	Lognormal	Normal	Normal
Molecular clock	Unlinked	Linked	Linked	Unlinked	Linked	Unlinked	Linked
Channa + Parachanna (Ma)	*Calibration 33 SD0.5*	*Calibration 33 SD0.5*	53.5 (27.0-87.2)	*Calibration 33 SD0.5*	*Calibration 33 SD0.5*	33.4 (22.5-45.2)	29.2 (20.6-38.6)
MRCA Mastacemblidae (Ma)	21.0 (13.7-31.1)	28.9 (16.7-47.4)	44.1 (27.4-64.7)	19.4 (14.3-25.3)	22.1 (16.2-29.9)	20.2 (16.0-25.2)	17.2 (13.8-20.9)
MRCA *Mastacembelus* (Ma)	13.8 (8.7-20.3)	21.3 (12.6-34.8)	32.7 (21.3-46.8)	18.0 (13.5-23.7)	20.5 (14.8-27.5)	18.9 (14.9-23.4)	16.0 (13.0-19.4)
MRCA Africa+Middle East (Ma)	9.9 (6.3-14.4)	16.2 (9.4-26.0)	25.1 (17.1-35.3)	15.5 (11.5-20.2)	17.5 (12.8-23.5)	16.3 (12.9-19.9)	13.7 (11.3-16.2)
MRCA Africa (Ma)	9.4 (6.1-13.9)	15.4 (8.8-23.9)	23.8 (16.0-33.0)	n.a	n.a	n.a	n.a
Lake Tanganyika radiation (Ma)	3.1 (1.8-4.7)	5.5 (3.0-9.2)	*Calibration 10.5 SD0.7*	6.5 (4.7-8.5)	7.6 (5.3-10.2)	*Calibration 10.5 SD0.7*	*Calibration 10.5 SD0.7*

TABLE 2 Comparison of unconstrained and constrained models using DEC. M0 (unconstrained—dispersal to and from the Congo basin); M1 (allowing only dispersal out of the Congo Basin; M2 (allowing only dispersal into the Congo Basin); dispersal (d); extinction (e); number of parameters (k); Akaike Weights (AW). The optimal model is highlighted in grey

		Parameter estimates			AIC analysis		
Constraints	Ln *L*	*k*	*d*	*e*	AIC	ΔAIC	AW
M0	−79.60	2	0.0035	0.0000	163.20	0.00	0.98
M1	−84.13	2	0.0033	0.0002	172.30	9.1	0.01
M2	−84.28	2	0.0052	0.0000	172.60	9.4	0.01

(Figure 1). Taking this into account, our results suggest that with the exception of the Congo Basin, all other geographical regions are remarkably conserved phylogenetically, and have likely been colonized once, indicating considerable geographic constraints (Figure 1, Appendix S3).

The only species included from the Quanza, *M. ansorgii* (Figure 1, see Appendix S3), may be pivotal in reseeding the Congo Basin during the more recent history of the group (clades D+E) leading to recent diversification of *Mastacembelus*. Conversely, in *Synodontis* catfish, the Quanza ichthyo-province was colonized by a Congolese ancestor and showed no further diversification (Day et al., 2013). Notably, the ancestor of clades D+E, reconstructed as Congo Basin in origin (Figure 1), seeded the East African rift lakes—Lake Tanganyika (containing a largely endemic radiation) and Lake Malawi (conversely containing a single endemic), as well as rivers within East Africa.

3.4 | Diversification dynamics and rates

Although a constant rate (pure birth [PB]) for the African radiation is best supported by ML analyses (Table 3), it is only 2.14 times more likely than the next best variable rate (exponential PB) model. This result is reflected by the pattern of diversification generated using BAMM that showed speciation rates gradually decreased through time (Figure 2), but for which zero core shifts (i.e. 100% of the posterior distribution was not assigned to a rate shift) received the best BF score (BF = 1). Visual inspection of LTT plots generated with and without missing taxa (see Appendix S3) supported the BAMM analyses and showed only a marginal trend for an initial early burst and slowdown in diversification. These results are further supported by the gamma statistic assuming 10 missing species ($\gamma = -2.01$), which although negative (indicating a decreasing diversification rate), is not significant ($p = 0.08$). This result does not change assuming additional possible cryptic species (based on results from the mtDNA tree) ($\gamma = -2.38$, $p = 0.17$). BAMM generated mean rates of speciation (λ) for the African radiation = 0.23 (0.16–0.33 upper/lower 95% highest probability density [HPD]) and extinction (μ) rate = 0.09 (0.05–0.25 upper/lower 95% HPD). In contrast to the African radiation, a variable rate model (exponential PB model) was best fit for the LT radiation when this clade was analysed separately using RPANDA (Table 3).

4 | DISCUSSION

4.1 | Into Africa: timing and biogeographic origins

Mastacembelids and their constituent genera originated in Asia, with *Mastacembelus* suggested to have diverged sometime during the Early Miocene 21.3 Ma (95% HPD: 34.8–12.6). A single colonization (Figure 1, nuDNA tree) of the African continent occurred later at 15.4 Ma (95% HPD 8.8–23.9) in which *Mastacembelus* subsequently radiated throughout the Afrotropics. Ancestral range estimation for the ancestor of the African radiation (M0) gave a strong preference

TABLE 3 Testing models of diversification. Models include: pure birth (PB); birth-death (BD); PB with an exponential rate (PBλexp); BD with a constant speciation rate and exponential extinction rate (BDλcst-μexp); BD with a exponential speciation rate and constant extinction rate (BDλexp-μcst); BD in which speciation and extinction rates are both exponential (BDλexp-μexp). Log-likelihood (AICc) and the difference in AICc with the best model (ΔAIC) are shown, along with Akaike Weights (AW). Parameters refer to the estimated rates at the tips and the corresponding time-variation parameter. The best model is indicated in grey. *f* = number of sampled taxa/total of that clade (see section on lineage diversification)

Model	λ parameter	μ parameter	Log L	AICc	ΔAICc	Aw
African radiation (*f* = 41/51)						
Constant rate models						
Pure birth (PB)	0.188		−108.509	219.121	0	0.480
Birth-death (BD)	0.188	<0.001	−108.509	221.334	2.213	0.159
Variable rate models						
PBλexp	0.160; 0.033		−108.164	220.643	1.5225	0.224
BDλcst-μexp	0.188	<0.001; −0.039	−108.509	223.667	4.5461	0.049
BDλexp-μcst	0.160; 0.033	<0.001	−108.164	222.976	3.8553	0.070
BDλexp-μexp	0.160; 0.033	<0.001; −0.071	−108.164	225.439	6.3178	0.020
Lake Tanganyika radiation (f = 15/17)						
Constant rate models						
Pure birth (PB)	0.293		−29.834	61.977	2.743	0.140
Birth-death (BD)	0.293	<0.001	−29.834	64.669	5.435	0.037
Variable rate models						
PBλexp	0.102; 0.442		−27.117	59.234	0	0.553
BDλcst-μexp	0.279	<0.001; −0.114	−32.827	73.653	14.419	<0.001
BDλexp-μcst	0.102; 0.442	<0.001	−27.117	62.416	3.182	0.113
BDλexp-μexp	0.103; 0.439	<0.001; −0.156	−27.117	66.234	7.000	0.018

for a broad West African conglomerate (Figure 1) supporting findings for several other freshwater Afrotropical radiations (Daniels et al., 2015; Day et al., 2013; Schwarzer, Misof, Tautz, & Schliewen, 2009). A more detailed biogeographic analysis within Day et al. (2013) that split West Africa into its ichthyo-provinces (i.e. N-S, UGF, LGF) supported the Nilo-Sudan region as ancestral. As *Mastacembelus* colonized from Asia, presumably through the Middle East, it is likely that they also dispersed into western Africa via the Nilo-Sudan, and that these species have since gone extinct from this region or have not yet been sampled.

4.2 | Multiple independent colonization events into the Congo Basin

We found strong evidence for repeated independent colonization into the Congo Basin, mostly likely due to availability of habitats and resources within this complex hydrological system. This has occurred throughout the evolutionary history of African *Mastacembelus* between different adjacent regions, in which several lineages have subsequently diversified, albeit to a limited extent. These inferred colonization events are consistent with dispersal being the primary cause of lineage divergence with respect to the Congo Basin. Based on our densely sampled tree, we however show that the original (unconstrained) model allowing dispersal into, as well as out of, the Congo Basin (Table 2) is strongly preferred over the Congo Basin acting purely as a macroevolutionary source (M1) or sink (M2) region.

Studies of other taxa including cichlid fishes and tigerfish (*Hydrocynus*) also highlight repeated colonization of the Congo Basin (e.g. Day, Santini, & Garcia-Moreno, 2007; Goodier et al., 2011; Schelly, Salzburger, Koblmüller, Duftner, & Sturmbauer, 2006; Schwarzer et al., 2009) but have not explicitly tested between alternative biogeographic models. The importance of dispersal in shaping broad-scale biodiversity patterns both temporally and spatially has recently been suggested (Pigot & Tobias, 2014) in which rates of transition from allopatry to sympatry were indicated to be faster in more strongly dispersive clades, although this has not been tested at a more local, that is, regional level, or in freshwater fishes.

While dispersal was likely to be an important process to the build up of the ichthyofauna of the Congo Basin, our findings otherwise show all other areas to be remarkably conserved phylogenetically indicating considerable geographic constraints. Aggregation of distinct biogeographic clades, including the Congo Basin, was also shown analytically for *Syndontis* (Day et al., 2013), in which these catfishes in contrast to *Mastacembelus* have diversified to a great extent in situ in this region.

Several of the recent independent colonizations of the Congo Basin by *Mastacembelus* are clades composed of Lower Congo River

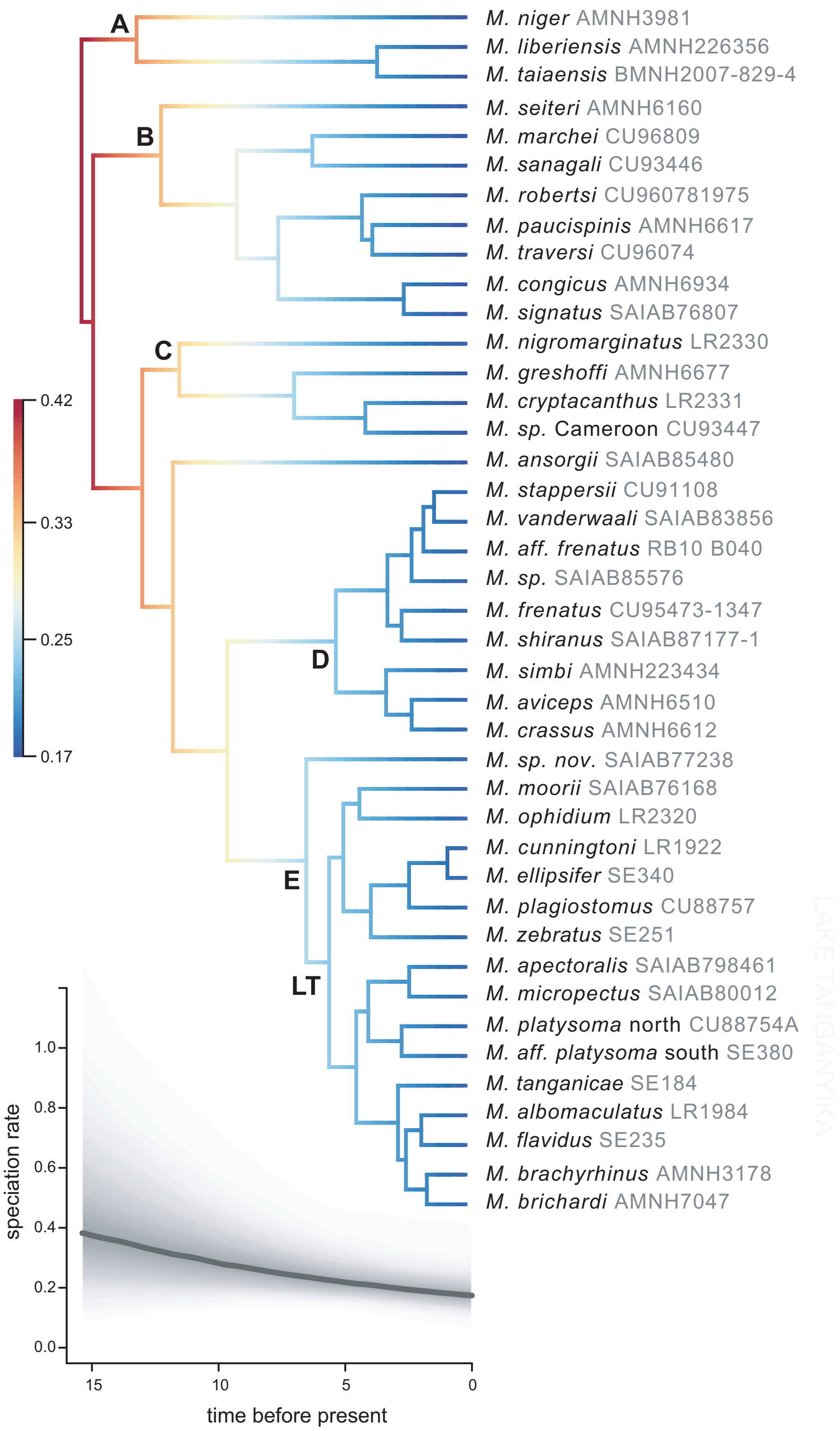

FIGURE 2 Diversification dynamics of the African *Mastacembelus* radiation inferred using BAMM. Top: Phylorate plot shows speciation rate along each branch ('linear' plot); warmer colours indicate faster rates; scale bar represents speciation rate per Ma. Bottom: Evolutionary rate variation through time (RTT plot); shading denotes confidence on rate reconstructions at any point in time

endemics (Figure 1, denoted in bold text; see also Alter et al., 2015). This high-energy flow environment differs from the low-energy environments (e.g. rift lake, mature rivers) of their ancestors. These independent colonizations of the Lower Congo River do not appear to be simultaneous (although there is some overlap in HPD values), with an initial event occurring 5.1 Ma (95% HPD: 9.1–2.5), while a second event occurred as recently as 2.4 Ma (95% HPD: 4.3–1.0), in which the ancestor diverged from a derived clade within the LT species flock. Both these ages are within the time frame of when the high-energy flow environment of the modern Lower Congo rapids was established (see Alter et al., 2015 and references therein). While LT *Mastacembelus* are endemic, the LT species *M. cunningtoni* has also been recorded in the Lukuga River (Kullander & Roberts, 2011). As such, it is plausible that the ancestor of the Lower Congo River species *M. brachyrhinus* and *M. brichardi* emigrated from LT via the Lukuga River, which is the only outflow of LT, and is part of the

Congo River system. Such emigration has been documented in other fishes, for example, lamprologine cichlids in which several species are suggested to have colonized the Congo River from LT (Day et al., 2007; Schelly et al., 2006). Notably, the colonization of LT from the Congo Basin contrasts to other evolutionary radiations from this system that have conversely been colonized from East Africa (e.g. Daniels et al., 2015; Day et al., 2013).

Applying a similar analytical framework to other freshwater continental clades will bring important insights into the processes underlying the high levels of diversity within the Congo Basin.

4.3 | Diversification dynamics of a continent-wide radiation

During the time period of diversification suggested by our findings, Africa experienced major climatic and geological events. For example, the Middle Miocene Climatic Optimum (MMCO *c.* 15–17 Ma) was a warmer period with a precipitation maximum (Zachos, Pagani, Sloan, Thomas, & Billups, 2001) that could have enhanced connectivity of African rivers, and therefore have facilitated diversification. However, despite fluctuating environmental conditions, and colonization and subsequent radiation by one lineage of an insular system (Lake Tanganyika), there is no evidence for rapid lineage diversification and our results suggest that diversification is near constant (Table 3, Figure 2, see Appendix S3). This contrasts to the early burst in diversification followed by clear declining rates demonstrated for some other continental clades across different animal groups (e.g. De-Silva et al., 2016; Rabosky & Lovette, 2008) that has been hypothesized to have been caused by rapid filling of available niches. South America, like Africa, has experienced major orogenic events, and for many of the South American groups that have been studied, these events are thought to have opened up vacant niches leading to density-dependent diversification (e.g. De-Silva et al., 2016 and references therein). However, a study of a truly continental South American radiation of ovenbirds identified a constant rate of diversification (Derryberry et al., 2011). That study and several others focusing on African continent-wide radiations (Day et al., 2013; Liedtke et al., 2016) have suggested that either these clades are too young to have reached their ecological limit, or that tropical continental diversification may not be as limited by ecological opportunities. The latter suggestion is particularly noteworthy considering that Africa, as the world's second large land mass, totals *c.* 30 million km^2. In contrast to our findings for the continental radiation, the LT clade (including non-endemics) was, however, supported by a variable rate model. Time variable models support a general trend for declining speciation rate over time (see Morlon, 2014 and references therein). This trend is often interpreted as a density-dependent effect from the saturation of niche space following adaptive radiation (Morlon, 2014), which is a common pattern identified in insular systems (e.g. Day et al., 2008; Reddy et al., 2012). Ultimately, further studies focused at the continental scale are needed to determine if these patterns are supported across other tropical groups, in addition to understanding the factors responsible for their diversification.

4.4 | Molecular divergence dates

Although our study employed a single fossil calibration that may bias age estimates (see Ho et al., 2015 and references therein), its inclusion produced relatively similar divergence estimates across mtDNA and nuDNA analyses considering 95% confidence intervals (Table 1), although we acknowledge that these were broad. We note that the time frame of the LT radiation is congruent with studies of other non-cichlid LT fish groups (at a similar taxonomic level) that have used limited fossil calibrations (e.g. Day et al., 2013; Peart, Bills, Wilkinson, & Day, 2014), providing validation of the selected calibration. In contrast, although the geologic calibration, placed on a shallow node, produced similar estimates for the mtDNA analyses to those generated from the fossil calibration based on a deep node, its effect on the nuclear data was striking in consistently estimating older divergence dates across all nodes. Calibrations at the root or deeper nodes are generally preferred to shallower nodes as substitution rate estimates are mostly based on the branches between the calibrating nodes and the tips, and as such deeper calibrations capture a larger proportion of the overall genetic variation (Duchêne, Lanfear, & Ho, 2014). This explains why the nuclear derived estimates using the geologic calibration have had a greater impact on node ages, as these branches between the calibrating node and the tips contain considerably less genetic variation than those for the mtDNA data. Nuclear loci from more rapidly evolving markers, and additional fossil calibrations, would allow future researchers to potentially generate more accurate divergence dates.

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DATA ACCESSIBILITY

Nexus files used in this study available from http://datadryad.org/ doi: 10.5061/dryad.1jq96.

AUTHOR CONTRIBUTIONS

J.J.D., K.J.B. and L.R. conceived the ideas; K.J.B. and A.F. generated and complied the sequence data; J.J.D., A.F. and L.R. analysed these data; J.J.D., wrote the paper; all co-authors contributed samples and helped revise the manuscript.

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BIOSKETCH

Julia J. Day is interested in investigating patterns and processes of diversification leading to the build up of diversity at local and regional scales.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

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