



RESEARCH ARTICLE

Going with the flow? Diversification of gastropods reflects drainage evolution in Africa

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Abstract

Aim: The roles of geodynamics, climatic variability and landscape evolution in shaping aquatic biodiversity patterns on the African continent remain poorly understood. We studied the geographical origin and phylogenetic relationships of an Afrotropical freshwater snail genus to examine the role of drainage evolution on diversification and range evolution. The relevance of fish provinces in bio-regionalization of invertebrates was explored, as well as the evolution of habitat specificity.

Location: Africa including Madagascar.

Taxon: *Lanistes* (Gastropoda: Ampullariidae).

Methods: Based on a sampling covering the entire geographical range, we reconstructed a fossil-calibrated multi-locus molecular phylogeny using maximum likelihood and Bayesian inference approaches. After applying species delimitation methods, we estimated ancestral areas and habitats and examined rates of diversification through time using lineage through time plots.

Results: RAXML and MrBayes analyses resulted in highly congruent topologies and a strongly supported phylogeny. Our BEAST analysis indicate that *Lanistes* probably originated in the Eocene about 50 Ma and the most recent common ancestor (MRCA) of all 23 *Lanistes* OTUs in our study may have inhabited an area including the Central African and adjacent Lower Guinean biogeographical regions. A steeper increase in species accumulation from the middle Miocene (c. 15 to 10 Ma), followed by a decrease towards the present was found. Sympatry and jump dispersal were the common cladogenetic events and only a single anagenetic dispersal event was detected. The biogeographical analyses further suggest that Madagascar was colonized from East Africa and that the Zambezi River was colonized at least twice independently. Seven species are confined to rivers and three live exclusively in lakes. The estimation of ancestral habitats suggested that the MRCA of all *Lanistes* probably evolved in a riverine habitat.

Main conclusions: The diversification of *Lanistes* started in the Eocene and occurred at a constant pace apart from a possible climate-related increase in the Miocene. This

Equal contribution: Björn Stelbrink and Christian Albrecht.

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study highlights the significance of temporal geographical isolation of river systems and subsequent reconnection in clade diversification and of jump dispersal in range evolution. More comparative analyses across various taxa are needed to obtain a better understanding of African freshwater biodiversity.

KEYWORDS

Ampullariidae, biogeography, dispersal, East African Rift System, fossil record, molluscs, radiation, river basin

1 | INTRODUCTION

Africa is renowned for its terrestrial megafauna and biodiversity hotspots in rainforest and savannah (e.g. Myers et al., 2000). Freshwater life is, however, also very diverse with, among others, >3000 species of fishes (Lévêque et al., 2008), and diverse invertebrates such as crabs, dragonflies and molluscs (Holland et al., 2012). This freshwater biodiversity is assumed to have largely arisen because of the very heterogeneous landscape and especially a hydro-landscape in which a mosaic of old and young rivers as well as lakes and extensive wetlands exists at various spatial scales (Thieme et al., 2005).

The current complexity of African aquatic ecosystems and their faunas partly traces back to the breakup of Gondwana (Moore & Larkin, 2001). However, the recent biodiversity represents an assemblage of species that originated on Gondwana, complemented by Cenozoic colonizers from Asia. Geological processes including dome formation and the rifting that created the East African Rift System (EARS) played a pivotal role in shaping the continent and its aquatic ecosystems (Ring et al., 2018; Salzburger et al., 2014). Rifting gave rise to large lakes such as Lakes Tanganyika and Malawi and modified the course and hydrographic connectivity of ancient river systems such as the Congo River (Stankiewicz & de Wit, 2006). However, the roles of rifting in shaping aquatic communities and biodiversity patterns over time are still largely unexplored.

Apart from geodynamics, climatic variability has also impacted terrestrial and aquatic biodiversity patterns profoundly (e.g. Lyons et al., 2015). Aquatic ecosystems might have been particularly impacted by Plio-Pleistocene climatic shifts (deMenocal, 2004), which altered water availability (Cohen et al., 2007), lake levels (Lyons et al., 2015), the presence of refugia and the hydrographic connectivity of rivers (Beadle, 1974). These environmental factors have influenced speciation and extinction and, as such, have shaped diversification processes (Salzburger et al., 2014). For example, the evolution of the major African drainage systems is hypothesized to have impacted evolutionary processes and large-scale biodiversity patterns in haplochromine cichlids (Genner et al., 2007). The few studies at the continental scale show partly contradictory patterns (Daniels et al., 2015; Day et al., 2013), which, however, hampers comparative studies of diversification across taxa.

Given the mosaic landscape evolution, one would expect complex dispersal events such as intra-basin dispersal across barriers

such as rapids and falls, inter-basin dispersal and, in rare cases, transoceanic dispersal as well as vicariance to have been involved (see, e.g. Daniels et al., 2015). Despite the potential of phylogenetic, biogeographical and phylogeographical studies for the reconstruction of landscape/drainage evolution, there is a significant bias towards studies on lakes rather than river systems. This bias is in part caused not only by the attractiveness of spectacular species radiations in the tectonic lakes but also the practical advantages of studying evolutionary processes in a comparatively small, insular system as opposed to vast drainages. Most of the few continental-scale studies on the evolution of aquatic biodiversity are based on fish such as haplochromine cichlids (e.g. Schwarzer et al., 2009), squeaker catfish (*Synodontis* spp.; Day et al., 2013) and spiny eels (*Mastacembelus* spp.; Day et al., 2017). Consequently, biogeographical regions are mainly delineated based on diversity patterns in fish (Lévêque et al., 2008). Given that most fish are highly mobile and may disperse over large distances, against the flow, across barriers or via short-term connections during wetter conditions, dispersal patterns may differ drastically from those in most invertebrates. In addition, fish translocations by humans may obscure biogeographical patterns (e.g. Van Steenberge et al., 2020). Biogeographical studies of less mobile invertebrates may allow reconstruction of hydrographic connectivity of drainages through time more reliably. However, beyond a detailed study on the crab family Potamonautidae (Daniels et al., 2015), which revealed historical drainage connectivity and transoceanic dispersal since the Eocene, invertebrate studies (Ortiz-Sepulveda et al., 2020; Schultheiß et al., 2014) are usually not at a fully continental scale, or not framed in an explicit biogeographical context (Jørgensen et al., 2008). Taxa endemic to Africa with a moderate number of species, a benthic lifestyle, omnipresence throughout continental freshwater systems, flexible ecology and life history are perhaps promising candidates to yield complementary information on evolutionary processes in deep time, especially if they have a well-preserved fossil record. Many freshwater gastropod groups possess all these characteristics (Brown, 1994).

Apple snails (Ampullariidae) are a diverse pantropical family of probably Gondwanan origin with several genera endemic to Africa (Brown, 1994). The family has been considered to represent an excellent model to study speciation, historical biogeography and adaptation (Hayes et al., 2009; Sun et al., 2019). We here focus on the endemic genus *Lanistes* as a potential model taxon to reconstruct drainage evolution. Fossils of *Lanistes* are well known

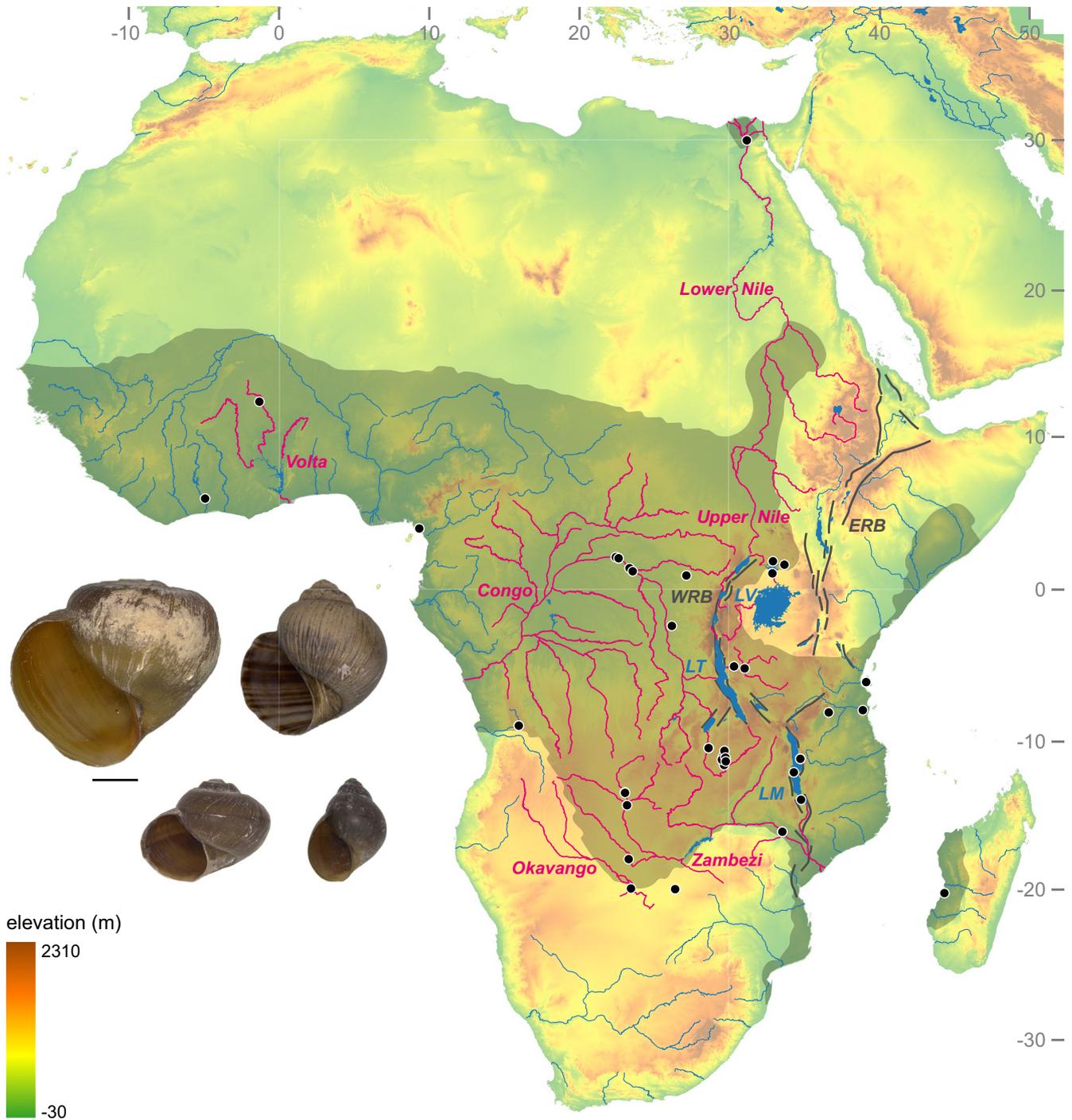


FIGURE 1 Digital elevation model (DEM) for Africa including major lakes and river systems. The shaded areas represent the range of *Lanistes* according to Brown (1994). Black dots indicate the sample sites of the present study. Important drainages discussed in the study are highlighted in magenta, large lakes are labelled (LV=Lake Victoria, LT=Lake Tanganyika, LM=Lake Malawi/Nyasa/Niassa). The East African Rift System (EARS) is shown with the eastern and western branches (ERB and WRB, respectively) that were redrawn from Ring et al. (2018). Shell images represent the morphological diversity of recent *Lanistes* species (from upper left clockwise: *L. nyassanus*, Lake Malawi, UGSB 23825; *L. libycus*, Cameroon, UGSB 20201; *L. purpureus*, Zanzibar, UGSB 22845; *L. carinatus*, Egypt, UGSB 20204). All shells are to scale (10 mm) [Colour figure can be viewed at [wileyonlinelibrary.com](https://onlinelibrary.wiley.com)]

from, among others, Eocene deposits in Egypt, Oligocene deposits in the Rukwa Rift and Miocene deposits from Rushinga Island and the Albertine Rift Valley (Epa et al. 2018; Harzhauser et al. 2017; Kat, 1987; Van Damme & Pickford, 1995). Additionally, *Lanistes*

shells were recently reported from the Zalumah Formation in the Salalah region of the Sultanate of Oman, the occurrence of *Lanistes* together with other species in the Tethys region suggest that the modern African–Arabian continental faunas can be

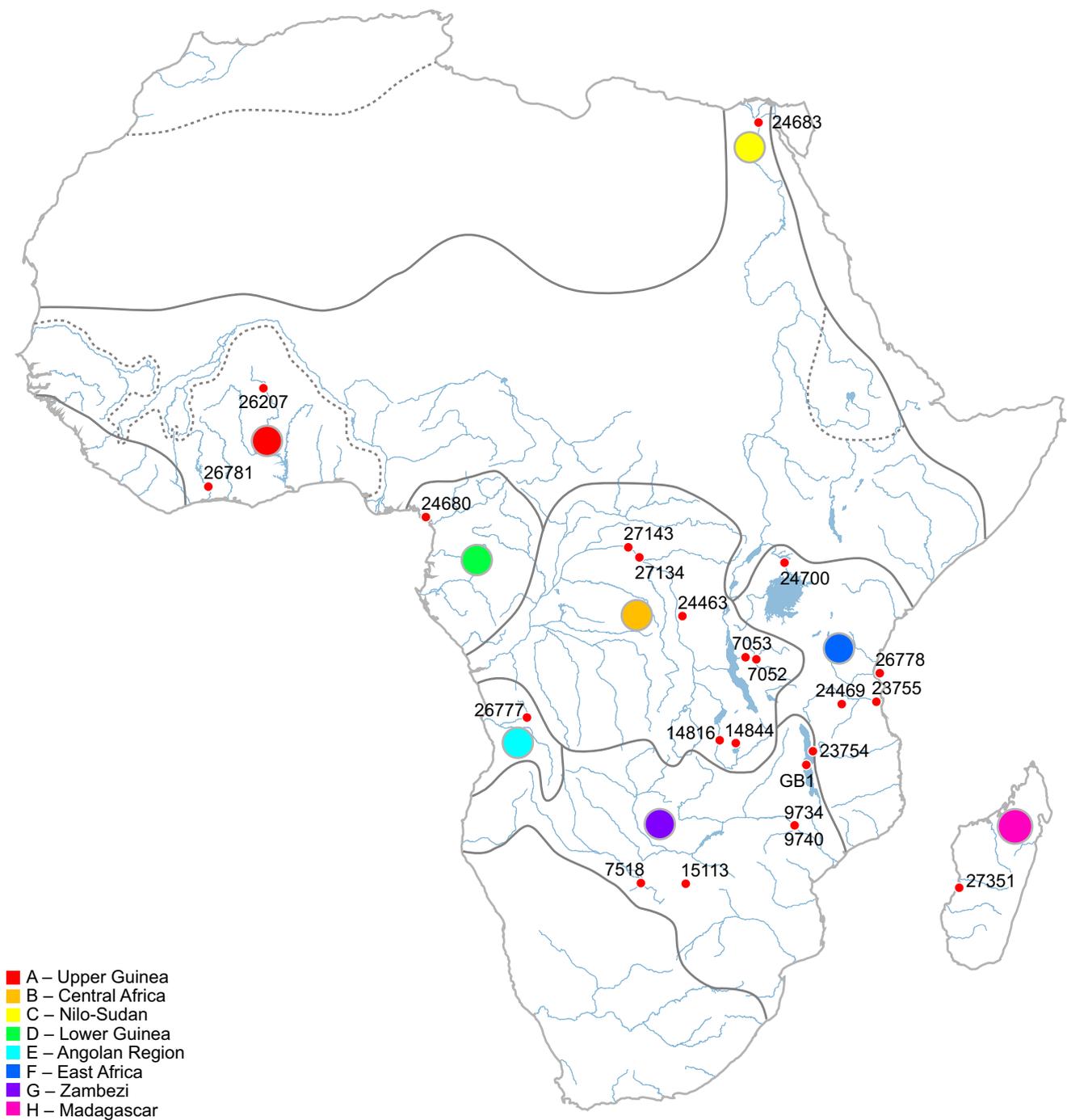


FIGURE 2 Map of Africa including major lakes and river systems and fish provinces according to Lévêque et al. (2008). Note that province A (Upper Guinea) is a modification of that of Lévêque et al. (2008) using new hydrological basin mapping published by the FAO, as recently used by Arroyave et al. (2020). It encompasses more Atlantic drainages that were previously considered to belong to the Nilo-Sudan or Eburneo-Ghanean Region. Provinces are colour-coded according to the subsequent biogeographical analyses. Red dots with numbers indicate specimens used for all downstream analyses representing the OTUs identified by the species delimitation analyses bPTP and GMYC [Colour figure can be viewed at wileyonlinelibrary.com]

partly traced back to the Eocene (Harzhauser et al. 2017), or even late Cretaceous times (Pickford, 2017). The group has an almost pan-African distribution including occurrences on Madagascar (Figure 1). Whereas 43 taxonomically valid species exist (Cowie, 2015), the estimated diversity of extant *Lanistes* species is

approximately 20 (Hayes et al., 2015; see also Berthold, 1991 and Brown, 1994). *Lanistes* species occur in almost all types of freshwater bodies ranging from small temporary ponds and streams to large lakes and rivers (Brown, 1994) because of their life history, adaptability, reproduction mode and ability to estivate (Berthold,



1991). Whereas most studies on *Lanistes* have focused on the relatively small adaptive radiation of the Malawi Basin (Schultheiß et al., 2009; Van Bocxlaer, 2017; Van Bocxlaer et al., 2020), comprehensive large-scale phylogenetic analyses of the genus are largely lacking (but see Jørgensen et al., 2008).

In this context, we conducted phylogenetic studies of *Lanistes* throughout its geographic range (Figure 1). Specifically, we aimed to: (1) identify the geographical origin of and phylogenetic relationships within *Lanistes*; (2) test the impact of drainage evolution and the role of the EARS on diversification of the group and, more generally, the roles of anagenetic versus cladogenetic events such as vicariance and dispersal on range evolution; (3) determine the role of palaeodrainage connectivity and to explore the relevance of fish provinces in bio-regionalization of invertebrates and (4) evaluate whether habitat specificity has changed within the genus over time and how it has been impacted by climate shifts.

To meet these aims we developed a fossil-calibrated multi-locus molecular phylogeny, further applied species delimitation methods to estimate ancestral areas and habitats and examined rates of diversification using lineage-through-time plots. To synthesize all of these results, we discuss diversification scenarios for *Lanistes* in the context of continental drainage evolution and large-scale biogeographical patterns in Africa. Finally, we compare our findings with patterns in fish and other freshwater invertebrates.

2 | MATERIALS AND METHODS

2.1 | Sampling

Specimens of *Lanistes* were collected between 2006 and 2019 throughout Africa including Madagascar covering most of the range of the genus, with less dense coverage in western Africa (Figure 1). This range includes West and Central African drainage systems, the Nilo-Sudan, Lower Guinea, Angola and East Africa, the Zambezi drainages and Madagascar (Tables S1 and S2; Figure 2). Obtaining recent samples from vast regions of the continent, especially western and Sahelian Africa but also parts of Central Africa, is challenging however, we have covered all bioregions. Snails were hand-picked or collected with a scoop net or dredge in perennial pools and ponds, marshes and swamps, lakes and a variety of lotic waters. Shells were partly cracked and specimens fixed in 80% ethanol. Vouchers are stored in the Systematics and Biodiversity Collection of the Justus Liebig University (UGSB).

2.2 | DNA extraction, amplification and sequencing

In most cases, DNA was extracted from two *Lanistes* specimens per locality with a CTAB protocol (Wilke et al., 2006). Fragments of two mitochondrial markers, COI and 16S rRNA, were amplified using the primers LCO1490 and HCO2198 (Folmer et al., 1994) and 16Sar and

16Sbr (Palumbi et al., 1991), respectively. Sometimes, internal primers were used to improve amplification. Fragments of two nuclear markers, histone 3 (H3) and 28S rRNA, were amplified with the primers H3F and H3R (Colgan et al., 2000) and C1 (Mollaret et al., 1997) and 28SR2 (Morgan et al., 2002) respectively. PCR conditions were as described by Stelbrink et al. (2016). Bidirectional sequencing was performed on an ABI 3730 XL sequencer (Life Technologies) at LGC Genomics, Berlin.

2.3 | Phylogenetic analyses

DNA sequences were edited using MEGA v.7.0 (Kumar et al., 2016) and complemented with previously published (Jørgensen et al., 2008; Schultheiß et al., 2009) sequences available from NCBI GenBank. The 43 ingroup specimens were supplemented with two ampullariid outgroups, namely *Afropomus balanoideus* and *Pila polita* (Table S1). The 16S rRNA and 28S rRNA fragments were aligned using the MAFFT web server (Katoh & Standley, 2013). We used PartitionFinder v.2 (Lanfear et al., 2016) with the greedy algorithm and codon-position data blocks to identify the best-fit substitution model for subsequent phylogenetic analyses based on the corrected Akaike Information Criterion (AICc). Bayesian inference (BI) was performed using MrBayes v.3.2.6 (Ronquist et al., 2012), with two independent Markov Chain Monte Carlo (MCMC) searches (each with four chains) for 10 million generations and sampling every 500th tree and applying a burn-in of 50%. Convergence of the two independent runs was examined *a posteriori* in Tracer v.1.5 (Rambaut & Drummond, 2007). A maximum likelihood (ML) analysis was conducted using the RAXML-HPC BlackBox v.8.2.10 (Stamatakis, 2014) with the standard GTR+ Γ substitution model for all partitions and a stop rule for the bootstrapping. For comparative purposes, RAXML analyses were also conducted on the mitochondrial and nuclear datasets separately. All phylogenetic and molecular-clock analyses (see below) were performed on the CIPRES Science Gateway (Miller et al., 2010).

2.4 | Species delimitation

We delimited molecular operational taxonomic units (OTUs) with the Poisson tree processes (PTP) model (Zhang et al., 2013) using the BI and the ML approaches and default settings as implemented on the bPTP web server with the MrBayes phylogram. In addition, BEAST was run with the full dataset of specimens and a single calibration point (see below). The resulting chronogram was analysed on the GMYC web server using the GMYC species delimitation approach (Fujisawa & Barraclough, 2013) with a single threshold. In parallel, specimens were identified based on shell morphology using the taxonomic literature (e.g. Brown, 1994; Cowie, 2015) to allow matching OTUs to species names. If a name existed for an OTU, we assigned it, otherwise the OTU was indicated as *Lanistes* sp.

2.5 | Estimation of divergence times and lineage-through-time plots

We estimated divergence times with BEAST v.1.8.4 (Drummond et al., 2012) using fossil calibration points, strict and lognormal relaxed-clock models and Yule and birth–death tree priors. We applied four fossil calibration points throughout the phylogeny, which were established based on extensive literature research and the examination of museum material. For all calibration points (CP), a gamma distribution was applied to the prior and we allowed the maximum (the 95% quantile) of the prior to be arbitrarily 25% older than the offset to account for the incompleteness of the fossil record and to further allow that the different calibration points can interact much more freely. CP1 marks the first appearance of hyperstrophic Ampullariidae in the fossil record. *Pseudoceratodes flandrini* originated in the Palaeocene of Algeria (Jodot, 1953), implying a hard minimal bound on the appearance of hyperstrophic Ampullariidae at 56.0 Ma (offset = 56.0, 95% quantile = 70.0, scale = 4.7). CP2 makes use of the Egyptian *L. antiquus* at the stem of the *L. carinatus-varicus* clade. Here, we follow the more conservative and original stratigraphic position provided by Blanckenhorn (1901), that is, we applied a hard minimum bound on the appearance of *L. antiquus* of 37.8 million years ago (Ma; offset = 37.8, 95% quantile = 47.25, scale = 3.2). The oldest *L. carinatus* was chosen for CP3. Verdcourt (1963) reported *Lanistes carinatus* from various localities at Rusinga Island, Kenya, more precisely in the Kiahera and Wayando Formations, which predate 18.0 Ma (Peppe et al., 2009). Kat (1987) reported an age estimate for this material of c. 23.0–18.0 Ma ago. Given the stratigraphic position, a hard minimum bound on the appearance of *L. carinatus* can be set to 20.0 Ma (offset = 20.0, 95% quantile = 25% of offset = 25.0, scale = 1.7). Finally, CP4 represents the split between *L. nsendweensis* and *L. congicus*. *Lanistes heynderycxi*, a small species of Palaeolake Obweruka (Uganda, DR Congo) occurs in mollusc association G2a, which has been reported to be from the middle Miocene (Van Damme & Pickford, 1995). However, its time range based on mammalian biostratigraphy has been dated to 9.0–6.5 Ma (Pickford et al. 1993), and Van Damme and Pickford (2003) also indicated a late Miocene age, c. 8.5 Ma (offset = 8.5, 95% quantile = 10.625, scale = 0.7).

Analyses were run in two replicates for 40 million generations sampling every 2000th tree. Marginal likelihoods were estimated using path sampling (PS) and stepping-stone sampling (SS) as implemented in BEAST (see Baele et al., 2012) to compare analyses with different combinations of clock models and tree priors. LogCombiner v.1.8.4 (BEAST package) was used to combine log and tree files of the replicates with a 50% burn-in. The maximum clade credibility (MCC) tree was generated with TreeAnnotator v.1.8.4 (BEAST package).

In order to assess whether rates of diversification were affected by Africa's dynamic geological and limnological history, lineage-through-time (LTT) and LTT95 plots were generated based on the BEAST MCC tree and the posterior tree distribution

using phytools v.0.6–44 (Revell, 2012) in R v.3.5.1 (R Core Team, 2019).

2.6 | Biogeographical analyses

We examined *Lanistes* from throughout its range in Africa and Madagascar based on literature records (Brown, 1994) (Figure 1). To perform biogeographical analyses, each OTU identified by the species delimitation method and subsequently validated was coded based on its occurrence in its corresponding fish province (Lévêque et al., 2008; Figure 2). Our sampled *Lanistes* individuals represent eight fish provinces which were matched to the OTUs: Upper Guinea (A), Central Africa (B), Nilo-Sudan (C), Lower Guinea (D), Angolan Region (E), East Africa (F), Zambezi (G) and Madagascar (H). Because Lake Malawi is either included in the East Africa or Zambezi provinces, it is here coded as belonging to both regions.

Six biogeographical models (DEC, DEC+j, DIVALIKE, DIVALIKE+j, BayAreaLIKE and BayAreaLIKE+j) were tested using the R package BioGeoBEARS v.1.1 (Matzke, 2013a, 2013b, 2014), in which lineages were allowed to occur in a maximum of two combined ancestral areas (i.e. A to H and AB to GH, but not, e.g. ABC or BCD). The best-fit model was identified using AIC comparison (Table S4). To identify the contribution of cladogenetic (sympatry, subset sympatry, vicariance and range-changing jump-dispersal sensu Matzke, 2014) and anagenetic (dispersal = range expansion, extinction = range contraction) events, biogeographical stochastic mapping (BSM; Dupin et al., 2017) was performed in BioGeoBEARS using the parameters obtained from the best-fit biogeographical model and 1000 simulations. To assess whether the estimation of both ancestral areas and particularly cladogenetic events was affected by the fish provinces we used, which may contain multiple drainage systems (e.g. Upper Guinea or East Africa), we performed a complementary analysis using 15 catchments that are independent of these bioregions (Figure S5, Table S5).

2.7 | Estimation of ancestral habitat types

We defined three habitat types: lake, river and swamp. Flood-plain habitats have been coded as 'river' because these form a continuous although dynamic habitat for snails; the category 'swamp' included all other types of wetlands. For OTUs that have an assigned species name, habitats were coded based on our empirical data and habitat descriptions from the literature as summarized by Brown (1994). For other OTUs, habitats were coded only based on our sampling.

Ancestral habitat types were estimated using stochastic character mapping (Huelsenbeck et al., 2003) as implemented in phytools. Three models of discrete character evolution available in phytools were tested, namely ARD (all rates different), SYM (symmetric rates) and ER (equal rates) using 1000 stochastic character maps. Model comparison was performed with AIC (Table S4).



3 | RESULTS

3.1 | Phylogenetic relationships and biodiversity patterns

According to the PartitionFinder analysis, the best-fit partition scheme and substitution models were as follows: GTR+I+ Γ for 16S rRNA, GTR+I+ Γ for 28S rRNA+H3^{2nd}, GTR+ Γ for COI^{1st}, GTR+I+ Γ for COI^{2nd}, GTR+I+ Γ for COI^{3rd}, GTR+ Γ for H3^{1st} and JC for H3^{3rd}. For the MrBayes analysis, effective sample size (ESS) values of >200 indicated adequate sampling of posterior distributions. Phylogenetic reconstruction with RAxML and MrBayes resulted in highly congruent topologies and an overall strongly supported phylogeny (Figure S1) comprising five major clades (Figure 3). The analysis of this total dataset was robust, given that it was compatible

to analyses based on only mitochondrial or nuclear (Figures S2, S3). Clade C1 is represented by *Lanistes libycus* from the Donger River (Cameroon), *L. congicus* from the Elila River (DR Congo) and Kalandula Falls (Angola), *Lanistes* sp. from the Malagarasi River (Tanzania) and *L. nsendweensis* from the Congo River (DR Congo). *Lanistes carinatus* from the Nile River (Egypt and Uganda) forms a clade C2 with *L. varicus* from the Bandama River (Ivory Coast) and Loumbila (Burkina Faso). Clade C3 comprises *L. grasseti* from Madagascar, forming the sister clade of Clades C4 and C5. Clade C4 contains endemics from Lake Malawi, their sister taxon *L. ellipticus* from the Zambezi River at Tete (Mozambique) and *L. purpureus* from Rufiji (Tanzania), Zanzibar and the Kilombero River (Tanzania). Clade C5 is complex and consists of *L. ovum* from the Nata River (Botswana), and undetermined specimens from Lake Sagara (Tanzania), the Luposhi River (Zambia), the Thamalakane

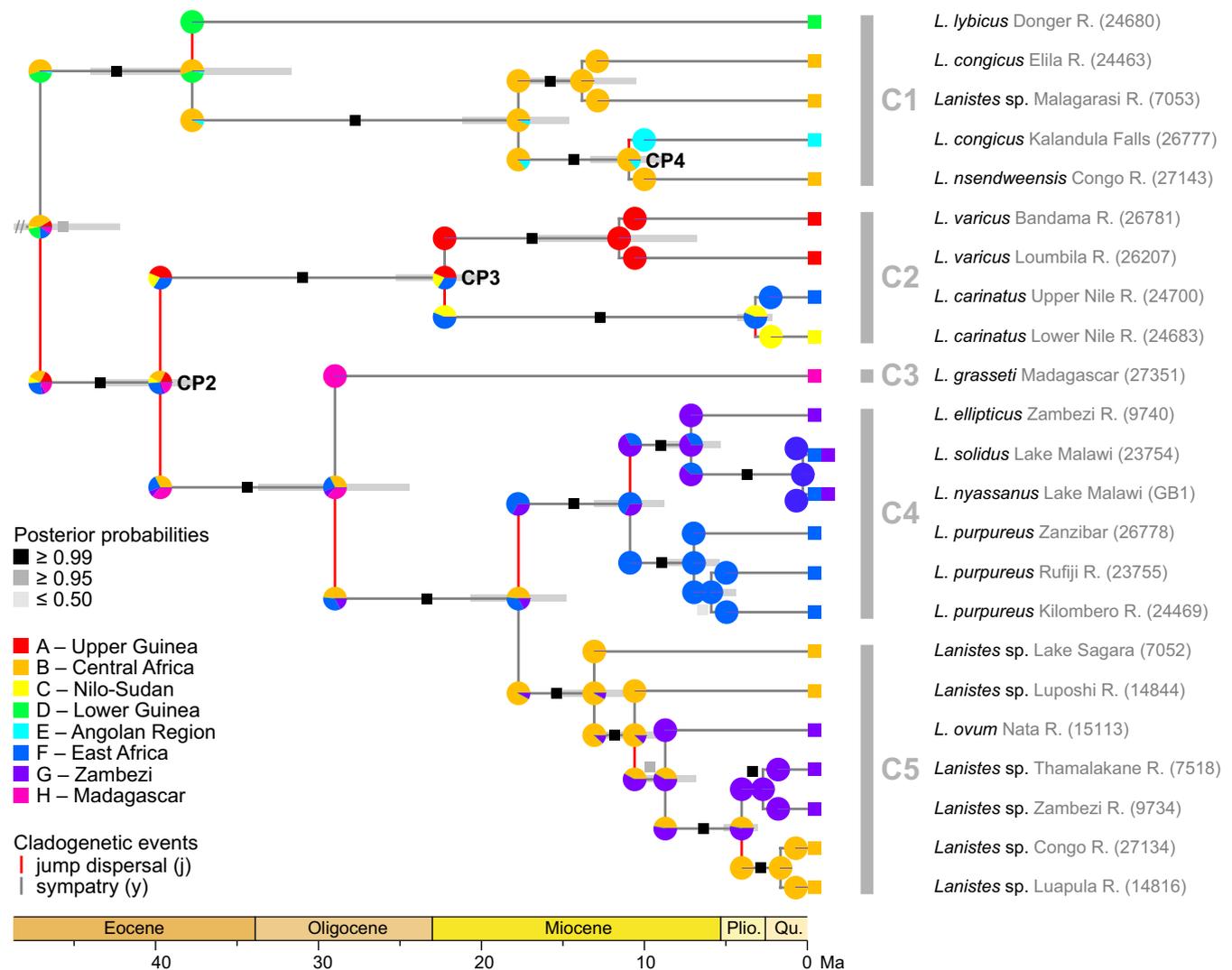


FIGURE 3 BEAST MCC tree including posterior probabilities (squares on branches), 95% highest probability densities (grey bars) and results from the biogeographical (BioGeoBEARS) analysis. Pie charts represent the probabilities of ancestral ranges at the respective nodes. Calibration points are indicated by CP2-CP4 (note that CP1 is not shown as it refers to the split between *Pila polita* and *Lanistes* spp.). Numbers represent specimen codes (see Table S1) [Colour figure can be viewed at wileyonlinelibrary.com]

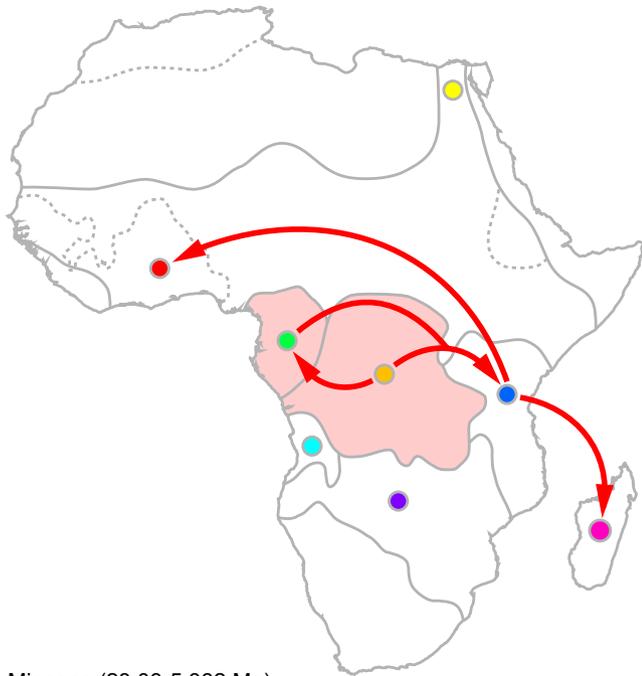
River (Botswana), the Zambezi River (Zambia), the Congo River (DR Congo) and the Luapula River (Zambia).

Our species delimitation methods both identified 22 OTUs for *Lanistes* (Figure 2, Figure S1), of which, however, we treated the two species endemic to Lake Malawi (*L. nyassanus* and *L. solidus*) as separate OTUs *a posteriori*. Including the two outgroup taxa, our final dataset used for subsequent analyses thus comprised 25 OTUs.

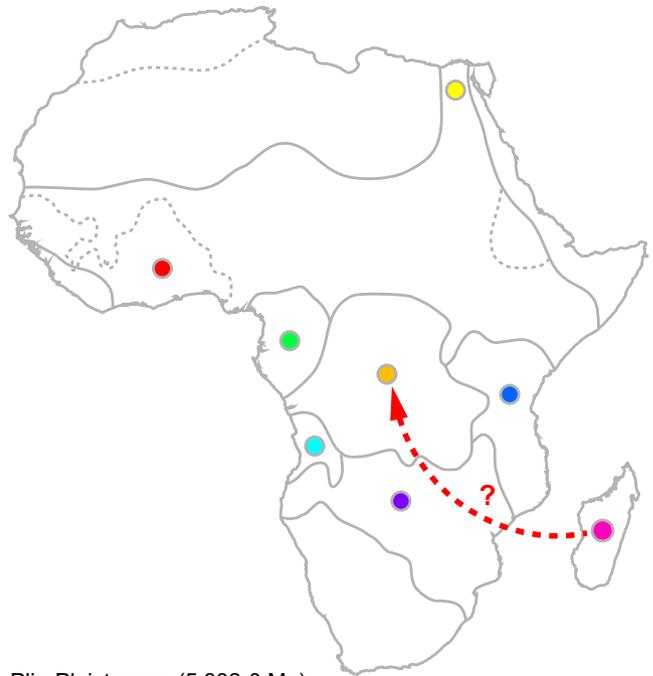
3.2 | Divergence times, diversification patterns and range evolution

Because of convergence issues with complex substitution models, we applied the less complex HKY model for all partitions, by which stationarity and considerably higher ESS values were reached for all parameters. The analysis with a strict-clock model and a birth–death

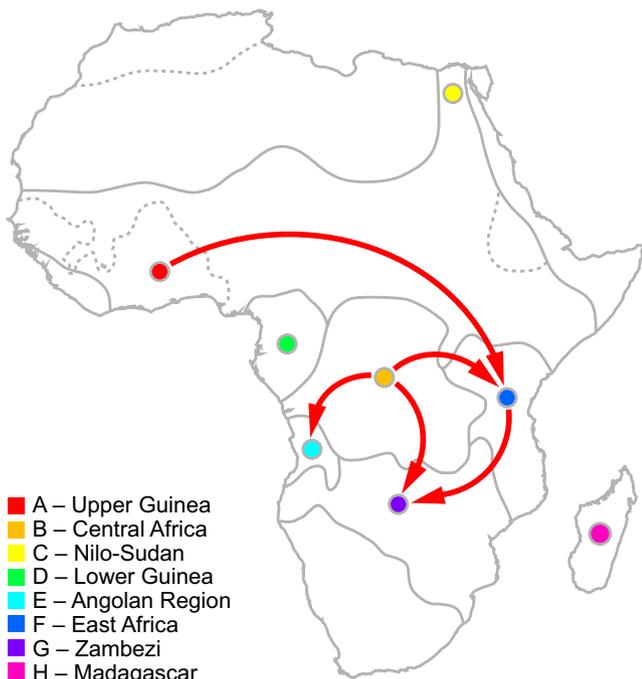
Eocene (55.8–33.9 Ma)



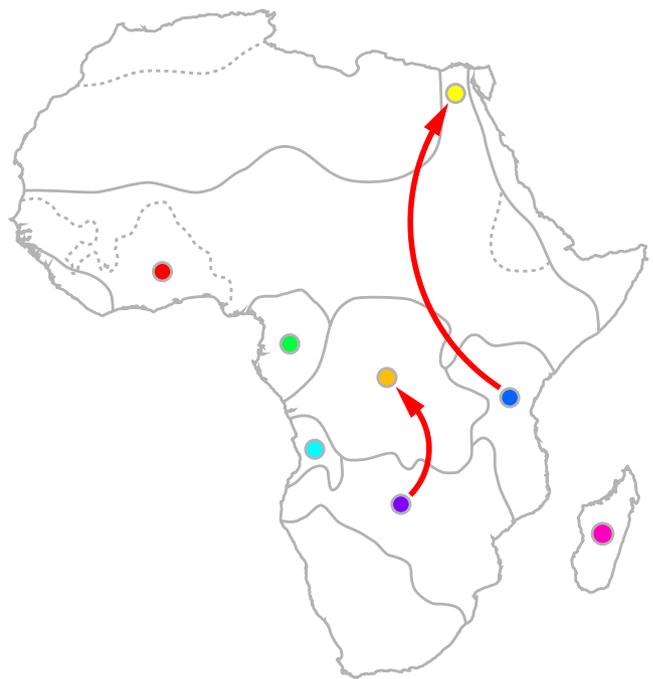
Oligocene (33.9–23.03 Ma)



Miocene (23.03–5.332 Ma)



Plio-Pleistocene (5.332–0 Ma)



- A – Upper Guinea
- B – Central Africa
- C – Nilo-Sudan
- D – Lower Guinea
- E – Angolan Region
- F – East Africa
- G – Zambezi
- H – Madagascar

FIGURE 4 Biogeographical history of *Lanistes* from the Eocene to the present. Map of Africa including fish provinces according to Lévêque et al. (2008). Cladogenetic jump-dispersal events and their directions are shown by red arrows. The estimated centre of origin is shaded in light red in the upper left map [Colour figure can be viewed at wileyonlinelibrary.com]



tree prior had the best marginal likelihoods (Table S3). Accordingly, *Lanistes* probably originated in the Eocene about 50 Ma (Figure 3). During the Eocene and Oligocene, clades C1 and C2 formed, whereas clades C4 and C5 originated much later in the middle Miocene. The LTT plot displays a slow increase in the number of lineages up to the middle Miocene (c. 15 to 10 Ma), and a subsequent steeper increase, followed by a decrease towards the present (Figure S4).

BayAreaLIKE+J was identified as the best-fit biogeographical model (Table S4). Using a probability threshold of 50%, sympatry (11 of 22 cladogenetic events) and jump dispersal (10 of 22 cladogenetic events) are the common cladogenetic events (Figure 3), and only a single anagenetic dispersal event has been detected (along the branch leading to the Lake Malawi endemics; not shown) by the BSM analysis. However, most jump-dispersal events are associated with multiple states in the ancestral range estimations, for example, towards the base of the tree (see Figure 3). Similarly, the complementary analysis using 15 catchments instead of the previously defined fish provinces identified both sympatry and jump dispersal for 11 of

22 cladogenetic events for the very same nodes, whereas vicariance was only rarely detected (best-fit model: DIVALIKE+J, $d = 0$, $e = 0$, $j = 0.106$; Figure S5, Table S5).

The most recent common ancestor (MRCA) of all considered *Lanistes* OTUs in our study probably inhabited an area including Central Africa (B) and adjacent Lower Guinea (D) (Figures 3, 5). This distribution persisted in clade C1, followed by sympatric diversification in Central Africa and a jump-dispersal event into the Angolan Region around 10 Ma. The MRCA of clades C2, C3, C4 and C5 may have originated in East Africa (F) (27%; Figure 3), although various other scenarios are plausible. Subsequently, *Lanistes* colonized Upper Guinea (A) via jump dispersal (clade C2). Assuming that the MRCA of clade C2 occurred in Upper Guinea (44%), a jump dispersal into East Africa occurred in the Early Miocene (branch leading to *L. carinatus*), from which the Nilo-Sudan (C) was colonized in the Pliocene. Despite the uncertain ancestral areas, the biogeographical analyses further suggest that the MRCA of clades, C3, C4 and C5 may have occurred in an ancestral area

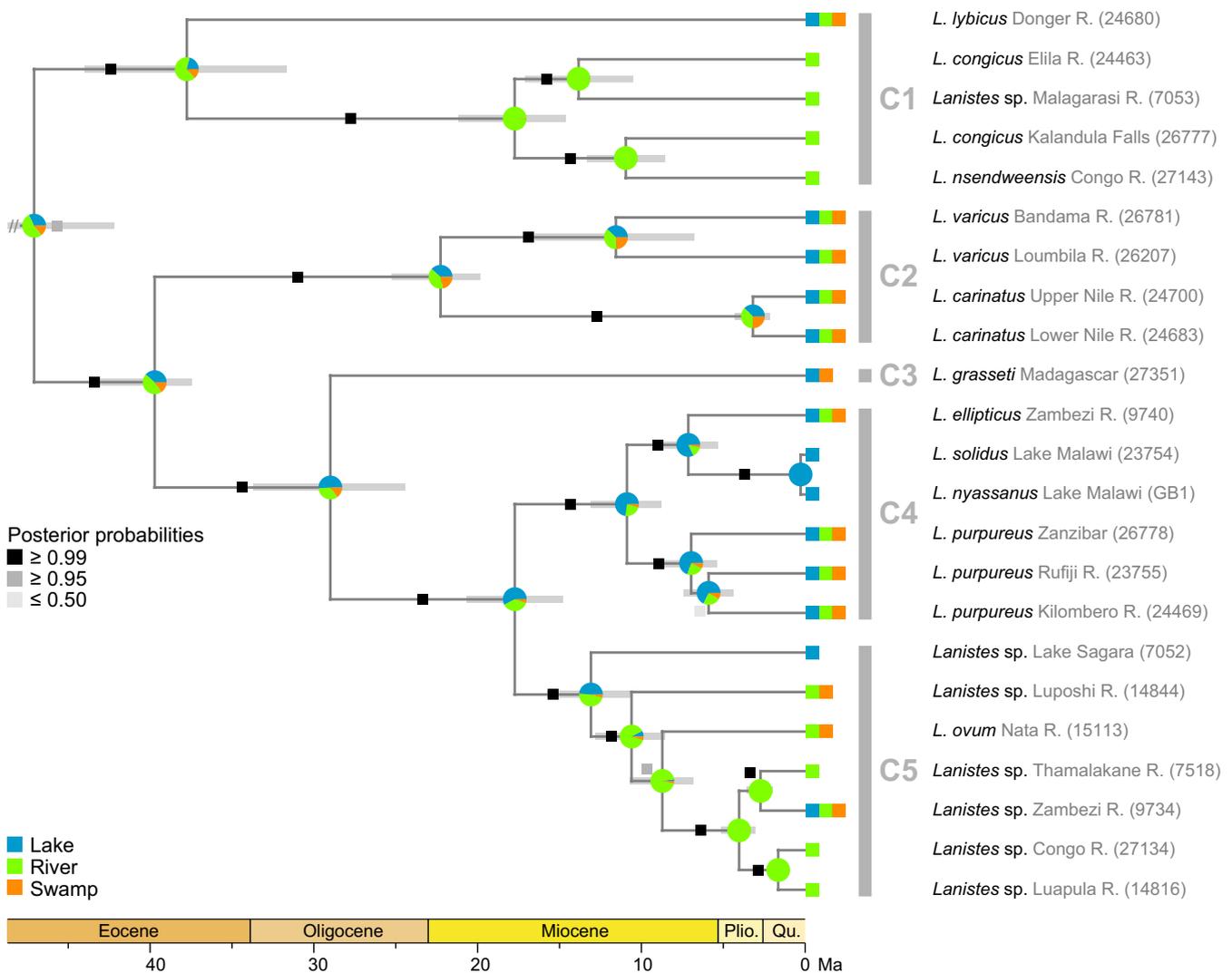


FIGURE 5 BEAST MCC tree including posterior probabilities (squares on branches), 95% highest probability densities (grey bars) and results from the analysis of habitat evolution. Pie charts represent the probabilities of ancestral states at the respective nodes [Colour figure can be viewed at wileyonlinelibrary.com]



including Madagascar (H), leading to clade C3 that established on Madagascar, whereas a jump-dispersal event into Central Africa (B) marked the ancestral area for the MRCA of clades C4 and C5. From this node, East Africa (F) was colonized via jump dispersal in the Early Miocene (clade C4). Around 10 Ma, and another jump-dispersal event gave rise to the colonization of the Zambezi Region (G) in clade C4, followed by the late colonization of Lake Malawi. The analyses further indicate that sympatric diversification prevailed in clade C5, except for a jump-dispersal event that led to the colonization of the Zambezi Region (G) in the late Miocene, followed by a re-colonization of Central Africa (B) via jump dispersal in the Pliocene (Figures 3, 4).

With regard to particular drainage systems, the MRCA of all *Lanistes* probably originated in the ancient Congo River system (Figure 3; clade C1). The directly descendent clade C1 contains populations from the Congo River, and tributaries such as the Elila River as well as the Malagarasi River east of Lake Tanganyika. Another specimen from the Malagarasi River (specimen 7052; Lake Sagara) belongs to a different clade (C5), which also comprises two other Congolese lineages (Lake Bangweulu and Luapula River). The Zambezi River has been colonized at least twice independently, the first lineage (9740; Tete, Mocambique) being sister to the Lake Malawi endemics in clade C4, whereas the second lineage, which also occurred at Tete, belongs to clade C5, together with another Zambezian lineage from the Nata River in the Makgadikgadi area. The geographically distinct populations of *L. carinatus* from Uganda and the Nile River delta in Egypt represent sister groups that diverged around 3 Ma. *Lanistes* populations in smaller Atlantic drainage systems are rather isolated and either share affinities with lineages from the Congo River (Donger and Cuanza Rivers) or those from independent river systems within the Upper Guinea province (White Volta, Bandama Rivers).

3.3 | Habitat evolution

The best-fit model for discrete habitat evolution was ER, which assumes that the transition rates among 'lake', 'river' and 'swamp' habitats are equal. A large share of the OTUs do not occur in a specific habitat type (Figure 5, Table S2). Seven species are confined to rivers, whereas three lineages live exclusively in lakes. The combination 'swamp'-'river' and 'swamp'-'lake' only occurred in two and one lineages, respectively. The estimation of ancestral habitats suggested that the MRCA of all *Lanistes* likely evolved in a riverine habitat, although the support for this scenario over others is not high (Figure 5). Clade C1 is dominated by riverine species, whereas clade C2 comprises species that are ecologically flexible. The MRCA of clades C3, C4 and C5 probably inhabited lakes and *L. grasseti* (clade C3) only occurs in stagnant waters. The only extant species that are exclusively distributed in lakes are the species from Lake Malawi/Nyasa/Niassa and perhaps another *Lanistes* species from Lake Sagara (clades C4 and C5). The MRCA of clades C4 and C5 appears to have diversified into a predominantly

lacustrine (clade C4) and a predominantly riverine (clade C5) group in the middle Miocene.

4 | DISCUSSION

4.1 | Diversity patterns and diversification dynamics

Studying the biodiversity of *Lanistes* on a continental scale, we recovered a robust phylogeny with 23 ingroup OTUs. Although we did not sample several narrow-range species from the Congo Basin (e.g. *L. neritoides*) or East Africa (e.g. *L. farleri*), our study indicates that more species exist than the 21 (Berthold, 1991) or 19 (Brown, 1994) that have been previously proposed. Additional species are likely to exist given that some drainage basins remain faunistically largely underexplored and given that our phylogeny lacks species names for several OTUs. Indeed, our result illustrates that several cryptic species exist, as is the case for other African freshwater molluscs (e.g. Elderkin et al., 2016; Mahulu et al., 2019), but given the lack of topotypic specimens for many of the 43 taxonomically valid species (Cowie, 2015) we prefer to refrain from providing formal revision.

The diversification of *Lanistes* started strikingly earlier than that of several other co-inhabiting freshwater molluscs such as *Bellamya* (Schultheiß et al., 2011, 2014) and *Coelatura* (Ortiz-Sepulveda et al., 2020). Whereas freshwater crabs of the Potamonautidae also originated in the Eocene (Daniels et al., 2015), most fish genera diversified only in the late Oligocene (e.g. *Distichodus*; Arroyave et al., 2020) or the Miocene (e.g. *Mastacembelus*; Day et al., 2017).

Our LTT plot (Figure S4) suggests a rather constant rate of diversification for *Lanistes*, as has been found also in *Synodontis* catfish (Day et al., 2013) and *Mastacembelus* spiny eels (Day et al., 2017). Under the assumption of diversity-dependent diversification (but see, e.g. Harmon & Harrison, 2015 and Rabosky & Hurlbert, 2015 for a debate), it suggests that these relatively young clades have not yet reached their ecological limits. Given that the *Lanistes* radiation is about twice as old as most freshwater genera that have been studied at a pan-African scale and potentially even older (Pickford, 2017), a comparatively geologically young origin becomes less compelling. Although the accumulation of lineages occurred at a comparatively constant rate, a slight increase in diversification was observed around 15 Ma. This increase coincides with the Middle Miocene Climatic Optimum (ca. 17–15 Ma), a period of pronounced precipitation under warm tropical conditions (Zachos et al., 2001). As for *Lanistes*, this epoch resulted in major diversification in crabs (Daniels et al., 2015), but also freshwater fishes (Day et al., 2013, 2017). It might even be related to the onset of diversification in other freshwater snails (*Bellamya* spp.; Schultheiß et al., 2014) and bivalves (Ortiz-Sepulveda et al., 2020). Intralacustrine diversification appears to have been rare in *Lanistes* in comparison to African representatives of Viviparidae and Cerithioidea. A single ongoing *Lanistes* radiation in the Lake Malawi Basin (Schultheiß et al., 2009; Van Bocxlaer, 2017; Van Bocxlaer et al., 2020) is the only current example and none of the species involved developed sculptured shells, as



is the case in other families such as Viviparidae (Stelbrink et al., 2020; Van Bocxlaer et al., 2021) or Thiaridae (Van Bocxlaer et al., 2015). Nevertheless, sculptured *Lanistes* shells have been described in fossil radiations (Van Damme & Pickford, 1995).

Spatial patterns of diversification in *Lanistes* show similarities to those in several fish taxa, suggesting that diversification is shaped by shared underlying factors. Palaeohydrological and palaeoclimatic changes have been hypothesized to have impacted landscape evolution and, as such, promoted diversification in freshwater organisms (e.g. Arroyave et al., 2020). *Lanistes* provides an ideal case to test this hydrogeological hypothesis. Our robust fossil-calibrated molecular phylogeny allows discussion of several scenarios in a historical biogeography framework. Continental-scale diversification is typically shaped by vicariance and dispersal events. However, no single (cladogenetic) vicariance event (e.g. AB→A+B) was inferred from the biogeographical analyses. Instead, jump dispersal (e.g. AB→AB+C) dominated in the first half of the evolutionary history of *Lanistes*, whereas sympatric diversification (e.g. A→A+A) occurred in roughly half of the cladogenetic events during the last 20 million years. Although some of the jump-dispersal events are simply inferred because ancestral ranges were identified with low probabilities (see Figure 3), our findings outline that (cladogenetic) jump dispersal can play a predominant role in diversification of freshwater invertebrates, particularly during dynamic limnological periods. Because our complementary analysis using 15 catchments (Table S2) resulted in a very similar proportion and distribution of cladogenetic events across the nodes in the phylogeny (Table S5), the present findings strongly suggest that our estimations based on fish provinces are robust. Ultimately, more invertebrate studies on the continental scale are needed to determine the prevailing modes of range evolution. Transoceanic dispersal and subsequent colonization of an island, here Madagascar (i.e. the cladogenetic event that gave rise to the MRCA of clades C3, C4 and C5; Figures 3, 4), have occurred only once in *Lanistes*, assuming that Zanzibar as a continental island was colonized when it was part of the continental landmass. This was also a rare event in potamonautid crabs (Daniels et al., 2015). Eastward flowing ocean currents from East Africa to the Gondwanan islands in the Indian Ocean during the Palaeogene and Miocene might have favoured dispersal from East Africa (e.g. Hawlitschek et al., 2017). At the time of colonization, Madagascar might have been closer to the continent and stepping stone islands might have existed, facilitating the dispersal. However, the relevant timeframe is much younger than the breakup of Madagascar and Africa. The exact dispersal mechanisms of *Lanistes* remains unknown; however, the capability of long-term estivation of ampullariids is well known and has probably facilitated the colonization of new areas globally, especially by some notorious invasive species (Berthold, 1991; Cowie & Hayes, 2012; Hayes et al., 2009; Sun et al., 2019).

4.2 | Landscape and ecosystem evolution

The results of the habitat analysis, being somewhat equivocal, are not interpreted further. Instead, the discussion focuses on the

biogeographical analyses. The ancestral area of all *Lanistes* species remains somewhat equivocal, but our results suggest that rivers in West Africa and the Congo Basin have played a pivotal role in the diversification of the genus and its distributional changes over time. These results corroborate previous findings suggesting a central role of the Congo Basin in the distribution of various other African freshwater biota, such as the tigerfish (*Hydrocynus*; Goodier et al., 2011), characins (*Distichodus*; Arroyave et al., 2020) and *Bellamya* snails (Schultheiß et al., 2014) but not squeaker catfish (*Synodontis*; Day et al., 2013) and spiny eels (*Mastacembelus*; Day et al., 2017). The colonization of the Congo Basin was related to the origin of *Lanistes*, but may have reached beyond the central parts of the basin also adjacent regions (Angolan and Lower Guinean; Figures 3, 4). A connection to the Angolan Region and the Congo drainage has generally been found in fishes, for example, cichlids (Musilová et al., 2013; Schwarzer et al., 2012), but not in crabs (Daniels et al., 2015). With the exception of Lake Sagara, which is hydrographically linked to the Congo River via the Malagarasi drainage, there is a more complex pattern of changing connections and breakups of rivers in the Miocene and Pliocene involving the Zambezi, Congo and western drainages in the vicinity of the Okavango Delta. Dynamic drainage interconnectivity is reflected in clade C5 containing Congo River specimens. The sister-group relationship of taxa from Lake Malawi and the Zambezi River within clade C4 as well as the sister relationship of this clade to a clade consisting of taxa from East African rivers and Zanzibar indicates previous hydrographic connectivity of the central part of the current Zambezi River and Tanzanian rivers. A strongly supported sister-group relationship between East African and Congo River species has also been shown for crabs and *Bellamya* snails (Daniels et al., 2015; Schultheiß et al., 2014).

The late Miocene drainage constellation has allowed dispersal between the Congo River catchment and the Okavango–Makgadikgadi regions to the headwaters of the Upper Zambezi (Moore et al., 2007; Stankiewicz & de Wit, 2006). Pliocene dispersal from the Okavango–Zambezi river system to the Congo River in clade C5 around 4 Ma relates to the phase when the Upper Zambezi River was still landlocked, that is, did not flow to the ocean (Moore et al., 2007). Frequent faunistic interchanges during this period have also been demonstrated for *Coelatura* bivalves (Ortiz-Sepulveda et al., 2020).

A pertinent pattern is the frequency of dispersal events inferred throughout the history of the genus, regardless of the geographical extent of biogeographical regions (Figure 4). This is in contrast to the gastropod genus *Bellamya* for which vicariance has been suggested as the main driver of continental-scale diversification (Schultheiß et al., 2014), although no formal biogeographical analysis was carried out. In *Bellamya*, even disjunct species ranges have been recovered, that is, distributional gaps of one or more drainage systems between more distant occupied drainages exist. In *Lanistes*, however, dispersal occurred predominantly from one bioregion (ichthyofaunal province) to an adjacent bioregion except for transoceanic dispersal to Madagascar (Figures 3, 4).

The bioregionalization based on fishes fits the distribution patterns of *Lanistes* very well. Our results shed light on some debated

borders or alternative regionalization based on the ichthyofaunal provinces. For example, our results support an inclusion of the Lake Malawi–Shire River systems into the Zambezi Region rather than to the neighbouring East African Region. The distinctness and close affinity of the Angolan Region (Cuanza River) to the Central African Region instead of the Zambezi Region is also supported by our findings. Our findings also support a north-eastwards extension of the Upper Guinean Region, which is traditionally often restricted to short Atlantic drainage systems (Lévêque et al., 2008).

The evolution of the EARS has been linked to biotic evolution and biodiversity of terrestrial and aquatic organisms as uplift and rifting have caused the redirection of rivers and the development of basins that subsequently harboured lacustrine systems (Couvreur et al., 2021; Salzburger et al., 2014). The rifting of the EARS has also been proposed as a vicariance event that led to four distinct groups of the extant viviparid gastropods (Schultheiß et al., 2014). Although the phase of elevated diversification coincides with the most intense Miocene rifting that progressively shaped the African continental surface, we found no indication of vicariance-driven diversification in our analysis. Even though diversification in many lineages intensified at the end of the major uplifting of the EARS 25–10 Ma (Ring et al., 2018), no particular large-scale imprint of a barrier (the EARS) was found in our phylogeny. Our findings are concordant with a continental study of overall freshwater mollusc biodiversity, where no straightforward barrier role of the EARS in shaping biodiversity patterns was found either (Hauffe et al., 2016). These results do not imply that habitat restructuring and increased habitat heterogeneity associated with the rift did not affect diversification in *Lanistes*. The ecological versatility of *Lanistes* and the mode of reproduction with egg clusters being attached to underwater aquatic vegetation have certainly contributed to effective dispersal along many major drainage systems, notably going with the flow during periods when extensive flooding connected otherwise isolated systems.

5 | CONCLUSIONS

The diversification of *Lanistes* probably started in the Eocene and may have exhibited a potentially climate-related increase in lineage splitting in the Miocene. This study highlights the significance in clade diversification of temporal geographical isolation of river systems and their subsequent reconnection. Jump dispersal is a major driver underlying continental diversification. Although this study sheds new light on patterns and processes of tropical continental diversification, more comparative analyses across various taxa are needed. However, the complexity of continental-scale evolution has been outlined by the similarities observed in biogeographical patterns among some fish taxa and *Lanistes*.

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DATA AVAILABILITY STATEMENT

All data are either available as Supplementary Information or from public databases (NCBI GenBank, accession numbers provided in the Supplementary Information).

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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