# Do sympatric catfish radiations in Lake Tanganyika show eco-morphological diversification?

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## Abstract

Adaptive radiation is characterised by eco-morphological differentiation, in which niche partitioning has been shown to be a central response to natural selection during the diversification of animal clades. This process is suggested to have generated the exceptional biodiversity in the East African rift lakes; however aside from the cichlid fishes, the nature of the divergence, over time, or among species is less clear in the other radiations. To address this, we focus on two distantly related sympatric Lake Tanganyika catfish clades, the genus *Synodontis*, considered to be Müllerian mimics, and the sub-family Claroteinae. We investigate to what extent, if any, these radiations have undergone eco-morphological diversification. We place these radiations in a common phylogenetic context, and test for morphological divergence and trophic niche partitioning using novel trait data and stable isotope signatures. Diversification of both catfish clades was recent, with the *Synodontis* radiation synchronised in time with individual genera within the claroteine radiation, suggesting initial diversification was facilitated by lake basin dynamics and/or lake level fluctuations. There is evidence for eco-morphological diversification within the claroteine radiation, as inferred from observed morphological disparity and divergence in diet both between and within genera; however, several species show significant overlap in dietary isotopic signatures. In contrast, the *Synodontis* radiation has greater overlap between taxa both in morphology and isotopic signatures potentially indicating niche conservatism, suggesting different selective forces maybe driving these radiations.

## Introduction

Radiations can be characterised as adaptive when genetic diversification is accompanied by a change in resource exploitation, with a concomitant change in physiological, behavioural or morphological traits enabling exploitation of new resources, producing an ecologically diverse array of descendent species (Schluter, 1996; Schluter, 2000). Adaptive radiation may occur as species diversify to fill different environments, as alternate food resources are more prevalent in different environments or may occur in syntopy as a result of niche partitioning. Trophic niche partitioning has been shown to be a central response of natural selection in various adaptative radiations including cichlid fishes (e.g., Ford *et al.* 2016; Muschick *et al.* 2012, Sturmbauer, *et al*. 1992), Darwin's finches (e.g., Grant and Grant, 2024), Hawaiian spiders (Kennedy *et al.* 2019), *Cyprinodon* pupfish (Martin and Feinstein, 2014) and Caribbean *Anolis* lizards (Losos, 2011). Not all radiations are necessarily adaptive under this definition however, for example, when genetic diversification results from sexual selection, hybridisation or geographic isolation (e.g., woodland salamanders, Kozak, *et al.* 2006) little or no divergence along ecological axes is observed, although hybridisation has been suggested to lead to novel traits in some radiations (e.g., Genner and Turner, 2012; Keller *et al.* 2013).

The East African rift lakes are hotspots of biodiversity, containing many endemic radiations, including the hyper diverse cichlid fishes, which have diversified into a multitude of different body forms and niche specialisms (Fryer and Iles, 1972). These fishes are by far the most intensively studied organisms of these lakes and multiple factors have been implicated in the generation of their diversity (e.g., Albertson, 2008, Wagner *et al.* 2012), including competition (Winkelmann et al., 2014), hybridisation (e.g., Genner and Turner, 2012; Irisarri, et al. 2018; Meier et al. 2017, 2023), intra-lacustrine allopatry (e.g., Koblmüller *et al*. 2011; Verheyen et *al*. 1996, Sturmbauer et *al.* 2001), sensory drive (Seehausen et al., 2008), and sexual selection (e.g., Albertson, 2008, Wagner *et al.* 2012). Many species co-exist in the littoral zone of these lakes, but the relevance of niche partitioning and the extent that this allows co-existence of multiple species, or has influenced their diversification has been largely over-looked in non-cichlid radiations, although see Marijnissen *et al.*, (2008). To address this, we focus on sympatric Lake Tanganyika catfish radiations, which in contrast to cichlids are considerably less well studied. Lake Tanganyika (LT) has a notably high diversity of catfish species (*ca.* 34), including three independent radiations. These include the Mochokidae genus *Synodontis* (*ca.* 11 species) (Day and Wilkinson, 2006; Koblmüller et al., 2006); the subfamily Claroteinae (15 described species, with additional diversity, Peart *et al.* 2014; Peart *et al.* 2018); and the genus *Tanganikallabes* (3 species, Wright and Bailey, 2012).

The adaptive character of the catfish radiations is unclear, as is the relative importance of factors driving their diversification. Previous studies have demonstrated common ancestry between endemic species within the three catfish clades (e.g. Day and Wilkinson, 2006; Day *et al.* 2013; Koblmüller *et al.* 2006; Peart *et al.* 2014) in which the onset of diversification is estimated to have occurred after the formation of the lake basin: Claroteine: 5.08 Ma (95% HPD: 3.61-6.84 Ma, Peart et al., 2014); *Synodontis*: 4.1-7.0 Ma (Day and Wilkinson, 2006) to 7.9 Ma (95% HPD: 5.7-10 Ma, Day *et al.* 2013). There is evidence that geographic restriction plays a role in at least two LT claroteine genera (*Phyllonemus* and *Lophiobagrus*) (Peart *et al.* 2014, 2018) and possibly in *Chrysichthys* (discussed in Hardman, 2008), with the latter taxon also suggested to be partitioned by habitat and depth (Coulter 1991), although many claroteine species can be collected in sympatry (pers. obs.). This geographic segregation is less clear for *Synodontis*, as while *S. multipunctatus* showed little spatial genetic structure (Peart *et al.* 2018), multiple species have been described from single localities (Wright and Page, 2006).

Unlike many cichlid species where sexual selection is proposed as an important driver in speciation (Wagner *et al.* 2012), there is no clear sexual dimorphism/dichromatism (used as a proxy for sexual selection) in LT catfishes, although mating strategies are varied across the clades. Several claroteine taxa are known paternal or bi-parential mouthbrooders (Ochi *et al.* 2000; 2001; 2002), which may provide a morphological constraint with respect to feeding, as shown in other non-cichlid fishes (e.g., Hoey *et al.*

2012), while *S. multipunctatus* is a brood parasite (Sato, 1986, Reichard *et al.* 2022). Notably endemic LT *Synodontis* are suggested to be Müllerian mimics based on experimental evidence of two species, and phylogenetic examination of colour patterns across the radiation (Wright, 2011). However, it is unclear whether these positive mutualistic associations outweigh competition for niche space.

Here, using an integrated approach we focus on the two more diverse LT catfish radiations, Claroteinae and *Synodontis*, allowing comparisons of the timing and mode of diversification. We place these radiations in a common phylogenetic context using a two-step dating process with multiple fossil calibrations. This allows investigation of patterns of morphological and ecological diversification, using morphological trait measurements, and stable isotope signatures of carbon and nitrogen to estimate dietary resource use. Using these data we address the following questions: 1) What is the age of origin of the two catfish clades, and did these independent radiations diversify concurrently? 2) Is there evidence for eco-morphological diversification, specifically trophic niche partitioning in these clades? and 3) Do they exhibit an adaptive signal? (following Schluter, 2000). We predict that as Müllerian mimics, the LT *Synodontis* are more likely to show constrained eco-morphological diversification than the LT claroteine catfishes.

## Material and methods

### Lake Tanganyika sampling

Specimens, including samples for molecular, and isotopic analysis (southern LT only) were collected using a variety of methods (e.g., scuba, snorkelling, fyke nets, rotenone, and for molecular samples only, also obtained from local fish markets) from sites in Burundi, Tanzania and Zambia. The isotopic samples in this study (collected February-March 2011) were mainly sampled from shallow (<18 m) rocky littoral habitats, with only *S. multipunctatus* sampled from greater depths from sites in the Southern Basin (Figure S1). In addition, specimens from museum collections were also measured for inclusion in the morphological analyses (Table S1). For detailed information regarding the taxonomic status of species, specifically species delimitation in *Synodontis*, refer to the Supporting Information (p1-2, Figure S2).

### Molecular sequence data

A total of 322 novel DNA sequences were generated and 252 sequences downloaded from GenBank to build the ostariophysian and 'Big Africa' phylogenies (Table S2a). In order to verify LT *Synodontis* taxa used in morphological and ecological analyses (Table S1) a further 106 novel sequences were generated, and 32 sequences downloaded from GenBank (Table S2b) for Cytochrome *b*. DNA was extracted using the Qiagen DNeasy kit. PCR reactions were conducted, cleaned, checked and sequenced following Peart et al. (2014) who used the primers and annealing temperatures from the following studies: RAG1 exon 3 (Sullivan et al., 2006), ENC1, Plagl2 (Li *et al.* 2007), RAG2 (Sullivan et al., 2006), Cytochrome *b* (Day and Wilkinson, 2006 and references therein). Chromatograms were checked and edited, and resultant sequences were aligned with Geneious 5.6 and translated to check for stop codons by eye. The sequences were trimmed to reduce missing data and match previously published sequences leading to 1371bp RAG1, 810bp ENC1, 680bp Plagl2, 922bp RAG2, 759bp (1st and 2nd positions *Cytb*) and 434bp (1st and 2nd positions CO1).

## Molecular dating analyses

To compare the timing of diversification events in both the LT *Synodontis* and claroteine radiations a two-step dating procedure was employed. This involved dating the onset of the 'Big Africa' catfish clade, which the focal clades (along with the majority of African catfishes) are members of (Sullivan *et al.* 2006), using a phylogeny including taxa and calibrations distributed throughout the ostariophysian phylogeny. The 'Big Africa' posterior probability estimate was subsequently used as a prior in a 'Big Africa' only analysis using additional molecular markers and denser taxonomic sampling, including the two LT radiations. This method was preferred since although the oldest catfish fossil found in Africa, in the genus *Nigerium*, is attributed to the family Claroteidae within the 'Big Africa' clade, its accurate placement is potentially problematic (see Peart *et al.* 2014). The rapid radiation of lineages at the base of the siluriform phylogeny, which often forms an unresolved polytomy (Lundberg *et al.* 2007; Sullivan *et al.* 2006), makes inferences on the timing of one clade from a fossil placed in another siluriform clade problematic.

### ***Ostariophysian phylogeny***

The ostariophysian tree was constructed using 203 specimens from across the ostariophysians in 146 genera, with two outgroup taxa from the Clupeiformes (Table S2a) and five fossil constraints. This analysis used the nuclear molecular markers RAG1 exon 3, ENC1 and Plagl2. The fossil calibrations were taken from Near *et al.* (2012) and applied with the same priors. For information on the calibrations and topological constrains, see Supporting Information (p3).

To determine the best partitioning scheme and nucleotide models, Partitionfinder v1.1 (Lanfear et al., 2012) was used, selecting a greedy algorithm and Bayesian Information Criteria (BIC). A scheme with two subsets, the first containing the first and second codon positions for all genes with the model GTR+I+G and the second subset containing the third codon partitions for all genes with the model SYM+I+G was preferred. A Bayesian phylogeny was generated with these constraints in BEAST 1.7.5 (Drummond et al., 2012) using a birth-death prior; the analysis was repeated three times with each analysis consisting of 175,000,000 generations. Preliminary analyses suggested that a relaxed clock for each gene over-parameterized the analysis, so each subset identified for the nucleotide model was assigned a separate relaxed lognormal clock. Burn in was determined in TRACER with a value of 10% (Rambaut and Drummond, 2009) and ESS values were all over 200. A maximum clade credibility (MCC) tree was calculated using Tree Annotator.

### *The 'Big Africa' phylogeny*

The 'Big Africa' only phylogeny was constructed using 72 taxa (Table S2a). Additional molecular markers were also used to provide greater resolution, the single copy nuclear markers RAG1 exon 3, ENC1 and Plagl2, RAG2 and the first and second codon positions of the mitochondrial sequences CO1 and *Cytb*. This phylogeny was calibrated using the posterior probability for the 'Big Africa' clade (as defined by Sullivan *et al.* (2006) with the addition of *Lacantunia enigmatica* (Lundberg *et al.* 2007) from the ostariophysian phylogeny as the prior on the root of this phylogeny. Partitionfinder v1.1 was again used to determine the partitioning scheme (selecting a greedy algorithm and BIC), in which the best scheme selected two subsets: one with the first and second codon positions for all genes (including the mitochondrial genes) with the model SYM+I+G, and the second subset containing the third codon position for all genes with the model SYM+G. The BEAST analysis (using a birth-death prior) was repeated three times and run for

100,000,000 generations, with each marker having a separate relaxed lognormal clock (the mitochondrial markers had the same clock). Burn in was determined in TRACER with a value of 10% and ESS values were all over 200. A MCC tree was calculated using Tree Annotator.

### Morphological data and analyses

Nine continuous head and body measurements and standard length (SL) were collected from individuals in each catfish radiation, totalling 198 specimens. These included: 1) head length from the upper lip to the posterior edge of the operculum, 2) the eye diameter, 3) head height through the centre of the eye, 4) eye position measured as the distance between the base of the head to the centre of the eye, 5) snout length from the upper lip to the centre of the eye, 6) gape width, 7) maximum body depth, 8) depth of the caudal peduncle, and 9) body width at pectoral fin inserts. These measurements capture shape changes and include measurements directly linked to feeding such as those that quantify the position of the mouth and eye, and have previously been used in studies of diverse fish systems including those investigating adaptive radiations (e.g., Alexandrou *et al.* 2011; López-Fernández *et al.* 2013; Montaña and Winemiller, 2013).

The claroteine dataset consisted of 135 specimens for 13 species, including three separate populations of *Lophiobagrus cyclurus* from Burundi, Kigoma and Zambia (Peart *et al.* 2014) totalling 15 sample groups (minimum 1, maximum 17, median 10 samples per group). The *Synodontis* dataset consisted of 63 specimens in eight species (minimum 2, maximum 14, median 8.5 samples per species). A full list of the specimens included in this study is given in (Table S1). A natural logarithm of each measurement was taken, and subsequently averaged within species. These trait species averages were corrected for size while taking into account phylogenetic history using phylogenetic size correction (phyl.resid command) in the phytools R package (Revell, 2009). These residuals were then used as the input for a phylogenetic PCA (pPCA) in the same R package (phyl.pca command). The phylogenetic component of these commands considers the non-independence of trait values. pPCA plots can be complex to interpret as the axes show the non-phylogenetic component of shape variation but the location of the points in this space still has phylogenetic covariance (Polly et al., 2013). Due to this and to investigate intraspecific variation each analysis was repeated without averaging within species and without the phylogenetic component in PAST (Hammer et al., 2001). Prior to the PCA size was removed as a component using the allometric vs. standard option. The latter analysis included species not present in the molecular phylogeny leading to a dataset of 144 specimens for 16 species, again including three separate populations of *Lophiobagrus cyclurus* from Burundi, Kigoma and Zambia (Peart *et al.* 2014) making 18 potential taxa (minimum 1, maximum 17, median 9.5 samples per group) for the claroteine radiation, and 97 specimens in 10 species (minimum 1, maximum 14, median 12 samples per species) for *Synodontis*.

## Stable isotope data and analyses

The dietary niche of each species from both radiations was approximated using nitrogen ($\delta^{15}N$) and carbon ($\delta^{13}C$) stable isotope values from white muscle tissue from 191 specimens (Table S1). $\delta^{15}N$ and $\delta^{13}C$ describe the relative position of trophic level, and position along the benthic–pelagic axis respectively (Post, 2002). A total of 113 claroteine specimens (minimum 1, maximum 22, median 10 specimens per species) and 78 *Synodontis* specimens (minimum 11, maximum 21, median 16 specimens per species) were euthanized using an overdose of clove oil (Neiffer and Stamper, 2009) immediately upon capture, and preserved in 70% ethanol (with tissue excised in the lab several months after collection). Sub-samples were taken as controls at this stage and dried immediately. A variety of pelagic and littoral basal resources were collected to investigate the composition of sources of primary productivity that each species used. Tissue samples were oven-dried (55°C) and homogenised using a pestle and mortar. Invertebrate samples that may have been contaminated with inorganic carbon e.g., in their shells were acidified using 0.4% HCL (with controls taken). Samples were accurately weighed to 0.6mg for fish and invertebrates and 1.5mg for plants and algae in tin capsules. To measure isotopic signatures of both nitrogen and carbon, samples were combusted in an ECS 4010 elemental analyser (Costech instruments) connected to a continuous flow Delta V Plus mass spectrometer (Thermo Scientific) at the National Environmental Isotope Facility (NEIF) Stable Isotope Ecology Laboratory, SUERC, East Kilbride, U.K. Secondary standards (agarose gel, $^{13}$C-enriched alanine, $^{15}$N-enriched glycine and tryptophan) were used at the beginning and end of each run and every eight samples during the run to monitor drift. The results are expressed in the standard delta notation ($\delta$; McKinney et al. 1950; Coplen 1994) showing the ratio of different isotopes relative to international standards (atmospheric nitrogen and Vienna-PeeDee Belemnite).

The role of preservation on isotopic signatures was also investigated, since preserved tissues have in some studies had an effect on nitrogen and/or carbon isotope composition (e.g., Correa, 2012; Kelly *et al*. 2006; Xu *et al.* 2011). We therefore included (dried) control samples from fish species from different trophic levels including cichlid fishes, mastacembelid spiny eels (Day, unpub.) plus claroteine and *Synodontis* catfishes. Baseline taxa were not included as preservation biases have been found to differ between fish and molluscs (Correa, 2012). Ethanol preservation was found to have a significant effect on $\delta^{15}$N (Wilcoxon test V=55, p=0.002, t-test t=4.34, df=9, p-value=0.002). Ethanol preservation had the effect of shifting $\delta^{15}$N by 0.454 ‰ (3 d.p.). The $\delta^{15}$N values were corrected through the use of a bootstrapped linear correction model (2000 bootstraps) which has previously been used to correct for ethanol preservation effects in a single species (Kelly et al., 2006), but was used in this context to correct for preservation effects across taxa with the model intercept 0.467 (3.s.f.) error 0.298, gradient 0.867 (3 s.f.) error (0.044). After this linear correction factor was applied there was no significant difference between the control and corrected values (Wilcoxon test, V=28 p=1, t-test t=0, df=9, p value=1, when this is plotted the intercept is not significantly different from 0 (p=1) and slope =1 (2 d.p.)). Preservation effects had no significant effect on the $\delta^{13}C$ (Wilcoxon test V=44, p=0.106, T-test t=1.07, df=9, p=0.314). As lipids are depleted in $^{13}C$ relative to other tissues, differences in lipid content can bias results (Kiljunen *et al.* 2006). However, the C:N ratio was not significantly different between control samples and those stored in ethanol (Wilcoxon test V=12, p=0.25, t-test t=-1.01, df=8, p=0.343).

As nutrient regimes differ from the north to south of LT (Plisnier et al., 1999), only samples from the southern (Zambian) sites were included in the isotopic analyses. These sites were sampled most intensively at Sumbu and Mpulungu. The isotope baselines between the two sites are similar, including those for bivalves, which are more depleted in $^{13}C$ at lower taxonomic levels than the other taxa sampled (Figure S3). Based on these values the samples from each location were pooled and analysed together. The resulting range of $\delta^{15}N$ was used to represent the trophic partitioning of the samples, as $^{15}N$ is enriched at higher trophic levels, and $^{13}C$ was used to estimate the contribution of littoral and pelagic basal resources (Post, 2002). The R package Stable Isotope Analysis in R (SIAR, Parnell *et al.*, 2010) was used to calculate standard ellipses for each species in order to assess isotopic niche space and overlap between species.

### Comparative analyses

Disparity through time (DTT) was used to investigate morphological diversification of the LT claroteine dataset (Harmon *et al.* 2003). We used the rank envelope method to compare the empirical data to the null model, using 5000 simulations (Murrell, 2018). This test ranks the DTT curves at each time point and then takes the most extreme rank across all time points as the 'global rank'. There is low power for DTT analyses in the *Synodontis* dataset due to the small number of *Synodontis* species (see Murrell, 2018). Although trait data was available for 18 LT claroteine taxa, only those in the MCC tree were included (n=15) due to issues with taxonomic placement (e.g. *Chrysichthys* is non-monophyletic). The scores from the non-phylogenetically-corrected PCAs were extracted for each of these taxa for PC axes 1–4 and combined with the MCC BEAST tree. DTT plots that have low subclade disparity than expected under Brownian motion (i.e., disparity <1) may indicate a signal of adaptive radiation where disparity is expected to be distributed between subclades rather than within them (Harmon et al., 2003). Conversely, high subclade disparity (i.e., disparity <1), in which there is greater disparity within subclades, has been interpreted as an Ornstein–Uhlenbeck (OU) model or an accelerating rate of trait evolution (Uyeda *et al.* 2015). The fit of each PC axis (1-4) to several likelihood models of continuous character evolution (Brownian motion, Ornstein-Uhlenbeck, Early Burst) was also assessed in the R package GEIGER v.2.0.11 (Harmon *et al.* 2008). The gamma (γ) statistic (Pybus & Harvey, 2000) was computed for the claroteine radiation to test if there was a departure from the pure birth model using R packages ape v.3.4 (Paradis et al., 2004) and phytools v.1.2 (Revell, 2012).

The phylogenetic signal for $\delta^{15}$N and $\delta^{13}$C signatures on the MCC tree was assessed for the claroteine radiation using Blomberg's K (Blomberg *et al.* 2003), implemented using the multiPhylosignal command in the R package picante v.1.6 (Kembel *et al.* 2010) with p-values calculated based on observed vs. random variance in phylogenetic contrasts. The relationship between morphology and ecology was also assessed using phylogenetic generalized least squares regression using GEIGER to simulate Brownian Motion correlation structure (Harmon *et al.* 2008) and R package nlme v.3.1 (Pinheiro *et al.* 2014) (gls command) with mean $\delta^{15}$N against retained PC axes 1-4 from the phylogenetic PCA and separately $\delta^{13}$C signatures against retained PC axes 1-4 from the phylogenetic PCA over 1000 trees pruned to include only taxa for which morphological and isotope data were available. The correlation between claroteine isotope signatures and morphology was further investigated using phylogenetic canonical correlation analysis (command phyl.cca in phytools v.0.4) to investigate correlations between PC axes 1-4 from the phylogenetic PCA and isotope signatures (both $\delta^{15}$N and $\delta^{13}$C signatures). This method finds the axes of largest correlation and assesses the significance of this correlation. The low sample numbers in *Synodontis* make testing for phylogenetic signal problematic using the isotope data.

## Results

### Divergence estimates

In the ostariophysian tree the posterior age estimate of the Siluriformes most recent common ancestor was 143.58 Ma (95% HPD 120.91-163.09) (Figure S4). This tree resolved the 'Big Africa' clade (Sullivan *et al.* 2006) with the addition of *Lacantunia enigmatica* (Lundberg *et al.* 2007) with a posterior age estimate of 83.83 Ma (95% HPD: 66.76-93.16), which is within the age estimated by Kappa *et al.* (2016) based on mitogenomes. This age estimate translated into a normal prior on the root of the 'Big Africa' only phylogeny with a mean of 80 Ma and a standard deviation of 8.0 Ma, truncated to only include 55-150 Ma (see Supporting Information p4 for discussion of these age estimates). From the 'Big Africa' analysis (Figure 1) the posterior age estimate for the claroteine LT radiation was 5.78 Ma (95% HPD 3.87-7.97) and the *Synodontis* LT radiation (including the LT non-endemic *S. victoriae*) was 2.70 Ma (95% HPD 1.67-3.94).

### Principal Component Analyses

There is more overlap between taxa on the Principal Component Analyses (PCA) of morphological variation plots in the *Synodontis* dataset than seen in the claroteines (Figure 2), with *Synodontis* species also packed into less morphospace (Figures 2-3). For the claroteine radiation pPC1-4 axes explained over 90% of the variation with 41.5%, 27.8%, 14.2% and 8.49% respectively. This is similar to the loading for the PCA for PC1 (though inverted), whereas those for PC2 correspond to the loadings on PC3 for the pPCA and vice versa (Figure 3). Species with negative values on pPC1 show greater relative head heights, higher eye heights, larger gape width and greater body depth, caudal peduncle depth and body width. Species with a positive score on pPC2 have a longer head length and smaller eye diameter while positive scores on PC3 mainly reflect a long snout length (Figure 3). In the claroteine radiation the different genera are separated in morphospace by PC1 and to a lesser extent by PC3 (Figure 3). *Chrysichthys sianenna* does not cluster with the other LT members of this genus in morphospace, whereas *Bathybagrus tetranema* occupies a similar area to them. The *Lophiobagrus* taxa are less clustered along PC2 and PC3 with *L. aquilus* occupying a similar area of morphospace to the *Phyllonemus* taxa (Figure 3). The two small species (*L. brevispinis,* 40.66mm max SL and *Phyllonemus* sp. C, 46.36mm max SL, in this study) do not occupy the same areas of morphospace based on these analyses but are like the other members of their respective genera on PC1. In general, intraspecific variation is small compared to interspecific variation, aside from *Lophiobagrus asperispinis* which shows by far the greatest intraspecific variation (Figure 2). Only four specimens were available for this species (with no sequence data) including the holotype and paratopotype, with all four widely distributed across PC1 and PC2 indicating considerable variation among specimens assigned this name. This result may be due to this species displaying allometric effects that are not well known or alternatively the samples collected may include more than one species. Unlike in the pPCA (Figure 3) *Chrysichthys sianenna* is not separated in morphospace on PC1 or PC2 with only a slight separation on PC3 (Figure 2). There are some general patterns with the genera clustering together on PC1 and PC2 though; *L. brevispinis* is separate from the remaining *Lophiobagrus* species and there is some separation between *Phyllonemus* sp. C and the other *Phyllonemus* species. *Bathybagrus tetranema* clusters with the LT *Chrysichthys* species (Figure 2) as it does in the pPCA (Figure 3).

For the *Synodontis* dataset pPC1-3 axes explain over 97% of the variation with 77.4%, 14.7% and 5.2% respectively, which are similar to the PCA axes. The positive scores on the first PC axis correspond to smaller head lengths and head height with a lower eye position, smaller eyes, shorter snout length and smaller body depth (Figure 3). On PC2 positive scores correspond to larger caudal peduncles with a lesser relationship to larger body widths. Positive scores on PC3 represent mainly larger gape width and larger eye diameter. In the pPCA, *Synodontis* taxa are spread evenly across PC1, whereas PC2 and PC3 show some distinct groups with *S. multipunctatus* distinct from other taxa especially on PC2 (Figure 3). Clade 3 and *S. granulosus* show very similar scores on PC2 with the remaining taxa tightly clustered on both PC2 and PC3. *Synodontis multipunctatus* shows the least intraspecific variation on the first two PC axes and only overlaps in morphospace with the two taxa to which it is proposed to be most closely related, *S. grandiops* and *S. granulosus*. *Synodontis granulosus* has the largest intraspecific variation overlapping in morphospace with five different taxa shown by convex hulls (Figure 2). In the PCA Clade 2 and Clade 5 overlap, as do Clade 6 and Clade 3 on PC1 and 2. There is less overlap of taxa in the pPCA for analyses including PC1, except for the comparison of PC2 vs. PC3, which shows considerable clustering of the unnamed clades, aside from Clade 3. (Figure 3).

### Disparity and diversification

Despite lower subclade disparity indicated for PC axis 1-2 in the DTT plots (Figure 4) for the claroteine radiation, our data (PC axes1-4) were not significant (the most liberal and most conservative p-values = 0.216 < *p* < 0.218 (Figure 4) and as such showed that disparity cannot be distinguished from Brownian Motion. Akaike weights also identify a model of Brownian motion as best supported for each PC axis on the MCC tree (Table S3), although no model was particularly strongly supported. The gamma (γ) statistic was slightly negative for the claroteine radiation (-1.51) but non-significant (p = 0.132, two-tailed test) even including missing species using the MCCR test (data not shown).

## Trophic partitioning

Although there is some overlap in isotopic signatures between each catfish radiation (Figure 5) the differences in $\delta^{13}C$ signatures suggest that they do not consume the same food resources. Niche overlap is far higher in the *Synodontis* species than the claroteine species (Figures 5-6, Table 1), with standard areas of the ellipses typically larger in *Synodontis* species (Table 2; Figure 5)

. There was greater spread in terms of carbon sources in the *Synodontis* species (Figures 5-6), which exploit more littoral resources, compared to the more pelagic resources mostly exploited by the claroteine, suggesting that near shore production, for example algae, is the dominant source of carbon in this radiation. In the claroteine radiation there are different patterns in resource partitioning, with evidence of both niche partitioning and niche overlap, both within and between genera (Figures 5-6). For example, there is evidence of niche partitioning within the genus *Phyllonemus* with *P. typus* not overlapping with either of the *Phyllonemus* species with which it was collected in sympatry (*Phyllonemus* sp. C and *P. aff. brichardi*). Conversely, a high level of niche overlap was identified between *Lophiobagrus aquilus* and *L. brevispinis* despite the difference in size between these species (max size *L. aquilus* 67.04 mm compared to max size *L. brevispinis* 40.66mm in this study). In contrast to *Synodontis* most claroteine species occupy a narrow range of $\delta^{13}C$ values (Figure 6) although *Chrysichthys platycephalus*, for which many samples were collected, had a large ellipse area (Table 2).

The K values of phylogenetic signal in the claroteine radiation were 0.755 for nitrogen and 0.844 for carbon and were non-significant based on observed vs. random variance in phylogenetic contrasts (p= 0.072 and p=0.083 respectively). The claroteine phylogenetic generalized least squares regression across 1000 trees showed no significant relationships between either $\delta^{15}N$ or $\delta^{13}C$ signatures with the correlation between $\delta^{13}C$ signatures and PC2 closest to significance (mean p=0.099, SD=0.042). These results are corroborated by the phylogenetic canonical correlation analysis, which was also not significant (p=0.402, p=0.809).

## Discussion

This study placed two sympatric lacustrine catfish radiations in a robustly calibrated tree, allowing for commonalities to be inferred regarding their diversification. We showed that there is evidence of synchronous diversification across independent lineages, as reported in other LT taxa (e.g., Brown *et al.* 2010, Salzburger *et al.* 2002), likely due to abiotic factors. In general, the catfish radiations showed contrasting signals regarding morphological divergence and resource partitioning. There is evidence for eco-morphological diversification in the claroteine radiation, as evidenced by morphological divergence between genera, and evidence of both trophic niche partitioning and niche overlap within genera. In contrast, the *Synodontis* radiation showed some evidence of morphological conservatism, in which constituent species also have larger isotopic niches, suggesting these two radiations may have been shaped by different selective forces.

### *Evidence of synchronous diversification of LT catfishes*

Our findings suggest that the LT catfish radiations were diversifying at similar times, with the origin of the *Synodontis* radiation coinciding with the origin of the LT claroteine genera, and diversification events in the *Synodontis* clades contemporaneous with diversification in LT claroteines *Chrysichthys*, *Phyllonemus* and *Lophiobagrus*. That there is evidence for synchronous diversification across evolutionary divergent radiations suggests this has been driven by an abiotic factor, such as lake basin dynamics and/or historic fluctuations in lake levels due to climatic shifts. Well documented recent (<1 Ma) lake level fluctuations have been postulated to drive allopatric speciation in African Great Lake cichlids (Sturmbauer *et al.* 2001), and such mechanisms from lake basin dynamics and/or lake level fluctuations have been postulated for the rapid divergence of lineages across various clades (e.g., Brown *et al.* 2010, Marijnissen *et al.* 2006, Salzburger *et al.* 2002).

Using a two-step dating process with multiple fossil calibrations, despite limitations, provides a way of comparing radiations separated over a large stretch of evolutionary time, for which a species level phylogeny is difficult to obtain. We showed that early diversification in *Synodontis* is potentially younger, at 2.70 Ma (95% HPD: 1.67-3.94 Ma), than previous estimates using a single fossil calibration, so that the age of origin estimated here is well after the onset of full lacustrine conditions 5-6 Ma (Tiercelin and Mondeguer, 1991). For example, a mtDNA only phylogeny generated estimates between 4.2-5.5 Ma (Day and Wilkinson, 2006), while a multi-locus densely sampled phylogeny of the genus estimated 7.9 Ma (95% HPD: 5.7-10.0 Ma, Day *et al.* 2013). Early diversification in the claroteines at 5.78 Ma (95% HPD: 3.87-7.97 Ma) was more similar to a previous estimate (5.08 Ma: 95% HPD: 3.61-6.84 Ma, Peart *et al.* 2014) using a different fossil calibration and only nuclear markers. Colonisation of LT in both catfish radiations, would thus appear to be much later than the LT cichlid radiation, dated at 9.7 Ma (95% HPD: 10.1–9.1 Ma) (Ronco *et al.* 2020), although diversification within the major cichlid tribes occurred later (e.g., Day *et al.* 2008; Salzburger *et al.* 2002, Ronco *et al.* 2020).

### ***Morphological diversification and resource partitioning of LT catfish radiations***

Morphological divergence is observed in the claroteine radiation, specifically between the genera, indicating that early divergence in traits likely occurred as these lineages filled different niches, as predicted in adaptive radiation (Schluter, 2000). Dietary divergence is most pronounced in the more fusiform species, which display signatures of a more pelagic diet. Divergence along a benthic/pelagic axis is often identified in adaptive radiations, as observed in East African cichlids (Cooper *et al.* 2010) and notothenioid icefishes (Wilson *et al.* 2013), and in examples of parallel speciation, such as three-spined sticklebacks (Willacker et al. 2010). Although most claroteine species occupy a narrow range of $\delta^{13}$C values (Figure 5), different patterns in resource partitioning in this radiation are observed i.e., both niche differentiation and niche overlap (Figures 4-5). Different patterns in trophic niche occupancy (as examined using stable isotopes) are also a feature of other LT radiations, such as cichlids (e.g., Muschick *et al.* 2012; Sturmbauer *et al.* 1992), and smaller scale radiations, such as *Platythelphusa* crabs (Marijnissen *et al.* 2008). While different genera were separated in morphospace, there was evidence that some distantly and closely related taxa overlapped in isotopic niche space. This suggests that morphological similarity is not explained simply by recent ecological pressures but that phylogenetic history within the LT radiation is important. This pattern holds when looking at species with similar characteristics such as the small-bodied *Lophiobagrus brevispinis* and *Phyllonemus* sp. C that overlap in isotopic niche (Table 1), but not in morphospace.

In contrast to the claroteine radiation, *Synodontis* taxa are much more similar morphologically and are more closely packed in morphospace. This is perhaps unsurprising given that they are suggested to be Müllerian mimics, where shared warning patterns across the endemic LT species are likely due to a selective influence, such as predation (Wright, 2011) and similarly, the conserved morphological forms may constitute a selective advantage. Certainly, constraints on morphology related to predator pressure may limit the ability of these species to specialise with respect to feeding. Our findings indicate that *Synodontis* in general showed greater trophic niche overlap than the claroteine, with typically larger isotopic niches (Figure 4). Despite these overlaps there is a range of ~1 trophic level between the ellipses in terms of $\delta^{15}$N for *Synodontis*. A comparison of gut contents of four *Synodontis* species (Day, unpub.) identified algae dominated diets of three closely related species (*S. petricola*, *S. lucipinnis* and *S. polli*) (see Figure S2 for corresponding clade names). These taxa showed a significant level of overlap but differed from the evolutionary divergent *S. multipunctatus* that was primarily insectivorous (although also consumes some algae), which explains the difference in trophic level identified from the staple isotope data. Further sampling of additional species would be needed to determine if diets across the LT radiation are conserved (along with seasonal data), lending support to this hypothesis.

The littoral habitat type, where the study fishes were sampled, comprises an exceptionally high diversity of species in the African rift lakes. Species from taxonomically unrelated adaptive radiations also overlap in ecological niche space within a radiation, including LT cichlids (Muschick *et al.* 2012) LT *Platythelphusa* crabs (Marijnissen *et al.* 2008), and Lake Malawi cichlids (Genner *et al.* 1999a). However, overlap of isotopic niches does not necessarily imply direct competition between species. For example, LT shrimp eating cichlids species consume overlapping size classes of shrimps (which would be predicted to cause identical isotopic signatures) but partitioning occurs via different capturing techniques (Yuma *et al.* 1998). Similarly, near identical isotopic niche space was found in two algivorous sympatric *Alcolapia* cichlids from Lake Natron, but which display differing foraging behaviours (Ford *et al.* 2016). In addition, facultative commensalisms are also prevalent in cichlids (Hori *et al.* 1993) that may also facilitate coexistence. Claroteine and *Synodontis* catfishes are thought to forage at different times of day, as the former are nocturnal and the latter diurnal (Sato, 1986), and therefore they may also not be in direct competition. Differences in $\delta^{13}$C signatures suggest that in general, they are not consuming the same food resources, however, further investigation into habitat partitioning and diet is warranted to determine this. Fine scale patterns including depth segregation have been shown to segregate LT *Platythelphusa* crabs (Marijnissen et al., 2008) and LT herbivorous cichlids of the same ecomorph (Hata and Ochi, 2016), and this has been hypothesised to lead to habitat segregation in LT *Chrysichthys* (Coulter, 1991), along with segregation by substrate type, although our sampling did not allow us to test this here.

### ***Is there evidence that the LT catfish radiations are adaptive?***

To identify adaptive radiation Schluter (2000) proposed four tests: recent common ancestry, phenotype-environmental correlation, trait utility, and rapid speciation. Although we and others find evidence of recent common ancestry in both LT catfish radiations (e.g. Day and Wilkinson, 2006; Day *et al.* 2009; Koblmüller *et al.* 2006; Peart *et al*. 2014; Day *et al.* 2013), there does not appear to be any evidence for rapid diversification at least for *Synodontis*. In a previous study of the family Mochokidae, including dense sampling, no speed up in rate was identified for the LT *Synodontis* (Day *et al.* 2023), although the radiation was estimated to be older. A similar study is needed to fully investigate the claroteine radiation as the gamma statistic, while negative was non-significant.

In radiations that are adaptive, taxa derived from a single common ancestor should also display morphological and/or physiological traits associated with the exploitation of differing resources (Schluter, 2000). There is evidence that the claroteine radiation is adaptive in this regard, specifically a lack of significant overlap in morphology between genera. There is also divergence in carbon and nitrogen isotope signatures (a proxy for trophic niche) both between and within genera, although a significant overlap in isotopic signatures is also identified between some species. Despite several axes (PC1 and PC2) displaying lower relative subclade disparity early in the radiation in the DTT analysis (Figure 4), our data was indistinguishable from Brownian Motion, which was also supported by the evolutionary models. Lower relative subclade disparity than expected under the null model at the beginning of a radiation (i.e., an early burst) is considered to be a signal of adaptive radiation (Harmon *et al.* 2003) and is observed in some empirical studies e.g., Neotropical cichlids (López-Fernández *et al.* 2013) and Madagascan vangas (Jønsson *et al.* 2012). However, DTT analyses have low power for smaller sample sizes (e.g., 10-20 species) as demonstrated by Murrell (2018, Fig. 3), which is an issue when studying small scale radiations as is the case for the LT catfish radiations. This is likely why even in classic cases of adaptive radiation such as Darwin’s finches (18 species), an early burst signature is not reported (Harmon *et al.* 2009).

We were also unable to demonstrate a correlation between morphology and ecology, despite significant differences in morphology and for some isotope signatures in LT claroteine catfishes. This does not necessarily rule out the possibility that this radiation is adaptive under this criterion. In our examples, it could be that the PC axes used to quantify morphology do not correlate with changes in either $\delta^{15}$N or

$\delta^{13}$C signatures. For example, barbel traits were not included here, but these features are important in detecting food, among other functions (Diogo *et al.* 2003), as are gill rakers, which have been shown to correlate with habitat in whitefishes for example (Bernatchez *et al.* 1996). In other lacustrine radiations, species feed opportunistically on a greater range of resources than those predicted by their specialised trophic morphologies (Genner *et al.* 1999b; Liem and Osse, 1975; Liem, 1980), suggesting that adaptive trophic specialisations can evolve without the exclusion of feeding generally on common resources. We also note that nitrogen stable isotope values can be affected in areas of human disturbance, as shown in the northern LT Basin (Britton *et al.* 2019), although we ensured reasonable levels of intra-specific sampling and baselines in our study to avoid such biases.

In contrast to the claroteines, the *Synodontis* radiation has greater overlap between taxa both in morphology and isotope signatures indicating niche conservatism and suggesting that different selective forces maybe driving these radiations. Not all radiations are adaptive (Kozak, *et al.* 2006; Wellenreuther and Sánchez-Guillén, 2015) and even some subclades of cichlids show no obvious ecological and morphological diversification (Sturmbauer and Meyer, 1992), although, ruling out ecological speciation is not easy (Matsubayashi and Yamaguchi, 2021). That *Synodontis* are Müllerian mimics (Wright, 2011) is supported here, both by their conserved morphology and by the general lack of differentiation in resource use in most of the species we studied, suggesting that sharing the costs of predator education is likely out-weighing competition for similar resources (Rowland *et al.* 2007). These findings appear to contrast to Amazonian *Corydoras* catfishes, where co-mimics of unrelated species typically differ in morphology (snout length and body size) and resource use (stable nitrogen isotopes), whereas closely related species are less likely to be co-mimics but are conserved in their eco-morphology (Alexandrou *et al.* 2011). Links between mimicry and microhabitat use have been identified in a group of closely related ithomiine butterflies, indicating that mimicry can cause ecological speciation (Elias *et al.* 2008). This could also be the case with *Synodontis,* for example, depth segregation is documented in herbivorous LT cichlids (Hata and Ochi, 2016), and this could also explain how co-mimics are exploiting similar resources.

## Conclusion

This study compared two independent LT catfish radiations to elucidate if there is an adaptive signal. Our findings indicated that the LT *Synodontis* radiation is younger than the claroteine radiation and is more equivalent in age to the different genera within the claroteines. That simultaneous diversification has occurred across divergent radiations suggests this has been driven by an abiotic factor, such as basin dynamics and/or fluctuations in lake levels via arid climatic periods as has been suggested in other taxa (e.g. Brown *et al.* 2010, Marijnissen *et al.* 2008, Salzburger *et al.* 2002). There is some evidence that the claroteine radiation is adaptive, as we showed a lack of overlap in morphology, notably between genera, and some divergence in diet both between and within genera, although significant overlap in isotopic signatures is observed between some species. In contrast, *Synodontis* showed greater overlap between taxa both in morphology and isotope signatures possibly indicating niche conservatism, suggesting that different selective forces maybe driving these radiations. Future work should consider detailed geometric morphometric analyses from CT scans, specifically of the head, and metabarcoding/metagenomics of gut contents, which may help to explicitly test for a correlation between trophic morphology and ecology at a finer scale, along with investigation of microhabitats e.g. depth segregation. Genomic-scale data would also enable more accurate molecular dating of these radiations, and test if hybridization has played a role in the diversification of these clades, as reported in other adaptive radiations (e.g. Meier *et al.* 2017; Irisarri *et al.* 2018).

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## Data Availability Statement

Data associated with this study (data matrices, linear trait measurements, and isotope data) are publicly available on the UCL Research Data Repository: https://doi.org/10.5522/04/c.7355620.v1

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**Table 1.** Overlap of standard ellipse values (carbon and nitrogen data) for A) Claroteinae and B) *Synodontis* taxa from the Southern Lake Tanganyika Basin.

| **Claroteinae** | *Bathybagrus tetranema* | *Chrysichthys platycephalus* | *Chrysichthys sianenna* | *Lophiobagrus aquilus* | *Lophiobagrus brevispinis* | *Lophiobagrus cyclurus* | *Phyllonemus aff. brichardi* | *Phyllonemus sp.* C | *Phyllonemus typus* |
|---|---|---|---|---|---|---|---|---|---|
| *Bathybagrus tetranema* | X | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Chrysichthys platycephalus* | | X | 0.000 | 0.000 | 0.363 | 0.000 | 0.208 | 0.869 | 0.000 |
| *Chrysichthys sianenna* | | | X | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| *Lophiobagrus aquilus* | | | | X | 0.969 | 0.508 | 0.101 | 0.000 | 0.751 |
| *Lophiobagrus brevispinis* | | | | | X | 0.237 | 0.804 | 0.367 | 0.000 |
| *Lophiobagrus cyclurus* | | | | | | X | 0.000 | 0.000 | 0.000 |
| *Phyllonemus aff. brichardi* | | | | | | | X | 0.310 | 0.000 |
| *Phyllonemus sp.* C | | | | | | | | X | 0.000 |
| *Phyllonemus typus* | | | | | | | | | X |

| ***Synodontis*** | Clade 3 | Clade 4 | Clade 5 | Clade 6 | *S. multipunctatus* |
|---|---|---|---|---|---|
| Clade 3 | X | 0.439 | 1.455 | 0.000 | 0.000 |
| Clade 4 | | X | 1.235 | 2.851 | 0.570 |
| Clade 5 | | | X | 0.065 | 0.267 |
| Clade 6 | | | | X | 0.000 |
| *S. multipunctatus* | | | | | X |

**Table 2.** Standard area of ellipses using both carbon and nitrogen isotope data for Claroteinae and *Synodontis* (grey shading) taxa from the Southern Lake Tanganyika Basin.

| **Taxon** | **Standard area of ellipse** |
|---|---|
| *Bathybagrus tetranema* | 0.09 |
| *Chrysichthys platycephalus* | 4.12 |
| *Chrysichthys. sianenna* | 0.28 |
| *Lophiobagrus aquilus* | 2.75 |
| *Lophiobagrus brevispinis* | 2.62 |
| *Phyllonemus. aff. brichardi* | 1.26 |
| *Phyllonemus* sp. C | 1.23 |
| *Phyllonemus typus* | 1.68 |
| Clade 3 | 1.70 |
| Clade 4 | 10.30 |
| Clade 5 | 4.58 |
| Clade 6 | 3.95 |
| *Synodontis multipunctatus* | 4.98 |

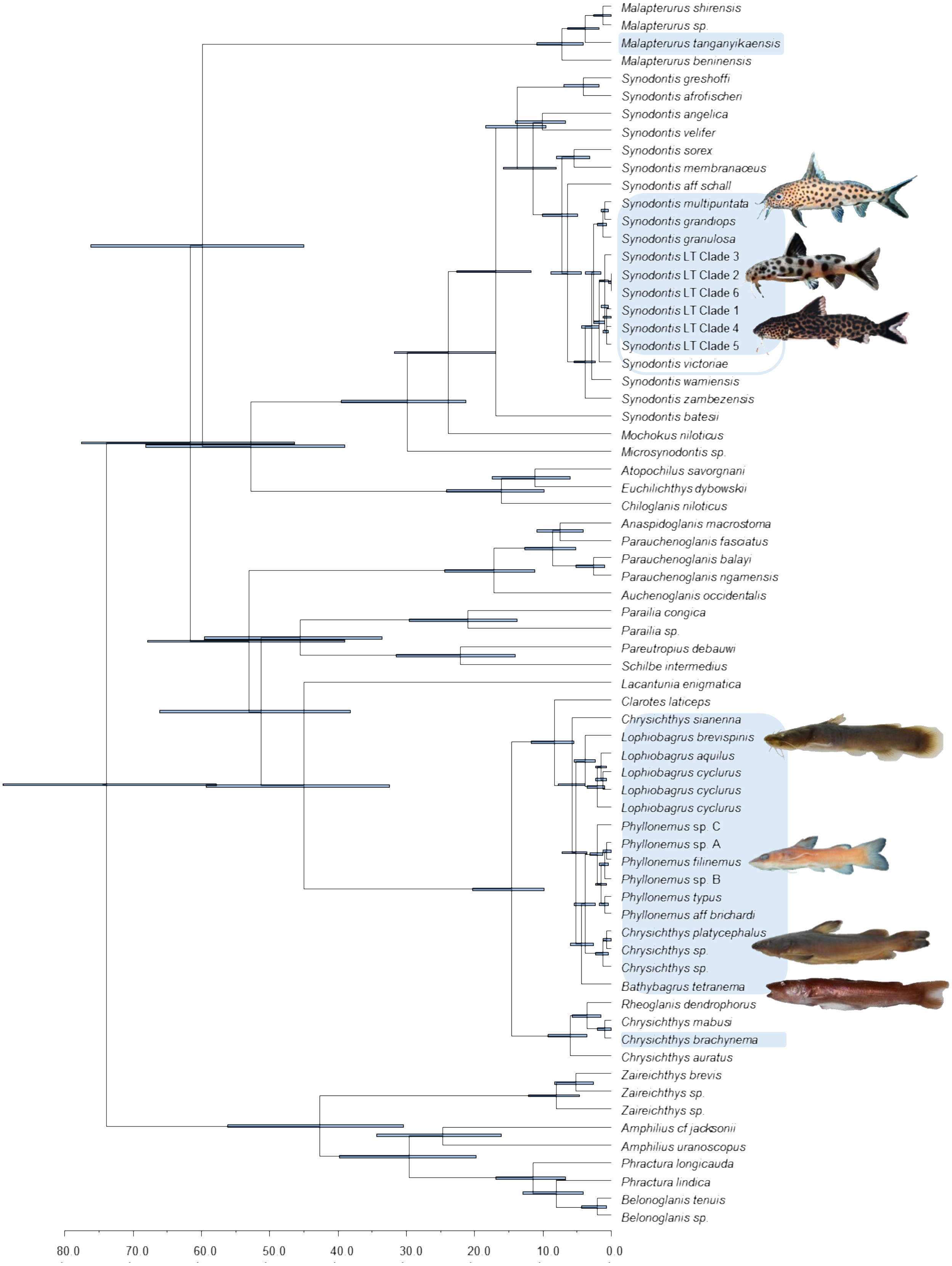

Malapterurus shirensis
Malapterurus sp.
Malapterurus tanganyikaensis
Malapterurus beninensis
Synodontis greshoffi
Synodontis afrofischeri
Synodontis angelica
Synodontis velifer
Synodontis sorex
Synodontis membranaceus
Synodontis aff schall
Synodontis multipuntata
Synodontis grandiops
Synodontis granulosa
Synodontis LT Clade 3
Synodontis LT Clade 2
Synodontis LT Clade 6
Synodontis LT Clade 1
Synodontis LT Clade 4
Synodontis LT Clade 5
Synodontis victoriae
Synodontis wamiensis
Synodontis zambezensis
Synodontis batesii
Mochokus niloticus
Microsynodontis sp.
Atopochilus savorgnani
Euchilichthys dybowskii
Chiloglanis niloticus
Anaspidoglanis macrostoma
Parauchenoglanis fasciatus
Parauchenoglanis balayi
Parauchenoglanis ngamensis
Auchenoglanis occidentalis
Parailia congica
Parailia sp.
Pareutropius debauwi
Schilbe intermedius
Lacantunia enigmatica
Clarotes laticeps
Chrysichthys sianenna
Lophiobagrus brevispinis
Lophiobagrus aquilus
Lophiobagrus cyclurus
Lophiobagrus cyclurus
Lophiobagrus cyclurus
Phyllonemus sp. C
Phyllonemus sp. A
Phyllonemus filinemus
Phyllonemus sp. B
Phyllonemus typus
Phyllonemus aff brichardi
Chrysichthys platycephalus
Chrysichthys sp.
Chrysichthys sp.
Bathybagrus tetranema
Rheoglanis dendrophorus
Chrysichthys mabusi
Chrysichthys brachynema
Chrysichthys auratus
Zaireichthys brevis
Zaireichthys sp.
Zaireichthys sp.
Amphilius cf jacksonii
Amphilius uranoscopus
Phractura longicauda
Phractura lindica
Belonoglanis tenuis
Belonoglanis sp.
80.0
70.0
60.0
50.0
40.0
30.0
20.0
10.0
0.0

**Figure 1**. Molecular dating (BEAST) analysis for the ‘Big Africa’ tree using both nuclear and mitochondrial markers. Scale bar is in Millions of years (Ma). Node bars represent 95% confidence intervals around the node ages.

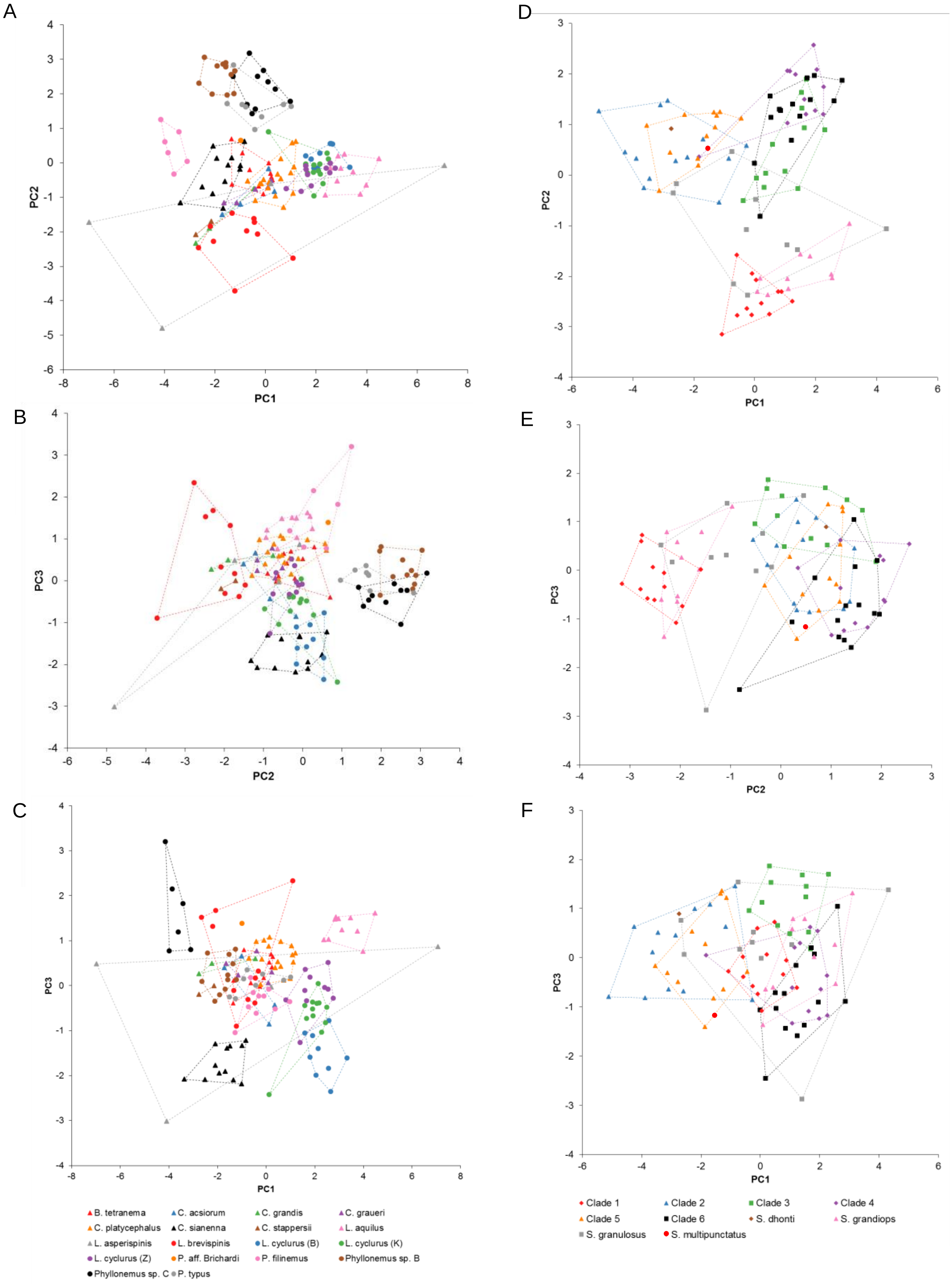
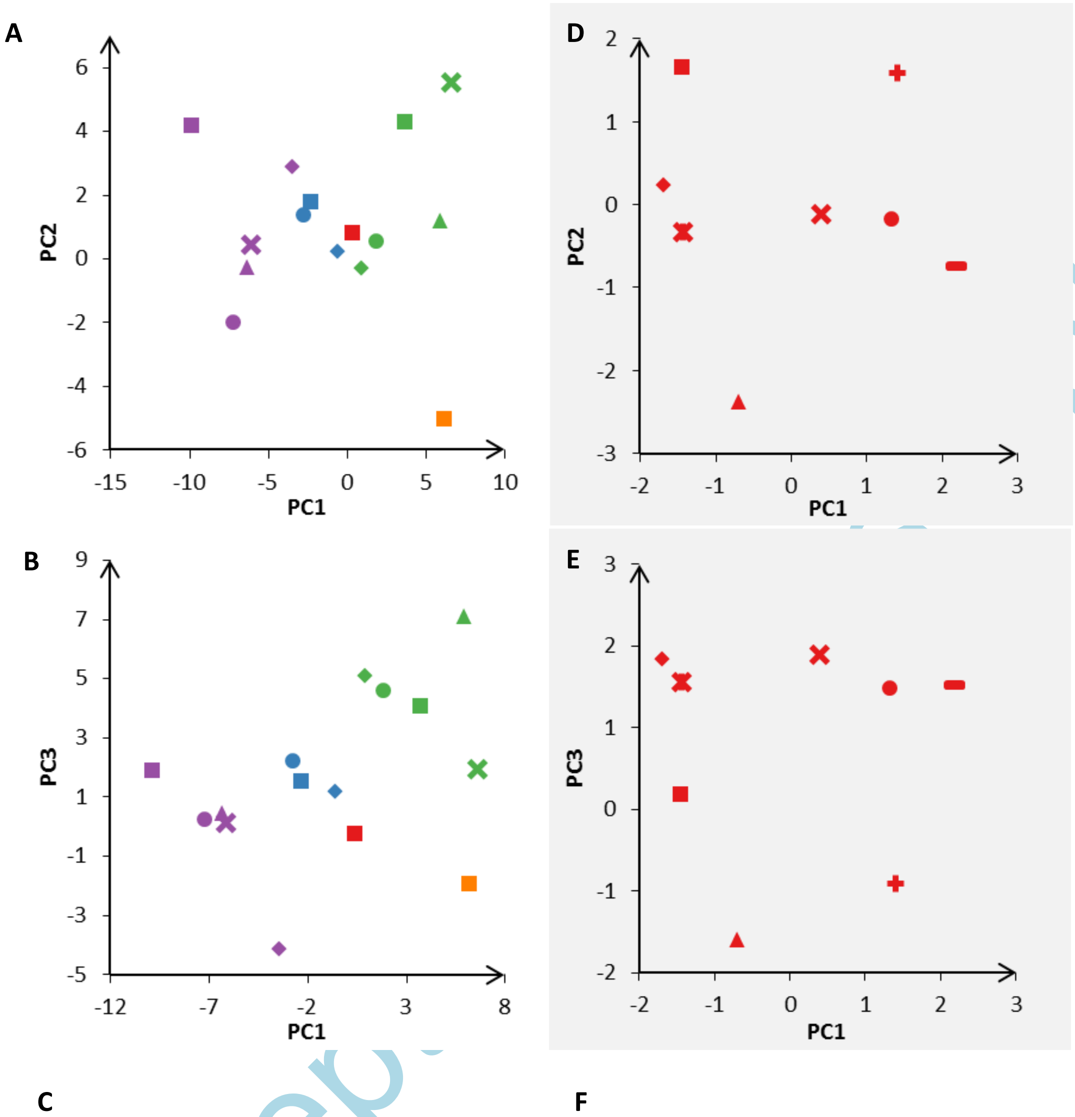

A
PC1
PC2
B
PC2
PC3
C
PC1
PC3
D
PC1
PC2
E
PC2
PC3
F
PC1
PC3
▲ B. tetranema
▲ C. acsiorum
▲ C. grandis
▲ C. graueri
▲ C. platycephalus
▲ C. sianenna
▲ C. stappersii
▲ L. aquilus
▲ L. asperispinis
● L. brevispinis
● L. cyclurus (B)
● L. cyclurus (K)
● L. cyclurus (Z)
● P. aff. Brichardi
● P. filinemus
● Phyllonemus sp. B
● Phyllonemus sp. C
● P. typus
◆ Clade 1
▲ Clade 2
■ Clade 3
◆ Clade 4
▲ Clade 5
■ Clade 6
◆ S. dhonti
▲ S. grandiops
■ S. granulosus
● S. multipunctatus

**Figure 2**. Principal Component Analysis (PCA) plots for all individuals showing the first three axes (A-C) for the claroteine and (D-F) for *Synodontis* radiations, with legends shown below the plots. Convex hulls are drawn for taxa with more than two specimens.


A
6
4
2
0
-2
-4
-6
PC2
-15
-10
-5
0
5
10
PC1
D
2
1
0
-1
-2
-3
PC2
-2
-1
0
1
2
3
PC1
B
9
7
5
3
1
-1
-3
-5
PC3
-12
-7
-2
3
8
PC1
E
3
2
1
0
-1
-2
PC3
-2
-1
0
1
2
3
PC1
C
F

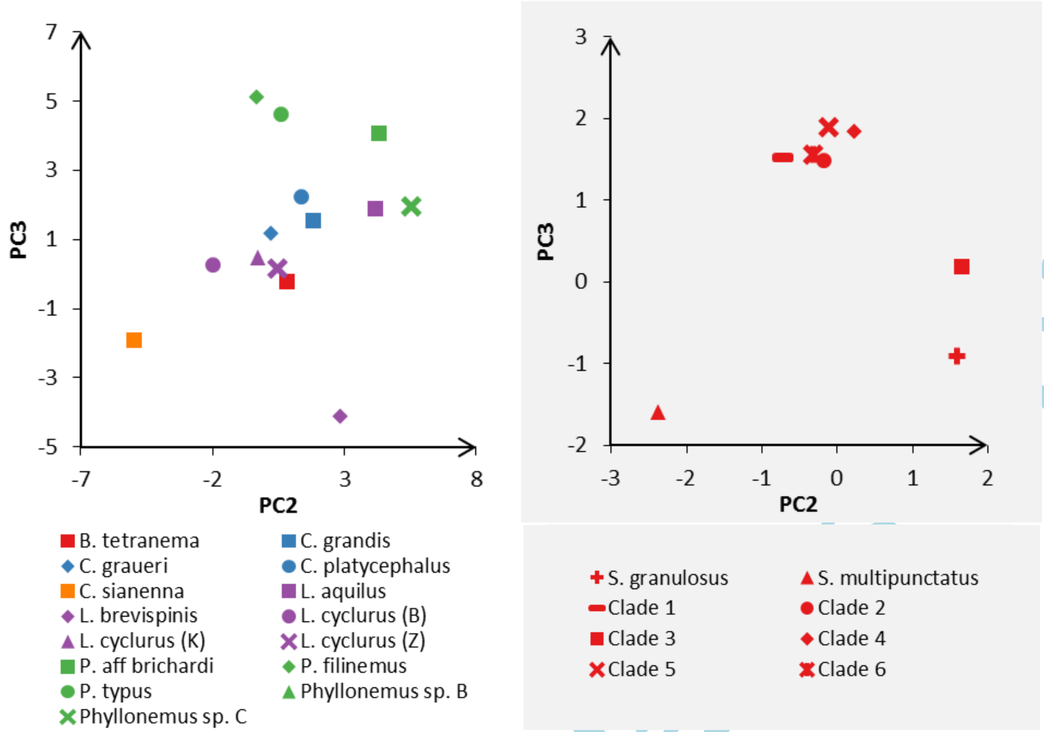


**Figure 3**. Phylogenetically corrected Principal Component Analysis (pPCA) plots showing the first three axes for the claroteine (A-C) and *Synodontis* (D-F, grey background) radiations, with corresponding legends shown below the plots.

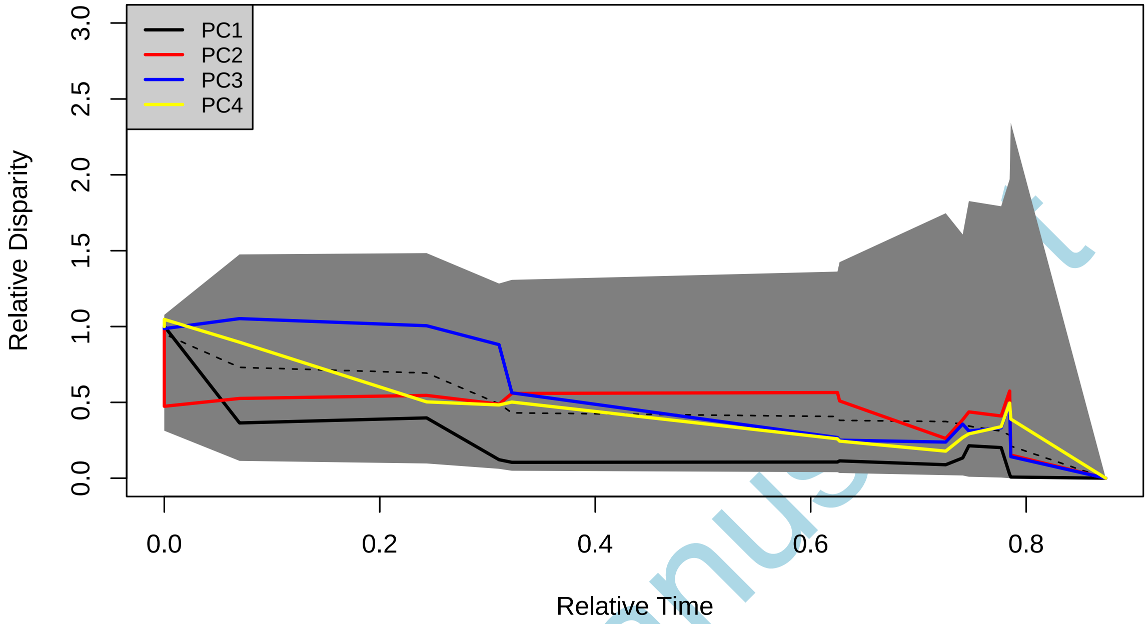


**Figure 4.** Disparity through time (DDT) plot using the rank envelope method for the LT claroteine radiation based on the MCC tree. Disparity profiles are indicated by solid lines (PC1-4), dashed line is predicted disparity under Brownian motion, and 95% confidence intervals from 5,000 simulations by the grey polygon. The relative time axis shows the proportion of time from the origin of the radiation (0) to the present.

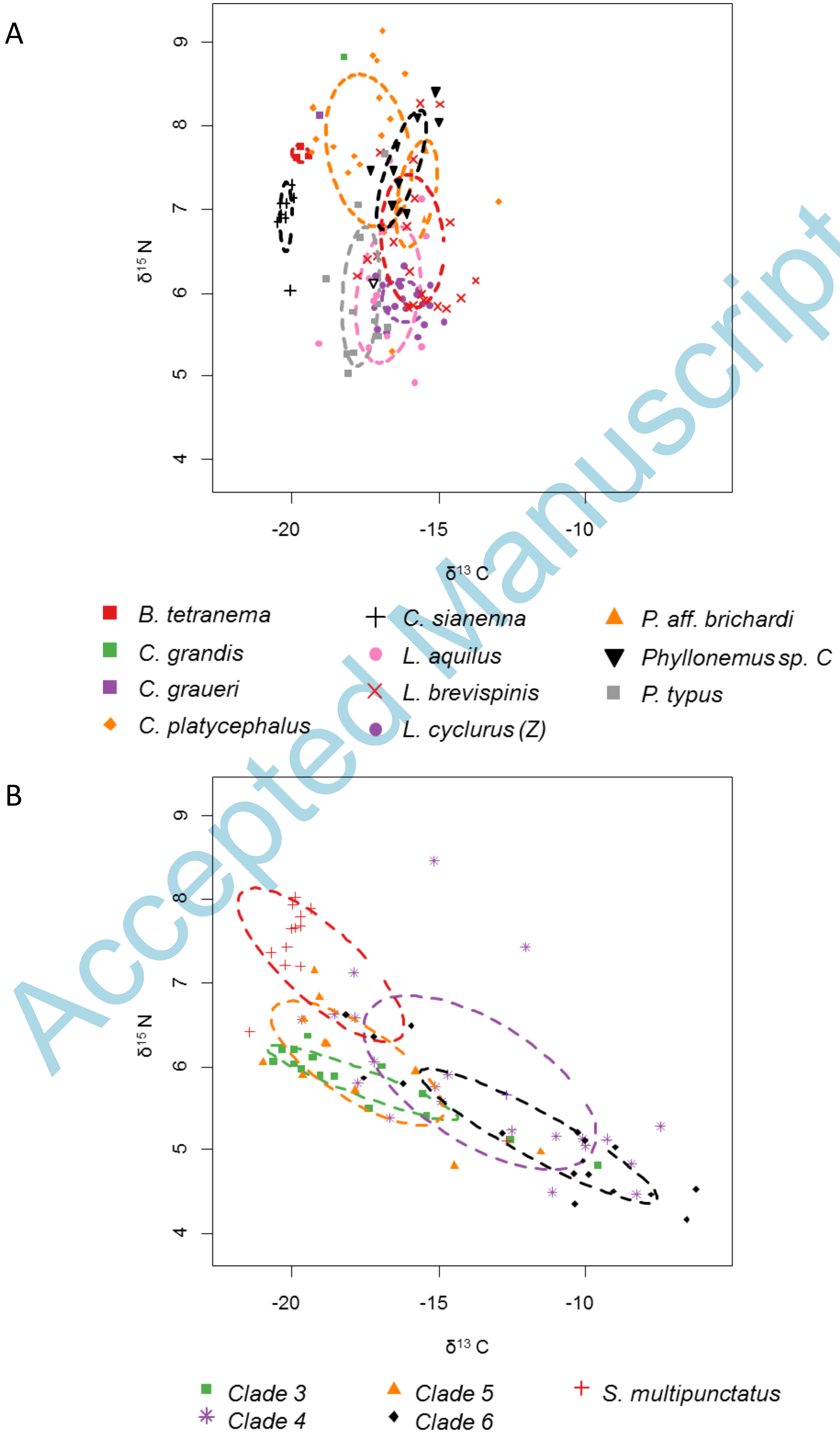
A
9
8
7
6
5
4
δ15 N
-20
-15
-10
δ13 C
B. tetranema
C. grandis
C. graueri
C. platycephalus
C. sianenna
L. aquilus
L. brevispinis
L. cyclurus (Z)
P. aff. brichardi
Phyllonemus sp. C
P. typus
B
9
8
7
6
5
4
δ15 N
-20
-15
-10
δ13 C
Clade 3
Clade 4
Clade 5
Clade 6
S. multipunctatus

**Figure 5.** Scatter plots of stable isotope values for the A) claroteines and B) *Synodontis* from the southern Lake Tanganyika basin. Ellipses show the standard ellipse area of each species.

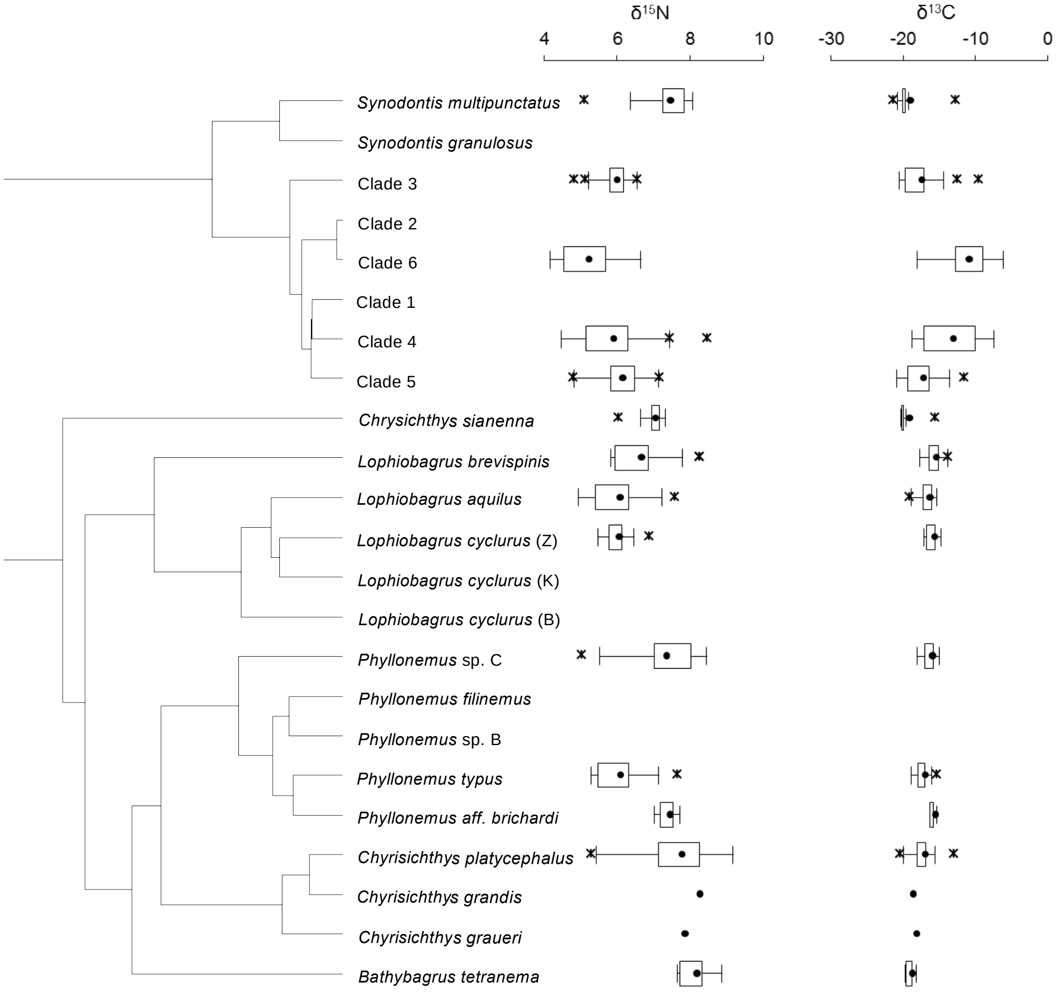


**Figure 6.** Phylogenetic tree of both the claroteine and *Synodontis* Lake Tanganyika (LT) clades (taken from Fig.1) showing the carbon and nitrogen values for each species sampled in the southern LT basin (Zambia). Dots show the mean value and crosses show outliers greater than 1.5 x interquartile range.