



Multigene fossil-calibrated analysis of the African lampeyes (Cyprinodontoidei: Procatopodidae) reveals an early Oligocene origin and Neogene diversification driven by palaeogeographic and palaeoclimatic events

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Abstract

Here, we present the first molecular and fossil-dated analysis focusing in the Procatopodidae, a widely distributed and little known African oviparous killifish family. The analysis included 36 species representing all Procatopodidae genera except the monotypic *Apticheilichthys*. Procatopodidae relationships were established through maximum likelihood and bayesian inference approaches based on fragments of one mitochondrial and five nuclear genes, a total of 5691 bp. The Procatopodidae is herein considered a monophyletic group, sister to the Old world Valenciidae and Aphaniidae. The genus *Plataplochilus* represent the most basal procatopodid lineage and the brackish water species *Aplocheilichthys spilauchen* is placed within the Procatopodidae. A clade including the morphologically distinct species of *Congopanchax*, *Lacustricola*, and *Lamprichthys* is herein suggested for the first time, and the genera *Micropanchax*, *Poropanchax*, *Lacustricola*, and *Hypsopanchax* revealed to be paraphyletic. A fossil-calibrated analysis, based on the same dataset, provided the first information about the evolution of the Procatopodidae in Africa. Our estimates indicate an early Oligocene origin for the Procatopodidae, as a consequence of the Eocene trans-Saharan epicontinental sea retreat, and also indicated that major Neogene paleogeographical and paleoenvironmental events influenced procatopodids diversification (e.g., increase activity in the African rift; late Miocene aridification; Pliocene and Pleistocene climatic instability).

Keywords Killifish · Africa · Cyprinodontiformes · Systematics · Procatopodidae

Introduction

The evolution of Africa landscape and its river drainages comprise an integrative research field with geological, palaeoclimatic,

palaeontological, and palynological studies resulting in a large database about Africa palaeohistory, essential to the understanding of biogeographical patterns in the continent (Beadle 1981; Bobe 2006; Burke 1996; Giresse 2005; Maley 1996; Plana 2004; Runge 2007; Sépulchre et al. 2006; Sommerfeld et al. 2016; Stankiewicz and de Wit 2006). Biogeographical studies of aquatic organisms inhabiting Africa freshwater environments also provide reliable information on landscape evolution (Day et al. 2013, 2017; Goodier et al. 2011; Pinton et al. 2013). The limited dispersion capability and the fact that freshwater aquatic fauna is ecophysiologically restricted to freshwater water bodies makes them historically linked to the freshwater environments (Albert and Carvalho 2011; Lundberg et al. 1998; Mayden 1988; Smith 1981). However, despite the advantages in incorporating aquatic organisms in large-scale biogeographical analysis, most studies related to Africa fish fauna were restricted to particular areas and drainages (Collier et al. 2009; Swartz et al. 2007). Special attention has been directed to Eastern Africa lacustrine diversification (Brown et al. 2010; Danley et al. 2012; Day and Wilkinson 2006;

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Kolbmüller et al. 2006; Peart et al. 2014; Salzburger et al. 2014), with few studies focusing on continental scale diversification (Day et al. 2013, 2017; Goodier et al. 2011; Pinton et al. 2013). The main challenges for such studies in Africa are probably related to its heterogeneous environments and large size, diverse and still poorly known fauna, political instability in some areas and the lack of local taxonomists (Day et al. 2013; Skelton and Swartz 2011; Swartz et al. 2008; Williams and Kniveton 2011).

The Procatopodidae (sensu Bragança et al. 2018) comprises about 100 small oviparous killifish species widely distributed over the African continent, occurring in Africa main river drainages and ichthyological realms (Ghedotti 2000). Species of Procatopodidae inhabit different environments, such as black water streams, swampy areas, small ponds, lakes, high altitude creeks, and even brackish water estuaries (Ghedotti 2000; Huber 1999), making them an interesting group for broad continental scale biogeographical studies. The African lampeyes, as they are popularly known due to the presence of an iridescent reflective coloration in the dorsal region of the eye are one of the morphologically most diverse families of the Cyprinodontiformes. The Procatopodidae includes some well-known and appreciated aquarium fish species, like the deep-bodied and colorful species of the genus *Procatopus* Boulenger, 1904, the pelagic *Lamprichthys tanganicus* (Boulenger, 1898) and the small species of the genus *Poropanchax* Clausen 1967. Some genera and species present more restrict distributions whereas others are more widespread. The miniature species of *Congopanchax* Poll, 1971 occurs in swampy areas and lakes of the Congo lowland rainforest (Poll 1971), whereas species of *Hypsopanchax* Myers, 1924 are restricted to high altitude rainforest streams in the western slope of the Albertinian rift and in the great lakes region (Poll and Lambert 1965). In contrast, *Micropanchax* Myers, 1924 is the most widespread genus, occurring in the sahelian and savannah areas along the Nilo-Sudanic region, West Africa, Eastern Africa, and Southern Africa.

Despite its great diversity and distribution, little is known about taxonomy and relationships between Procatopodidae genera (Ghedotti 2000). The first species and genera descriptions were brief, with few illustrations, and were based only on external morphology and teeth structure (Ahl 1924, 1928; Myers 1924, 1931, 1938). Most taxonomic acts, such as genera and species descriptions and synonymizations, included few comparative taxa and relied uniquely on characters related to body size and shape, fins position, and cephalic sensory system (Clausen 1967; Huber 1982; Poll and Lambert 1965; Poll 1971). Thus, there is no consensus on literature about generic classification of the Procatopodidae, the number of valid genera and species is uncertain, with most genera waiting an updated formal definition

(Ghedotti 2000). Procatopodids were included in few morphological and molecular phylogenetic analyses, but poorly sampled, mostly as outgroups (Bragança et al. 2018; Costa 1996, 1998; Ghedotti 2000; Helmstetter et al. 2016; Parenti 1981; Pohl et al. 2015; Pollux et al. 2014; Reznick et al. 2017). The only phylogenetic study directed to establish procatopodids internal relationships (Huber 1999) was based on external morphology, morphometrics, and ecological features, without including molecular and osteological data.

Historically, a close relationship between the African lampeyes (procatopodids) and the oviparous miniature Amazon killifish genus *Fluviphylax* Whitley, 1965 has been suggested due to a similarity in fin position and the presence of a bright coloration on the dorsal region of the eye (Myers 1955; Roberts 1970). In the first morphology-based cladistic analysis of the Cyprinodontiformes (Parenti 1981) as well as in subsequent morphological analyses (Costa 1996; Ghedotti 2000), the African lampeyes and *Fluviphylax* were grouped in a more comprehensive Poeciliidae, including also the American livebearers poeciliines. Parenti (1981) and Costa (1996) grouped all procatopodins together in the same subfamily, but Ghedotti (2000) considered *Aplocheilichthys spilauchen* (Duméril, 1861) to be sister to a clade including other African lampeyes, *Fluviphylax*, and poeciliines, thus recognizing the procatopodins as a paraphyletic assemblage. Recent molecular studies refuted the monophyly of the Poeciliidae (sensu Parenti 1981), and the African lampeyes were considered to be more closely related to the Aphanidiidae and Valenciidae, both comprising old world oviparous cyprinodontoids, whereas *Fluviphylax* was supported to be a distinct lineage (Fluviphylacidae), sister to a clade comprising American livebearers (Poeciliidae) and the Anablepidae (Helmstetter et al. 2016; Pohl et al. 2015; Pollux et al. 2014; Reznick et al. 2017; Bragança et al. 2018; Bragança and Costa 2018). However, few Procatopodidae species were incorporated into the aforementioned molecular analysis, and the monophyly of the African lampeyes have never been investigated in a more broad and inclusive study.

Here, in order to test the monophyly of the Procatopodidae and establish the phylogenetic relationships among the African lampeyes genera, we provide the first multigene phylogeny, comprising five nuclear and one mitochondrial gene for 36 species representing all procatopodid genera except the monotypic *Apticheilichthys* Huber, 2011. In addition, a fossil time-calibrated analysis provides for the first time information on the evolution and diversification patterns of the Procatopodidae in Africa, representing one of the few contributions to the understanding of continental-scale evolution in African riverine fishes. The results of the dated analysis are interpreted in view of the major studies about African landscape evolution and African biogeography.

Materials and methods

Taxon sampling

Thirty-six species of African lampeyes representing all genera except the monotypic *Apticheilichthys* were included in the analysis (information on the representativeness of this dataset is present in Online Resource 1). It was not possible to obtain suitable material of *A. websteri* (Huber 2007) for molecular studies, but for most procatopodid genera, type species were included in the analysis (see Online Resource 1). Molecular data were obtained from specimens conserved in 98% ethanol and later preserved in the same fixative. In addition, 17 outgroup species representing the main lineages of the Cyprinodontoidei suborder were analyzed. The outgroup comprises species of the Valenciidae and Aphaniidae, that are considered to be sister to the Procatopodidae (Bragança et al. 2018), species of the Fluviphylacidae, Anablepidae, and Poeciliidae, considered to be sister to the Procatopodidae, Aphaniidae, and Valenciidae clade (Reznick et al. 2017; Bragança et al. 2018), and species of Fundulidae and Cyprinodontidae, representing a basal Cyprinodontoid clade (Reznick et al. 2017). The analyses were rooted in the Aplocheiloid killifish *Aplocheilus lineatus* (Valenciennes, 1846) (Aplocheilidae). All sequenced specimens used in this study are deposited in the Ichthyological collection of the Institute of Biology, Federal University of Rio de Janeiro, Rio de Janeiro (UFRJ) and in the Royal Museum for Central Africa, Tervuren (MRAC). A list of species with their respective localities and GenBank accession numbers appears in Online Resource 2.

Classification remarks

Species from a genus not clustering in the same clade of the genus type species will be referred to the same generic name between quotation marks (e.g., “*Hypsopanchax*,” “*Lacustricola*”). The use of quotation marks to distinguish between species not grouping with the genus type species and congeners of the type species will help further discussion on the placement of Procatopodidae genera and their relationships.

DNA extraction and sequencing

The genomic DNA was extracted from muscle tissue of the right side of the caudal peduncle or from fin clips using DNeasy Blood & Tissue Kit (Qiagen, Hilden) according to the manufacturer instructions. Sequences were taken from a single individual per species. Primers used to amplify the fragments of DNA are listed in Table 1. Polymerase chain reactions (PCR) were performed in 30 µl reaction mixtures containing 5 × Green GoTaq Reaction Buffer (Promega,

Madison), 3.6 mM MgCl₂, 1 µM of each primer, 50 ng of total genomic DNA, 0.2 mM of each dNTP, and 1 U of Taq polymerase. The thermocycling profile was (1) 1 cycle of 4 min at 94 °C; (2) 35 cycles of 1 min at 92 °C, 1 min at 47–62 °C (varying according to the primer and the sample) and 1 min at 72 °C; and (3) 1 cycle of 4 min at 72 °C. In all PCR reactions, negative controls without DNA were used to check contaminations. Amplified PCR products were purified using the Wizard SV Gel and PCR Clean-Up System (Promega). Sequencing reactions were made using the BigDye Terminator Cycle Sequencing Mix (Applied Biosystems, Foster City). Cycle sequencing reactions were performed in 20 µl reaction volumes containing 4 µl BigDye, 2 µl sequencing buffer 5 × (Applied Biosystems), 2 µl of the amplified products (10–40 ng), 2 µl primer, and 10 µl deionized water. The thermocycling profile was as follows: (1) 35 cycles of 10 s at 96 °C, 5 s at 54 °C, and 4 min at 60 °C. The sequencing reactions were purified and denatured and the samples were run on an ABI 3130 Genetic Analyzer.

Data partition, evolution models, and alignment

The dataset included the following genes: Cytochrome C Oxidase Subunit I, COI (682 bp); Glycosyltransferase, GLYT (910 bp); Ectodermal Neural Cortex 1, ENC1 (863 bp); Recombination Activating gene 1, RAG1 (1495 bp); Brain Super Conserved Receptor 2, SREB2 (953 bp); Myosin Heavy Chain 6, MYH6 (788 bp); comprising 5691 characters. Sequences were aligned using ClustalW (Chenna et al. 2003). The DNA sequences were translated into amino acids residues to test for the absence of premature stop codons or indels using the program MEGA 6 (Tamura et al. 2013). In the alignment, gaps were coded with a dash (–) and missing data with a question mark (?), but during analyses, both of them were treated as missing data. For each gene, Measure Substitution Saturation tests were performed in DAMBE5 (Xia 2013) according to the algorithm proposed by Xia et al. (2003) considering only fully resolved sites. The third codon position of MYH6 gene appeared to be saturated relative to the first and second codon positions, suggesting a considerably different evolution rate. Thus, the first and second codon positions and the third codon position of MYH6 were included in the analysis under different evolutionary models. The best-fit evolutionary model was calculated for each gene, the first and second codon positions of MYH6 and the third codon position of MYH6, using the corrected Akaike Information Criterion (AICc) determined by the jModelTest 2.1.7 (Darriba et al. 2012). The best model for each gene is presented in Table 2. The data was partitioned according to the delimited evolution models, and five partitions were defined: (1) COI; (2) GLYT, RAG1, and the first and second codon positions of MYH6; (3) ENC1; (4) SREB2;

Table 1 Primers used in this study

Gene	Primer	Sequences	Author	Annealing temperature
Glyt	Glyt_F577	5' ACATGGTACCAGTATGGCTTTGT 3'	Li et al. (2007)	
	Glyt_F559	5' GGACTGTCMAAGATGACCACMT 3'	Li et al. (2007)	
	Glyt_R1562	5' CCCAAGAGGTTCTTGTTAAAGAT 3'	Li et al. (2007)	
	Glyt_FProc	5' CTKGTCAGCATGCAYGGAGCTCAGCT 3'	This study	60 °C
	Glyt_RProc	5' GACCAGGGTGGACYGTGCTGGC 3'	This study	60 °C
MYH6	myh6_F507	5' GGAGAATCARTCKGTGCTCATCA 3'	Li et al. (2007)	
	myh6_F459	5' CATMTTYTCCATCTCAGATAATGC 3'	Li et al. (2007)	
	myh6_R1325	5' ATTCTCACCAACCATCCAGTTGAA	Li et al. (2007)	
sreb2	sreb2_F10	5' ATGGCGAACTAYAGCCATGC 3'	Li et al. (2007)	
	sreb2_R1094	5' CTGGATTTTCTGCAGTASAGGAG 3'	Li et al. (2007)	
	sreb2_R1082	5' CAGTASAGGAGCGTGGTGCT 3'	Li et al. (2007)	
	sreb2_RPan	5' TAGAACATYCTRCTAATCCTCTTCTC 3'	This study	59 °C
RAG1	RAG1_F1	5' CTGAGCTGCAGTCAGTACCATAAGATGT 3'	López et al. (2004)	
	RAG1_R1	5' CTGAGTCCTTGTCAGCTTCCATRAAYTT 3'	López et al. (2004)	
	H3405	5' GCNGAGACTCCTTTGACTCTGTC 3'	Hrbek et al. (2007)	
ENC1	ENC1_F85	5' GACATGCTGGAGTTTCAGGA 3'	Li et al. (2007)	
	ENC1_F88	5' ATGCTGGAGTTTCAGGACAT 3'	Li et al. (2007)	
	ENC1_R982	5' ACTTGTTTRGCMACCTGGGTCAAA 3'	Li et al. (2007)	
	ENC1_R975	5' AGCMACCTGGGTCAAACCTGCTC 3'	Li et al. (2007)	
	ENC1_FPan	5' ATGCTGYTWCTGTCTGAYGCCACCAAGTG 3'	This study	59 °C
	ENC1_RPan	5' GCYTTBGGRTATKATCTCTTTGGC 3'	This study	59 °C
	LCO1490	5' GGTCAACAAATCATAAAGATATTGG 3'	Folmer et al. (1994)	
	HCO2198	5' TGATTTTGTGGTCACCCTGAAGTTTA 3'	Folmer et al. (1994)	

(5) and third codon position of MYH6. Partitions were concatenated manually.

Phylogenetic analysis

The phylogenetic analyses were conducted through Bayesian inference (BI), using the program MrBayes v3.2.5 (Ronquist et al. 2012) and Maximum Likelihood (ML), using the program Garli 2.0 (Zwickl 2006). When performing MrBayes v3.2.5, BI was conducted using two Markov chain Monte Carlo (MCMC) runs of four chains each for three million

generations, a sampling frequency of 1000. The quality of the MCMC chains, stationarity and the respective ESS values of analysis parameters were checked in Tracer 1.6, and the analysis was finished when parameters were above 200. A 25% burn-in was removed in MrBayes v3.2.5. A Maximum Likelihood stepwise-addition starting tree was performed in Garli 2.0, with 100 attachment branches for each taxon and ten independent search replicates. The support values of the ML analysis were calculated by 1000 bootstrap replications (Felsenstein 1985). Phylogenetic analysis including only nuclear partitions and analysis considering both the mitochondrial and nuclear partitions were performed, testing the influence of mitochondrial data over the analyses. Maximum Likelihood and BI analyses including both nuclear and mitochondrial partitions are presented in Online Resource 3.

Time-calibrated tree

The time-calibrated analysis was performed in BEAST v.1.8 (Drummond et al. 2012), including the same concatenated dataset and partitions as above described, and an uncorrelated relaxed clock model (Parham et al. 2012). Bayesian Inference

Table 2 Substitution models for each gene/partition according to JModeltest 2.1.7

Partition	Model (AICc)
COI	HKY+I+G
ENC1	K80+I+G
GLYT	TrN+I+G
1st2nd MYH6	TrN+I+G
3rd MYH6	K80+G
RAG1	GTR+I+G
SREB2	HKY+I+G

was performed with 100 million generations with a sampling frequency of 1000. The value of parameters of the analyses, convergence of the MCMC chains, sample size, and the stationary phase of chains was evaluated using Tracer 1.6. A Yule speciation process for the tree prior (Gernhard 2008) was used, establishing two fossil calibration points. Fossil choice for calibration followed Costa et al. (2017), where the fossil *Prolebias stenoura* Sauvage, 1874, from the Lower Stampien (Lower Oligocene) from Puy-de-Dôme, France, was placed at the stem of the clade comprising Aphaniidae, Valenciidae, and Procatopodidae, corresponding to the origin of the crown Old World cyprinodontoid clade (Pohl et al. 2015; Bragança et al. 2018), estimated to have occurred at least 33 Mya (prior setting: lognormal distribution, mean = 33, and standard deviation = 0.5), and the fossil *Aphanius chebrianus* (Obrhelová, 1985), of the Ottnangian (Lower Miocene) of the Cheb basin, Czech Republic, placed at the node where *Aphanius* Nardo, 1827 and *Valencia* Myers, 1928 diverge, corresponding to the most ancient record of *Aphanius*, with an estimated age of 17 Mya (prior setting: lognormal distribution, mean = 17, and standard deviation = 0.5). A lognormal distribution is indicated for calibration points based on fossils, because it emphasizes the minimum age considering the incompleteness of the fossil record (Forest 2009). In a comprehensive publication on the Cyprinodontoidei Oligocene fossil species from Europe, *Prolebias stenoura* was considered the oldest identifiable member of the Valenciidae (Costa 2012); and *Aphanius chebrianus* was recognized as the oldest identifiable *Aphanius* species (Gaudant 2009a, 2009b; Costa et al. 2017). For information on osteological diagnostic characters of both *P. stenoura* and *A. chebrianus*, see Gaudant (2009a, 2009b) and Costa (2012). For additional information on Europe fossil Cyprinodontiformes from Neogene, see Reichenbacher and Kowalke (2009). The time-calibrated analysis including both nuclear and mitochondrial partitions is present in Online Resource 4. Posterior probability values of the time-calibrated analysis including only nuclear partitions are present in Online Resource 5.

Results

Phylogenetic relationships

The ML and the BI analyses presented the same topology (Fig. 1). A monophyletic Procatopodidae was strongly supported in both analyses, as well as a clade comprising Procatopodidae, Aphaniidae, and Valenciidae that received high support values. *Plataplochilus* Ahl, 1928 was supported to be sister to all other African lampeyes and presented maximum posterior probability value and high bootstrap values (ML = 90), whereas *Aplocheilichthys spilauchen* was considered to be sister to all remaining Procatopodidae, being

supported by maximum support values. In the ML and BI analyses, a clade including *Procatopus*, “*Hypsopanchax*,” *Hypsopanchax*, and *Hylopanchax* Poll and Lambert, 1965 was recovered, being highly supported. All internal nodes of this clade presented maximum posterior probability values and bootstrap values above 90, except the node representing *Hypsopanchax modestus* (Pappenheim and Boulenger, 1914) and *H. platysternus* (Nichols and Griscom, 1917) (ML = 53; BI 0.87).

Both analyses strongly supported a close relationship among the genera *Congopanchax*, *Lacustricola* Myers, 1924, and *Lamprichthys* Regan, 1911 (ML = 84; BI = 1), and highly supported their internal nodes. The clade comprising the three genera was supported as sister to a clade containing *Poropanchax*, “*Poropanchax*,” “*Lacustricola*,” *Rhexipanchax* Huber, 1999, *Micropanchax* and “*Micropanchax*.” In all analyses, the aforementioned clade was strongly supported, but some relationships among included taxa received low support values. In the ML and BI analyses, “*Poropanchax*” *normani* (Ahl, 1928) was considered to be sister to all remaining species of the clade, and despite the low support values, a relationship between a clade comprising *Poropanchax luxophthalmus* Brüning, 1929, *P. stigmatopygus* Wildekamp and Malumbres, 2004, and “*Micropanchax*” *scheeli* (Roman, 1970) was indicated. *Poropanchax* species were recovered as sister taxa, presenting maximum support values. In addition, two well-supported clades, “*Lacustricola*” (including “*Hypsopanchax*” *jubbi*) and *Micropanchax*, were recovered in all analyses, as well as their internal relationships. In contrast, the internal relationships between the well-supported “*Lacustricola*,” *Micropanchax*, *Poropanchax*, and *Rhexipanchax* were poorly supported in the ML, with bootstrap values between 66 and 75.

The inclusion of mitochondrial gene COI in the BI analysis does not result in significant changes regarding the topology and the posterior probability support values. A sister species relationship between *Hypsopanchax modestus* and *H. platysternus* supported by a low support value (PP = 0.87) in the analysis including only nuclear partitions was strongly supported in the analysis including COI (PP = 1), and the placement of “*Micropanchax*” *scheeli* as sister to *Poropanchax* was not recovered in the BI analysis including COI. In fact, it was not possible to establish the phylogenetic position of “*Micropanchax*” *scheeli* among species of the *Poropanchax*, *Micropanchax*, “*Lacustricola*,” and *Rhexipanchax* clade. Despite the maximum support value in the analysis without the COI, the placement of “*Lacustricola*” *johnstoni* as sister species to “*Lacustricola*” *katangae* and “*Lacustricola*” aff. *katangae* was not recovered, and it was considered sister to the species “*Lacustricola*” *centralis*, also being supported by a maximum posterior probability value. In contrast, the inclusion of the COI in the ML analysis strongly affected bootstrap support values. Most recent diversification between species within the same genus received higher support values in the analysis including the mitochondrial gene, but nodes

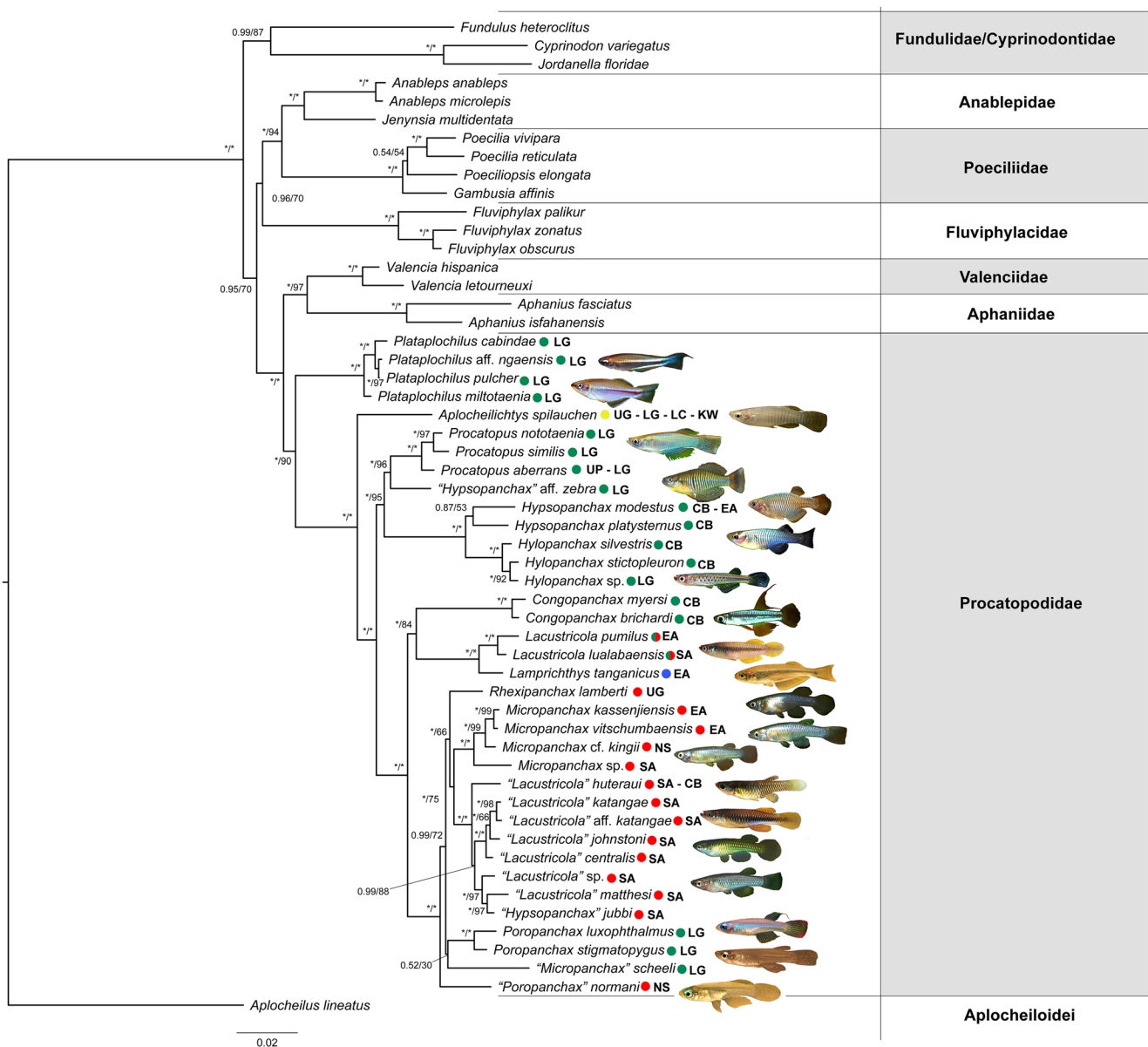


Fig. 1 Phylogenetic relationships among 36 species of Procatopodidae, including all genera but *Apticheilichthys*, inferred by using partial sequences of the nuclear-encoded genes *GLYT1*, *ENC1*, *RAG1*, *MYH6*, and *SREB2*, a total of 5009 bp. Numbers left to the bar indicate posterior probability values and in the right are bootstrap support values taken from the maximum likelihood analysis. Asterisk means maximum values. The green dot next to species name refers to species occurring in rainforests, red dot refers to species occurring in savannahs, green/red dot refer to

species present in swampy humid areas in savannah environments, blue dot refer to species present in Lake Tanganyika, and the yellow dot is a reference to species occurring in brackish environments. Abbreviations next to species name are a reference to Africa biogeographic provinces (modified from Roberts 1975): CB, Congo basin; EA, East Africa; KW, Kwanza; LC, Lower Congo; LG, Lower Guinea; NS, Nilo-Sudanic; SA, Southern Africa; UG, Upper Guinea

relative to the deeper relationships between *Poropanchax*, *Micropanchax*, “*Micropanchax*,” “*Lacustricola*,” and *Rhexipanchax* were little supported. Similar to the change in topology seen after the inclusion of the COI in the BI analysis, in the ML, the species “*Lacustricola*” *johnstoni* was also considered sister to “*Lacustricola*” *centralis*, and “*Micropanchax*” *scheeli*, despite the low support value, was placed as sister to all species of the *Poropanchax*, *Micropanchax*, “*Lacustricola*,” and *Rhexipanchax* clade.

Divergence dates

Divergence time estimates indicate that the Procatopodidae diverged from its sister group, the clade comprising the Aphaniidae and the Valenciidae, in the Eocene/Oligocene transition (32.3 Mya, 95% HDP: 30.9–34.9 Mya) (Fig. 2). The most ancient lineage of Procatopodidae comprising *Plataplocheilus* split from the clade including all other procatopodids in the Oligocene (30.1 Mya, 95% HDP 26.8–33.5 Mya), but intrageneric lineage

diversification of *Plataplochilus* happened only in the late Miocene-Pliocene. During the early Miocene, the *Aplocheilichthys spilauchen* lineage diverged from a clade containing the other procatopodids (α clade) (23.1 Mya, 95% HDP 19.1–27.2 Mya), which also split during the early Miocene (20.1 Mya, 95% HDP 16.3–24.0 Mya), originating two diversified clades, one comprising the genera *Procatopus*, *Hypsopanchax*, “*Hypsopanchax*,” and *Hylopanchax* (β clade), and the other comprising the remaining procatopodids (γ clade). The former clade first diverged in the early Miocene (17.7 Mya, 95% HDP 13.6–21.9 Mya), forming two groups, one with *Procatopus* and “*Hypsopanchax*” and the other with *Hypsopanchax* and *Hylopanchax*. The lineage comprising *Procatopus* and “*Hypsopanchax*” split in the transition between the early and middle Miocene (15.1 Mya, 95% HDP 10.1–19.9 Mya), whereas *Hypsopanchax* and *Hylopanchax* split in the late Miocene (8.6 Mya, 95% HDP 5.5–12.2 Mya). Species-level diversification within these genera occurred between the late Miocene and early Pliocene. Another major split happened during the early Miocene (16.3 Mya, 95% HDP 12.8–20.0 Mya), giving origin to two groups, one comprising *Congopanchax*, *Lamprichthys*, and *Lacustricola* (δ clade) and the other including “*Poropanchax*,” *Poropanchax*, *Micropanchax*, “*Micropanchax*,” “*Lacustricola*,” and *Rhexipanchax* (ϵ clade). In the first group, *Congopanchax* diverged from the *Lacustricola*-*Lamprichthys* clade in the middle Miocene (14.4 Mya, 95% HDP 10.7–18.2 Mya), but *Congopanchax* diversification occurred only during the Pleistocene. *Lamprichthys* split from *Lacustricola* during the late Miocene (5.8 Mya, 95% HDP 3.3–9.0 Mya), and further species-level diversification in *Lacustricola* happened in the Pliocene-Pleistocene transition. All lineages of the “*Poropanchax*,” *Poropanchax*, “*Micropanchax*,” *Micropanchax*, “*Lacustricola*,” and *Rhexipanchax* clade (ϵ clade) originate in the transition between the middle and late Miocene and most species-level diversification in this clade happened between the Pliocene and the Pleistocene.

The inclusion of the mitochondrial gene COI does not result in differences considering the topology of the nuclear genes time-calibrated analysis, but most cladogenic events among the Procatopodidae received a slightly older age. However, these changes do not compromise the interpretations of palaeogeographic and palaeoclimatic events.

Discussion

Monophyly and phylogenetic position of the Procatopodidae

The present study agrees with recently published molecular phylogenies in refuting monophyly of the Poeciliidae (sensu

Parenti 1981) (Helmstetter et al. 2016; Pohl et al. 2015; Pollux et al. 2014; Reznick et al. 2017; Bragança et al. 2018; Bragança and Costa 2018). Procatopodids are indicated to be more closely related to the Aphaniidae and Valenciidae, both comprising old world oviparous cyprinodontoids, whereas *Fluviphylax* is supported to be a distinct lineage (Fluviphylacidae), sister to a clade comprising American livebearers (Poeciliidae) and the Anablepidae, as supported in previous molecular studies (Helmstetter et al. 2016; Pohl et al. 2015; Pollux et al. 2014; Reznick et al. 2017; Bragança et al. 2018; Bragança and Costa 2018). In the present analysis, the more inclusive study of the African lampeyes, the Procatopodidae was recovered as a monophyletic assemblage, also agreeing with the aforementioned but less inclusive molecular studies.

Internal relationships among the Procatopodidae

In contrast to the internal procatopodid relationships proposed in Huber (1999) and Ghedotti (2000) morphology-based analyses (Fig. 3), *Plataplochilus* is here considered the most basal lineage of the Procatopodidae, and *Aplocheilichthys spilauchen* is the only member of a lineage sister to a clade comprising all other Procatopodidae except *Plataplochilus*. According to Huber (1999), *Lamprichthys* was sister to all other African lampeyes, and *Plataplochilus* was sister to *Procatopus*, whereas *A. spilauchen* was considered sister to a clade comprising *Lacustricola*, “*Lacustricola*,” *Pantanodon*, *Rhexipanchax*, *Hypsopanchax*, *Hylopanchax*, *Plataplochilus*, and *Procatopus* (Fig. 3). Ghedotti (2000) otherwise considered the Amazon genus *Fluviphylax* as the most basal lineage of the Procatopodinae, which also included all African lampeyes except *Aplocheilichthys spilauchen*, establishing two main clades among the African procatopodins, one including “*Hypsopanchax*,” *Procatopus*, *Pantanodon*, *Lamprichthys*, *Hypsopanchax*, and *Rhexipanchax*, and the other including *Micropanchax*, *Lacustricola*, “*Lacustricola*,” and *Poropanchax* (Fig. 3).

A clade comprising *Hylopanchax*, *Hypsopanchax*, “*Hypsopanchax*,” and *Procatopus* is highly supported in the present study. In contrast, Huber (1999) recognized a deep-bodied lineage grouping not only the aforementioned genera but also *Rhexipanchax* and *Plataplochilus*, whereas Ghedotti (2000) does not recognize a close relationship between *Hypsopanchax modestus* and species of *Procatopus* and “*Hypsopanchax*” (Fig. 3). Another clade herein supported includes the genus *Congopanchax*, with two of the smallest freshwater fish species in Africa (Conway and Moritz 2006; Roberts 1972), the monotypic *Lamprichthys* and *Lacustricola*. Historically, the phylogenetic position of *Congopanchax* is confused and unclear. Parenti (1981) considered *Congopanchax* a subgenus of *Aplocheilichthys* Bleeker, 1863, whereas Huber (1999) considered it a

Fig. 2 Time-calibrated phylogeny of the Cyprinodontoidei and Procatopodidae obtained from the Bayesian dating analysis in BEAST v.1.8. Bars represent maximum and minimum date estimates for each node and the numbers are nodes divergence mean ages. Arrows indicate the nodes calibrated by the age of cyprinodontiform fossil record. The colors and letters in the time bar are a reference to the proposed time extension of Africa main palaeogeographic and palaeoclimatic events during Procatopodidae evolution: the blue bar represent the Eocene trans-Saharan sea; the green bar represent the Miocene climatic optimum; the orange bar represent the late Miocene aridification; the red bar represent the Pliocene-Pleistocene climatic instability. Letter A is a reference to the increase in African Rift tectonic activity; B is a reference to Tanganyika lake formation; C is a reference to the reactivation of the Cameroonian volcanic line; D is a reference to the rise of the Rwenzori mountains and the disruption of the Paleo Obweruka Lake; and E is a reference to Malebo Pool formation. Posterior probability values are present in Online Resource 5. In view of contributing to a better understanding and discussion of the results, some clades were identified by α , β , γ , δ , and ϵ

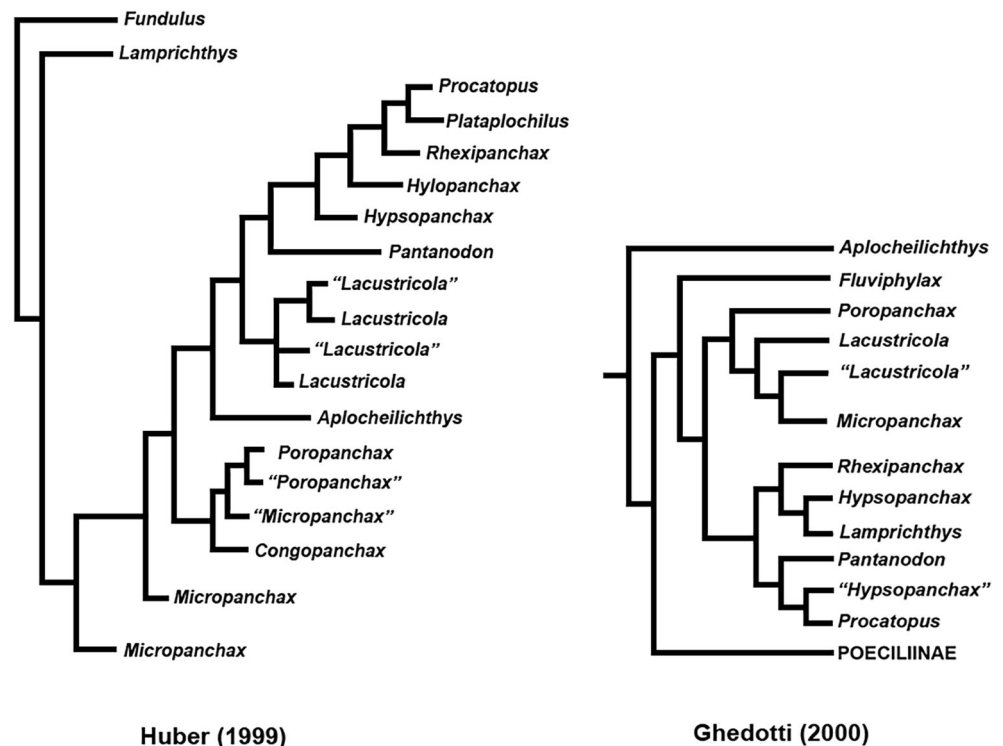
genus *Hypsopanchax* Myers, 1924 was erected to include only the Congolese deep-bodied species *H. platysternus* (Nichols and Griscom 1917), but other deep-bodied species occurring in the Congo and adjacent Lower Guinea coastal river drainages were later moved to or described as *Hypsopanchax* species (Myers 1938; Poll and Lambert 1965). Recently, monophyly of *Hypsopanchax* has been challenged by Van der Zee et al. (2015) due to differences on the cephalic sensory system pattern among included species, suggesting that it could represent a non-monophyletic assemblage. In the present study, the clade comprising *Hypsopanchax platysternus* and *Hypsopanchax modestus* is

considered to be closely related to *Hylopanchax* Poll and Lambert, 1965, whereas “*Hypsopanchax*” aff. *Zebra* revealed to be sister to *Procatopus* Boulenger, 1904.

A paraphyletic *Lacustricola* is herein suggested for the first time, in which *Lacustricola pumilus* (Boulenger, 1906) and *L. lualabaensis* (Poll, 1938) are nested within the *Congopanchax* and *Lamprichthys* clade and the other “*Lacustricola*” species are placed in the same clade of *Poropanchax*, “*Poropanchax*,” *Micropanchax*, “*Micropanchax*,” and *Rhexipanchax*. The genus *Lacustricola* Myers, 1924 was firstly erected to include *L. pumilus* (Boulenger, 1906) but the absence of clear morphological characters diagnosing it resulted in taxonomic instability and probably contributed to the grouping of non-related species (Myers 1938). However, in the present analysis, the “*Lacustricola*” clade was also well supported and the deep-bodied species “*Hypsopanchax*” *jubbi* was considered to be part of that clade.

Following the present topology, *Micropanchax* and *Poropanchax* were also recovered as paraphyletic genera. *Micropanchax* Myers, 1924 was described to include *M. schoelleri* (Boulenger, 1904), a junior synonym of *M. loati* (Boulenger, 1901), and *Poropanchax* Clausen, 1967, to include *P. macrophthalmus* Meinken, 1932 a junior synonym of *P. luxophthalmus* Brüning, 1929. Despite the low support values in both ML and BI analyses, “*Micropanchax*” *scheeli* (Roman, 1970) seems to be more closely related to *Poropanchax* than to other *Micropanchax* species, and “*Poropanchax*” *normani* (Ahl, 1928) is herein considered

Fig. 3 Phylogenetic relationships between African lampeyes genera as depicted in Huber (1999) and Ghedotti (2000)



the unique member of a clade, sister to *Poropanchax*, *Micropanchax*, “*Micropanchax*,” “*Lacustricola*,” and *Rhexipanchax*. Despite not including *R. nimbaensis*, the type species of *Rhexipanchax*, we considered *R. lamberti* a member of the same lineage of *R. nimbaensis* since they share external morphology, head scales pattern and cephalic lateral system characters.

Origin of the African lampeyes

Around 37 Mya, at the end of the Bartonian time, during the late Eocene, a trans-Saharan epicontinental sea advanced over the low relief northern region of Africa due to high eustatic sea level (Bonne 2014). The extension of this epicontinental sea is still being debated (Bonne 2014; Giraud et al. 2005; Otero 2010), but fossil data support a connection between the Tethys and the Atlantic ocean isolating the western region of Africa from the main continent (Bonne 2014). The presence of an epicontinental sea during the middle Eocene is also in accordance with time-calibrated analysis of marine derived African freshwater pufferfish and herring that supposedly have entered African freshwater environments during that period (Wilson et al. 2008; Yamanoue et al. 2011). According to our dated analysis, we suggest that the common ancestor of the Procatopodidae, Aphaniidae, and Valenciidae dispersed from Europe over northern Africa during the late Eocene, between 33 and 42 Mya, and subsequently diversified as a result of the retreat of the trans-Saharan marine connection. Despite most species of the Procatopodidae inhabit freshwater environments, we consider that the common ancestor of the African lampeyes, Aphaniidae, and Valenciidae was a salt-brackish water tolerant species. This assumption relies on the fact that extant Valenciidae and Aphaniidae fauna are represented by several salt-brackish water tolerant species inhabiting estuary regions in the Mediterranean, Middle East, and Central Asia (Freyhof et al. 2014; Hrbek and Meyer 2003). Procatopodid lineage diversification started during the early Oligocene (30.1 Mya, 95% HDP 26.8–33.5 Mya), just after the epicontinental sea retreat.

Procatopodidae diversification

Procatopodidae basal lineages and the early and middle Miocene moist-wet climate

Plataplochilus lineage originated in the early Oligocene, but subsequent species diversification started only in the Pliocene (3.2 Mya, 95% HDP 1.7–5.6 Mya), whereas *Aplocheilichthys spilauchen*, a salt-brackish water tolerant species, distributed over Africa west coast (Fig. 4), diverged in the early Miocene (23.1 Mya, 95% HDP 19.1–27.2 Mya). Despite all, *Plataplochilus* species are strictly freshwater; it is not possible to conclude if during the Procatopodidae evolution, two independent

freshwater invasions occurred, one with *Plataplochilus* and the other with the remaining African lampeyes except *A. spilauchen*, or if the latter species represents a reversal to the salt-brackish water tolerant plesiomorphic condition.

Genera representing lineages that diversified during a moist-wet stable climate phase between the early and middle Miocene are today restricted to rainforests (*Procatopus*, “*Hypsopanchax*,” *Hypsopanchax*, and *Hylopanchax*) or swampy humid areas (*Congopanchax* and *Lacustricola*), and all genera except *Lacustricola*, *Lamprichthys*, and one species of *Hypsopanchax* occur only in the Lower Guinean and Congo river ichthyological provinces (Figs. 1 and 4) (Wildekamp et al. 1986). During this warm time, rainforests were broadly distributed in Africa, forming a continuous area, extending to the present East African coast and to the Sahara, in the north (Lovett 1993; Maley 1996; Plana 2004; Stanley et al. 2005).

Procatopus and the Cameroon volcanic line

Procatopus species are presently distributed over dense rainforest areas, occurring in Cameroon coastal river drainages (*P. nototaenia* Boulenger, 1904 and *P. similis* Ahl, 1927) as well as in forest patches in arid areas of Nigeria (*P. aberrans* Ahl, 1927) revealing a disjunct distribution for the genus (Fig. 4) (Clausen 1959; Wildekamp et al. 1986). According to the present analysis, the split between the Nigerian *P. aberrans* and the Cameroonian clade containing other congeners in the late Miocene-Pliocene transition (4.6 Mya, 95% HDP 2.3–7.6 Mya) is probably related to the increasing aridification in that period, fragmenting a previously continuous forest to form forest refugia and to the reactivation of the Cameroon volcanic line during the Miocene and Pliocene (Fig. 4) (Fitton 1987; Obaje 2009; Ubangoh et al. 1998). The Cameroon volcanic line with its volcanoes and highlands is considered the main geological feature in the region, acting as a forest refugia during arid times and as a zoogeographical barrier for many taxa such as crocodiles, small mammals, bats, anurans, birds, and the tigerfish genus *Hydrocynus* (Fjeldsø and Bowie 2008; Goodier et al. 2011; Koubínová et al. 2013; Nesi et al. 2013; Nicolas et al. 2012; Shirley et al. 2013; Zimkus 2009).

The albertinian rift and *Hypsopanchax* distribution

Hypsopanchax is the only procatopodid genus to occur in high altitude rainforest streams in the western slope of the African rift and in the great lakes region (Fig. 4) (Poll and Lambert 1965; Wildekamp et al. 1986). *Hypsopanchax platysternus* is known to occur in the rainforest streams east to Kisangani whereas *H. modestus*, its hypothesized sister species, is distributed in the upper Ituri river, that drains west into the Congo river basin, and in the Semliki river and other streams flowing

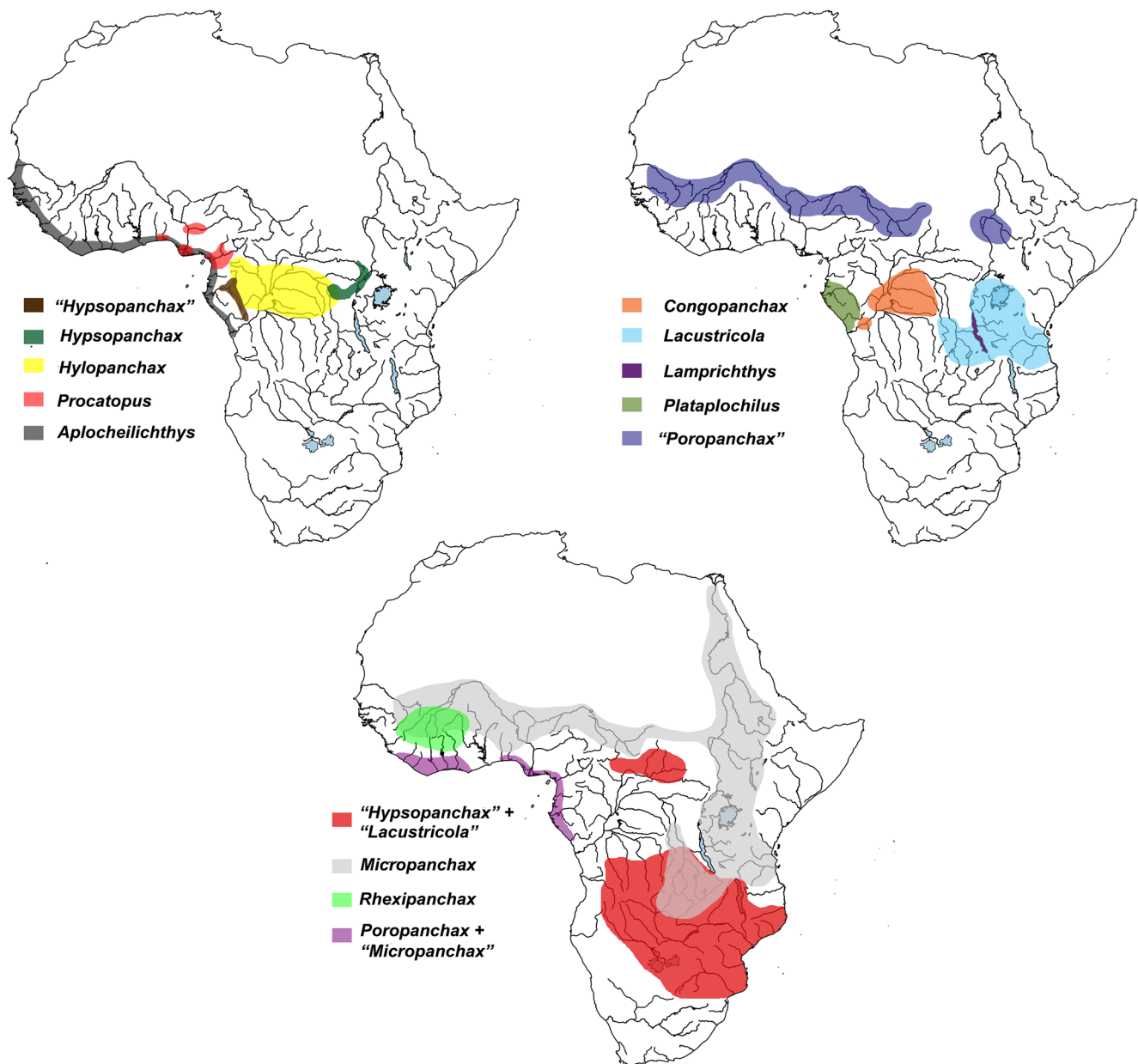


Fig. 4 Estimated distribution extension of Procatopodidae genera based on information present in Wildekamp et al. (1986), Wildekamp (1995), and Wildekamp (2004)

into Lake Edward (Wildekamp 2004). The area presently occupied by *H. modestus* nearly corresponds to the Miocene-Pliocene Paleo Obweruka Lake, which was a large rift lake draining into the Congo basin through the proto-Aruwimi river. The rise of the Rwenzori mountains in the Pliocene ended this connection and split the Paleo Obweruka Lake into the present Albert and Edward lakes (Danley et al. 2012; Ring 2008; Salzburger et al. 2014; Schultheiß et al. 2014; Van Damme and Pickford 1995). According to our analysis, the split between *H. platysternus* and *H. modestus* is estimated to have occurred in the late Miocene (6.7 Mya, 95% HDP 3.7–10.1 Mya) (Fig. 2), therefore before the rise of the Rwenzori mountains (Ring 2008). *Hypsopanchax modestus* is the only

fish species occurring both in the western slope of the Albertine Rift, in drainages flowing west into the Congo river, and in the eastern slope in rivers draining into Lake Edward, a distribution pattern shared with freshwater molluscs of the Viviparidae and Ampullaridae (Schultheiß et al. 2014; Van Damme and Pickford 1995; Van Damme and Pickford 1999). However, cichlid species related to the Eastern African haplochromine clade, known for its high diversity in Africa great lakes where they have undergone unparalleled diversification, have been recently collected in the Congo river tributaries draining the western slope of the African rift (Decru et al. 2017; Schwarzer et al. 2012). The new records have been considered either an evidence of past connections between the

Congo and the great lakes (Schwarzer et al. 2012) or the result of recent introduction events (Decru et al. 2017).

The African rift and its impacts over the Congo river and east African procatopodids

The clade comprising the genera *Congopanchax*, *Lacustricola*, and *Lamprichthys* is unique in grouping species from the Central Congo river basin (*Congopanchax*), southern Congo, and East Africa (*Lacustricola* and *Lamprichthys*) (Fig. 4). It diverged from its sister group in the middle Miocene (14.4 Mya, 95% HDP 10.7–18.2 Mya) (Fig. 2), thus coinciding with the Miocene wet phase, when Africa rainforest was widespread and an east-west drainage connection between the Congo and East Africa river drainages through the proto-Malagarasi river was established (Cohen et al. 1997; Goodier et al. 2011; Weiss et al. 2015). During humid periods with high rainfall, increase in river and stream water level probably facilitated connections between river drainages and freshwater fauna dispersion between adjacent river drainages (Lévêque 1997, 2006; Roberts 1975). However, the continuing uplift of the East African rift during middle Miocene and the late Miocene aridification resulted in the fragmentation of the rainforest and disruption of the east-west drainage connection between the Congo and East Africa river drainages (Bobe 2006; Maley 1996; Plana 2004; Sépulchre et al. 2006; Sommerfeld et al. 2016), probably promoting isolation between *Congopanchax* and the *Lacustricola-Lamprichthys* lineage (Fig. 2). However, the suggested palaeogeographic pattern is only supported if we take into account the distribution of the other *Lacustricola* species inhabiting East Africa (e.g., *L. bukobanus* (Ahl, 1924), *L. maculatus* (Klausewitz, 1957), *L. lacustris* (Seegers, 1984), *L. omoculatus* (Wildekamp, 1977), and *L. usanguensis* (Wildekamp, 1977)). The present distribution of *L. pumilus* in the Lake Tanganyika and in the Malagarasi river, and the connection of both Tanganyika and Malagarasi with the Congo through the Lukuga river, as well as, the presence of *L. lualabaensis* in the upper Lulua and Lualaba river drainages, both draining into the Congo river, probably indicates a subsequent vicariant or dispersion event within *Lacustricola*. Unfortunately, it was not possible to include other *Lacustricola* species in the present study, not making possible to date and better understand *L. pumilus* and *L. lualabaensis* actual distributions in face of other *Lacustricola* species distribution and the complex African rift activity. Ongoing studies on the morphology and osteology of the Procatopodidae, strongly indicate a sister relationship between *L. pumilus* and *L. lualabaensis* and all other *Lacustricola* species restricted to East Africa. A similar vicariant pattern was proposed as an explanation for the cladogenic event involving species of the tigerfish genus *Hydrocynus* inhabiting the Congo river basin and species from the

Tanzanian plateau (Goodier et al. 2011). Later, tectonic activity in the African rift led to the origin of the Lake Tanganyika, the oldest and the deepest of the East African rift lakes, between 9 and 12 Mya (Chorowitz 2005; Cohen et al. 1997; Macgregor 2015). However, Weiss et al. (2015) argued that the estimated time for the lake formation was not based on direct dating, whereas other studies applying direct sedimentology and thermochronology indicated that the lake reached deep lacustrine conditions much later, only around 5.5 Mya (Capart 1949; Banister and Clarke 1980; Scholz and Rosendahl 1988). The monotypic genus *Lamprichthys* includes only *L. tanganicus*, a pelagic species endemic to the Lake Tanganyika (Fig. 4), that according to our dated analysis originated in the late Miocene (5.8 Mya, 95% HDP 3.3–9.0 Mya), partially agreeing with the proposed age for the establishment of full lacustrine conditions in the lake (Fig. 2). In contrast to the Lake Tanganyika evolutionary radiations of cichlid fishes (Danley et al. 2012; Salzburger et al. 2014), mastacembelid eels (Brown et al. 2010); and catfishes (Day and Wilkinson 2006; Kolbmüller et al. 2006; Peart et al. 2014), *Lamprichthys* did not diversify, but evolved drastic morphological changes adapting to pelagic environments. Unlike the other procatopodids, *Lamprichthys tanganicus* have a marked sexual dimorphism, an extremely elongated anal and dorsal-fin, more gill rakers, an elongated and compressed body resembling a pelagic clupeomorph and also present a complex courtship behavior and inhabits deep areas near the rocky substrate, where females place their eggs in narrow crevices in the rock (Huber 1999).

The Malebo Pool as Pliocene-Pleistocene refugia

Interpretation of sedimentary and geological data suggest that during the late Miocene-Pliocene, due to tectonic reactivation of the west Atlantic margin, the Congo river became dammed as an endorheic system and that the Lake Mai-Ndombe and Lake Tumba could be remnants of this endorheic system (Beadle 1981; Burke 1996; Giresse 2005; Runge 2007; Stankiewicz and de Wit 2006). However, there is still no consensus on the origin of the Mai-Ndombe and Tumba lakes, with some authors arguing that both of them are more probably the result of submergence of fluvially formed lows or the damming of an affluent of the Congo river (Veatch 1935; Malier 1958; Wiese 1980; Runge 2001). Later, in the late Pliocene, the dammed lake was probably captured by a coastal drainage, establishing the modern high energy lower Congo (Beadle 1981; Burke 1996; Giresse 2005; Runge 2007), agreeing with a proposed Pliocene origin of cichlid and mastacembelid eel species inhabiting the lower Congo rapids (Alter et al. 2015; Schwarzer et al. 2011). Although many studies pointing to the formation of an endorheic system and its subsequent capture by a coastal river drainage, dates and the extent of these events are far from established. However,

this palaeogeographic pattern is congruent with the proposed ages for the origin and diversification of the miniature *Congopanchax*. Despite its middle Miocene origin, species diversification in *Congopanchax* happened only in the Pleistocene (2.0 Mya, 95% HDP 0.7–3.9 Mya), probably due to the formation of the Malebo Pool during the Pliocene-Pleistocene transition, following the capture of the endorheic system by a coastal drainage (Fig. 2) (Beadle 1981; Burke 1996; Giresse 2005; Runge 2007; Stankiewicz and de Wit 2006). Here, we suggest for the first time that the Malebo Pool acted as a refugia, as have already been previously proposed for the Mai-Ndombe and Tumba lakes (Schliewen and Stiassny 2006). Curiously, during most of time since *Congopanchax* lineage diverged, proposed lowland forest refugia before the Pleistocene may have acted as “museums,” conserving ancient lineages instead of promoting its diversification (Fjelds  and Lovett 1997). There are only two species of *Congopanchax*, *C. myersi* (Poll, 1952a) restricted to the lowland rainforest swamps and streams draining the Malebo Pool area and *C. brichardi* Poll, 1971 known only from the Mai-Ndombe and Tumba lakes and lowland rainforest swamps in the Cuvette Centrale (Poll 1971).

Late Miocene-Pleistocene climate instability and Procatopodidae diversification

Late Miocene aridification and subsequent Pliocene and Pleistocene climate instability were determinant in the diversification of African taxa (Bobe 2006; deMenocal 1995; deMenocal 2004; Dorn et al. 2014; Plana 2004) and probably played a main role in the diversification of the clade comprising *Poropanchax*, “*Poropanchax*” *Micropanchax*, “*Micropanchax*,” *Rhexipanchax* and “*Lacustricola*”. This clade includes the most widespread African lampeyes genera, “*Lacustricola*” and *Micropanchax*, and all of them but *Poropanchax* and “*Micropanchax*” *scheeli* includes species adapted to live in arid environments such as the savannahs of Eastern and Southern Africa and the arid Sahel (Figs. 2 and 4) (Wildekamp et al. 1986; Wildekamp 1995). “*Poropanchax*” *normani*, the only member of the most basal lineage of this clade, diverged from its sister group in the transition between the middle and late Miocene (11.6 Mya, 95% HDP 8.7–14.7 Mya), during the onset of a more arid climate (Fig. 2). It is the most widespread African lampeye species, inhabiting the Nilo-Sudanic ichthyofaunal province, being recorded from the Senegal river to the Tchad lake, besides occurring in isolated oasis of the middle part of the Sahara desert (Fig. 4) (L  v  que et al. 1991; Trape 2013). Considering that the clade including *Congopanchax*, *Lamprichthys*, and *Lacustricola*, is sister to the clade comprising *Poropanchax*, “*Poropanchax*” *Micropanchax*, “*Micropanchax*,” *Rhexipanchax* and “*Lacustricola*” and

inhabit the rainforests of the Congo basin, we tentatively assume that “*Poropanchax*” *normani* ancestor moved from the later region into more arid lands in the north, probably through a past drainage connection with the Chiari-Chad drainage (L  v  que 2006). A connection between the Congo and the Nilo-Sudanic region during the Miocene, followed by isolation due to reactivation of the Central African Shear zone have already been suggested for explaining vicariant patterns in *Hydrocynus* and *Synodontis* (Goodier et al. 2011; Pinton et al. 2013). A similar drainage connection was proposed to explain the close relationship among species of the Aplocheiloidei killifish genus *Epiplatys* Gill, 1862, with *E. bifasciatus* (Steindachner, 1881) and *E. spilargyreus* (Dum  ril, 1861) inhabiting the Nilo-sudanic savannah, and the miniature species *E. duboisi* Poll, 1952b restricted to the Congo rainforest (Collier et al. 2009). At last, this drainage connection could also explain the disjunct distribution between the cichlid genus *Limbochromis* Greenwood, 1987, inhabiting rainforest in West Africa and the Congolese widespread genera *Congochromis* Stiassny and Schliewen, 2007 and *Nanochromis* Pellegrin, 1904 (Schwarzer et al. 2014).

The most widespread Procatopodidae clades are “*Lacustricola*”/“*Hypsopanchax*” and *Micropanchax*. The former clade occurs in Southern Africa, including tributaries of the Congo river in the Katanga region, few areas in East Africa and with only one species, “*L.*” *hutereaui*, occurring in savannahs in both Southern Africa and northern Congo. Species of *Micropanchax* inhabit extreme arid environments, occurring in the Nilo-Sudanic region, East Africa, southern Congo tributaries, and Southern Africa (Figs. 2 and 4). According to the dated analysis, both “*Lacustricola*”/“*Hypsopanchax*” and *Micropanchax* originated during the arid late Miocene conditions (5.4 Mya, 95% HDP 3.7–7.5 Mya) and (5.1 Mya, 95% HDP 3.1–7.2 Mya) respectively, and further diversification occurred during the Pliocene and Pleistocene climatic instability (Fig. 2). Intense aridification in that period is related mainly to palaeoclimatic and geological events, such as the increased tectonic activity and consequent orogenesis in the African rift (deMenocal 1995, 2004; S  pulchre et al. 2006; Sommerfeld et al. 2016). Fossil data and phylogeographic studies of large mammals revealed that during the arid climates, when savannah vegetation spread, clade diversification intensified, and arid corridors were established (Bobe 2006; deMenocal 2004; Lorenzen et al. 2012). Arid corridors extended from Southern to Eastern Africa, and from Eastern Africa to the west in the Sahel, allowing connections between mammalian fauna (Bobe 2006; deMenocal 2004; Lorenzen et al. 2012). The extension of the proposed arid corridors agrees with *Micropanchax* and “*Lacustricola*” distributions (Fig. 4), suggesting that dispersion and isolation associated with aridification and climatic instability played a main role in shaping the present diversity and distribution of the aforementioned taxa. Recently, a similar distribution and diversification pattern

have been described for *Nothobranchius*, a genus of African seasonal aplocheiloid killifishes belonging to a clade occurring in dry savannas (Costa 2018; Dorn et al. 2014), a phenomenon also reported for South American seasonal aplocheiloid killifishes living in similar conditions (Costa et al. 2017).

Finally, despite the few *Micropanchax* species included in the analysis, it was possible to identify the influence of a vicariance event during the Pleistocene in *Micropanchax* diversification. The divergence between *M. vitshchumbaensis* (Ahl, 1924), from the Edward lake, and *M. kassenjiensis* (Ahl, 1924) from the Albert lake (0.8 Mya, 95% HDP 0.2–1.7 Mya) (Fig. 2), agrees with the disruption of the Paleo Obweruka Lake in the end of the Pliocene due to the rise of the Rwenzori mountains (Ring 2008).

Conclusion

This study shows that time-calibrated analysis of riverine well-distributed fish groups in Africa can provide reliable information about Africa landscape evolution, and we consider that similar efforts on other African fish groups are needed for a more clear comprehension on Africa evolution. Also, the time-calibrated analysis suggested the influence of paleogeographical and paleoenvironmental events on the procatopodids diversification, but further studies including more terminals are desired. At last, a monophyletic Procatopodidae was supported as well as its position as sister to the Aphanidiidae and Valenciidae agreeing with previous molecular studies.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Submission declaration and verification We declare that the manuscript is not under consideration for publication elsewhere and that it was approved by both authors that completely agree with its content. Also, if the manuscript is accepted it will not be published elsewhere in the same form or in another language.

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