Supporting Information

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

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Taxonomic rationale of included taxa

Claroteinae

Within the claroteine radiation there is taxonomic uncertainty over the species status of *Chrysichthys grandis* and *Chrysichthys graueri* with conflicting designations in different taxonomic keys (Bailey and Stewart, 1984; Hardman, 2008), and both species were synonymised in the catalogue of the Royal Museum of Central Africa, Tervuren. Following the taxonomy of Bailey and Stewart (1984) specimens with a longer lower jaw are assigned to *Chrysichthys platycephalus* whereas following the key of Hardman (2008) a longer lower jaw is a feature of *C. graueri*. It is worth noting that neither of these studies examined the type specimens of *C. graueri* and Hardman cites no specimens at all. Peart et al., (2014) included tissues from one specimen with a longer lower jaw (CUMV95204) which resolved within *C. platycephalus* (which have jaws of equal length) in an analysis based on nuclear and mitochondrial genes. Therefore, in this study specimens with a longer lower jaw are classed as *C. platycephalus*. There are two *Chrysichthys* clades with longer upper jaws in the molecular phylogeny (Peart et al., 2014) including CUMV95203 which has been measured. This specimen is accessioned as *Chrysichthys acsiorum* and has the small teeth of this species, however, it does not fit the other measurements used to describe *C. acsiorum*. The ratios of different measurements, however, do fit *C. graueri* as described in Bailey and Stewart. As such in this study the key of Bailey and Stewart (1984) is used along with the addition of the more recently described species *C. acsiorum* from Hardman (2008).

In the genus *Phyllonemus* there is additional diversity that has not yet been formally described (Bills, unpub.), with five species supported by Bayesian species delimitation methods (Peart et al., 2014). Of the two putative species that were not supported, this study excludes *Phyllonemus* sp. A, which had very low support in the species delimitation analysis (range from 0.25-0.32), but includes *Phyllonemus* sp. D (support from 0.8-0.9) in an attempt to include the most recent divergences. In addition, samples of *L. cyclurus* from multiple localities are included (from Burundi, Kigoma in Tanzania and Zambia) as these show genetic divergence (Peart et al. 2018). These taxa are included in order to encompass as much on-going divergence in the radiation as possible.

Synodontis, including the use of clade names

The taxonomic key for the LT *Synodontis* radiation Wright and Page (2006) was published after the first molecular phylogenies (Day and Wilkinson, 2006; Koblmüller et al., 2006) and described a further three species. This key provides some useful diagnostic features, including for the first time, the axillary pore which has not previously been tested to assess its use as an informative character. However, molecular phylogenies suggest that some of the features in the key are not sufficient to diagnose species. Molecular data from specimens used in the key are rare. However, CUMV88758 and BMNH 2005.9.26.18[1] are both in the Wright and Page, (2006) key as *S. polli* and are included in a phylogeny constructed using Cytochrome b (*Cytb*) sequences (Figure S2). In this phylogeny, these specimens resolve in distinct clades and within the LT clade are not closely related. The key separates *S. petricola* and *S. lucipinnis* by the difference between a very small and absent axillary pore and the absence/presence of light patches at the base of black triangles on rayed fins. In the *Cytb* phylogeny the clade that contained specimens with these light patches also included specimens without them suggesting that this character may not be useful in species diagnostics or may be affected by preservation. In contrast the specimens which key out as *S. petricola* or *S. lucipinnis* cluster by sampling location, in either the northern or southern basin. In addition, white colour spines and papillae shape are also used as diagnostic features but the colours can fade during preservation and some papillae shapes are delicate and do not preserve well. These difficulties in accurately identifying specimens suggest that the LT *Synodontis* key requires refinement and there is a problem in relating specimens from museum collections to the taxonomy suggested by the molecular phylogeny.

Several *Synodontis* species are very distinctive (*S. granulosus*, *S. multipunctatus*) and so can be accurately identified from museum collections. However, for the other species, this study has included only specimens for which *Cytb* sequences were available (32 from GenBank, 106 generated for this study, Table S2b) and investigated clades in this phylogeny rather than named species (Figure S1). *Cytb* sequences were used as this marker has been found to provide resolution in this genus (Day and Wilkinson, 2006; Day et al., 2009; Koblmüller et al., 2006) and to date there has been no evidence of nuclear-mitochondrial discordance in this group (Day et al.,

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[1] referred to in Wright and Page (2006) as BMNH 2005.9.26.17-18, however BMNH 2005.9.26.17 using the same key is the morphologically distinct *S. irascae.*

2009). The only *S. grandiops* specimens for which *Cytb* sequences are available is CUMV91902 which resolves within *S. multipunctatus*, however, this specimen was not measured for this study so its identity could not be established. Due to this, this specimen was not used to place *S. grandiops* in the molecular phylogeny, but this species was still included (using measurements from the type series) in the non-phylogenetically corrected analyses. All of the *S. multipunctatus* specimens used in this study do not conform to the description of the more recently described *S. grandiops* (Wright and Page, 2006).

Calibrations used in the Ostariophysian phylogeny

The fossil genus †*Rubiesichthys* (Poyato-Ariza, 1996), which resolves in a clade with the genus *Chanos* in an analysis based on morphological characters (Poyato-Ariza et al., 2010), was used as a stem *Chanos* calibration. This calibration was applied as a lognormal prior, mean = 1.51 and SD = 0.8 which gives 133.9 Ma as the minimal age offset and 150.8 Ma as the 95% soft upper bound. In Novel et al., (2012) this calibration was used to date the most recent common ancestor (MRCA) of *Chanos* and *Cromeria*. In our analysis there is increased taxon sampling from the Gonorynchiformes with the genera *Chanos*, *Cromeria*, *Grasseichthys*, *Gonorynchus*, *Kneria*, *Parakneria*, and *Phractolaemus* included. There is conflict in the placement of the family Gonorynchidae between molecular (*Gonorynchus* sister to a clade containing *Chanos*, *Cromeria*, *Grasseichthys*, *Parakneria* and *Kneria*) (Lavoué et al., 2005) and morphological analyses (Gonorynchidae and Kneriidae in a clade sister to Chanidae) (Poyato-Ariza et al., 2010). In this analysis the calibration prior was applied to the MRCA *Chanos*, *Cromeria*, *Grasseichthys*, *Kneria*, *Parakneria*, and *Phractolaemus* with no monophyly constraint. This allows the calibration to represent a stem *Chanos* lineage in both topology hypotheses. The fossil †*Astephus* (Lundberg, 1975) which resolves as sister to the Ictaluridae in phylogenetic analyses based on morphological characters (Lundberg, 1992) is used to date the MRCA of Ictaluridae (*Ameiurus, Ictalurus, Noturus, Pylodictis*) in this analysis and *Cranoglanis*. The calibration prior was applied with a lognormal prior, mean = 1.135 and SD = 0.8 leading to 59.0 Ma as the minimal age offset and 70.6 Ma as the 95% soft upper bound. The fossil genus †*Amyzon* (Bruner, 1991; Wilson, 1993) resolves as sister to a clade containing *Ictiobus* and *Carpiodes* (Smith, 1992). This genus was used to date the MRCA of the Ictiobinae (*Ictiobus* and *Carpiodes*) and its sister clade containing *Catostomus*, *Erimyzon*, *Hypentelium* and *Moxostoma*. A lognormal prior, mean = 0.764 and SD = 0.8 was used to set 49.4 Ma as the minimal age offset and 57.0 Ma as the 95% soft upper bound.

Two additional calibrations were also used, *Ameiurus pectinatus* (Lundberg, 1975) was used as a stem lineage calibration for the genus *Ameiurus* (*A. natalis* and *A. nebulosus*) using the include stem option in BEAST with the lognormal prior, mean=1.9 and SD=0.8 with 34.1 Ma as the minimum offset and 59.03 Ma as the 95% soft upper bound. The upper bound corresponds to the minimal age offset used in the calibration of stem Ictaluridae. This fossil was described from the Oligocene Florissant Lake Beds in Colorado, USA. It is assigned to *Ameiurus* based on the broad snout and premaxillae, and the shape of the anteroventral crest of the dentary which is prominent and extends to the symphysis. It is considered to lie Novel the base of *Ameiurus* because the proximal posterior dentations of the pectoral spine arise from the posterior groove which is found in living *Ictalurus*. Other species of *Ameiurus* have these proximal dentations attached to the dorsal half of the spine shaft (Lundberg, 1975).

The fossil kneriid, †*Mahengichthys singidaensis* (Davis et al., 2013) was also used as a calibration. This fossil was collected from the Mahenge deposits in Tanzania which based on recovered fish fossils were assigned a Paleogene (possibly Oligocene) age (Greenwood and Patterson, 1967). A zircon crystal hypothesized to be from the eruption that created the lake has been dated using a ^{206}Pb/^{238}U age of 45.83± 0.17 Ma (Harrison et al., 2001) leading to estimates of the age of the fossils at 45-46 Ma. The fossil †*Mahengichthys singidaensis* is resolved as sister to the genus *Kneria* within the tribe Kneriini using a morphological matrix and using a combined morphological and mitogenome matrix (Davis et al., 2013). Synapomorphies that support the placement of this fossil within the tribe Kneriini (extant genera *Kneria* and *Parakneria*) include the shape of the opercular bones in lateral view (squarish or square), the first six anterior epicentral bones being highly modified and larger than the posterior ones, and the lateral line not piercing the supracleithrum. This calibration is applied with the lognormal prior mean = 2.1 and SD = 1.22 with an offset of 46 Ma and 109.1 Ma as the 95% soft upper bound.

In addition to the dating constraints, topological constraints were applied to this phylogeny to aid convergence. The Ostariophysians, Gonorynchiformes, Otophysi, Gymnotiformes, Cypriniformes, Characiformes and Siluriformes were each constrained to be monophyletic. The root of the phylogeny was constrained with a normal prior, mean = 245.5 and SD = 10.8, a wide prior that reflects the clade age in a phylogeny of teleost fishes (Novel et al., 2012).

Results

Divergence estimates - ostariophysian and 'Big Africa' phylogenies

The posterior age estimate for Siluriformes generated here (143.58 Ma: 95% HPD 120.91-163.09) is similar to a fossil calibrated mitogenome phylogeny (133.1 Ma: 95% HPD 113.95–143.98, Kappa et al. 2016), but is older than several estimates from fossil calibrated phylogenies of ray-finned fishes based on nine nuclear markers (106.1 Ma: 95% HPD 89.9-123, Novel et al., 2012) and 21 molecular markers (117 Ma, Betancur-R et al. 2013). Our age estimates along with these studies are, however, younger than an estimate based on a fossil calibrated Otophysi phylogeny built from mitogenomes (180Ma: 95% HPD 162-198, Nakatani et al., 2011). We note that *Lacantunia enigmatica*, a Mesoamerican catfish species that resolves within the 'Big Africa' phylogeny, is dated at 54.32 Ma (35.11-70.62) in the ostariophysian phylogeny in this study. Using the same sequence data for *L. enigmatica*, Lundberg et al., (2007) dated this species at 75-94 Ma. However, the claroteid calibration used in that analysis is problematic (see Peart et al. 2014), therefore the different constraints used in our study perhaps provide a more robust age estimate of its divergence from its African relatives despite the wide prior used to calibrate the root of the 'Big Africa' phylogeny (reflecting the uncertainty in the ostariophysian analysis).

References

Bailey RM, Stewart DJ. Bagrid catfishes from Lake Tanganyika, with a key and descriptions of new taxa. *Miscellaneous Publications Museum of Zoology, University of Michigan* 1984; 168**.**

Betancur-R. R, Broughton RE, Wiley EO, et al. The Tree of Life and a New Classification of Bony Fishes. *PLOS Currents Tree of Life*. 2013; Apr 18:Edition 1.

Bruner J. Comments on the genus *Amyzon* (Family Catostomidae). *Journal of Paleontology*, 1991;65:678–686.

Davis MP, Arratia G, Kaiser TM. The first fossil shellear and its implications for the evolution and divergence of the Kneriidae (Teleostei: Gonorynchiformes), In: Arriata G, Schultze, H-P, Wilson MVH. (eds.), *Mesozoic Fishes* 5*-Global Diversity and Evolution* 2013;325–362.

Day JJ, Bills R, Friel JP. Lacustrine radiations in African *Synodontis* catfish. *Journal of Evolutionary Biology* 2009;22:805–817.

Day JJ, Wilkinson M. On the origin of the *Synodontis* catfish species flock from Lake Tanganyika. *Biology Letters* 2006;2:548–552.

Greenwood PH, Patterson C. A fossil osteoglossoid fish from Tanzania (E . Africa). *Zoological Journal of the Linnean Society*, 1967;47:211–223.

Hardman M. A new species of catfish genus *Chrysichthys* from Lake Tanganyika (Siluriformes: Claroteidae). *Copeia*;2008:43.

Kappas I, Vittas S, Pantzartzi CN, et al. A Time-calibrated mitogenome phylogeny of catfish (Teleostei: Siluriformes). *PLoS ONE* 2016;11:e0166988.

Koblmüller S, Sturmbauer C, Verheyen E, et al. Mitochondrial phylogeny and phylogeography of East African squeaker catfishes (Siluriformes: *Synodontis*). *BMC Evolutionary Biology* 2006;6:49.

Lavoué S, Miya M, Inoue JG, et al. Molecular systematics of the gonorynchiform fishes (Teleostei) based on whole mitogenome sequences: implications for higher-level relationships within the Otocephala. *Molecular Phylogenetics and Evolution* 2005;37:165–177.

Lundberg JG. The fossil catfishes of North America. Claude W. Hibbard Memorial, Volume 2. *University of Michigan, Papers on Paleontology* 1975;11:1–51.

Lundberg JG. The phylogeny of ictalurid catfishes: A synthesis of recent work. In R. Mayden (Ed.), *Systematics, Historical Ecology, and North American Freshwater Fishes.* Stanford :Stanford University Press, 1992;392–420.

Lundberg JG, Sullivan JP, Rodiles-Hernández R, et al. Discovery of African roots for the Mesoamerican Chiapas catfish, *Lacantunia enigmatica*, requires an ancient intercontinental passage, 2007;156:39–53.

Nakatani M, Miya M, Mabuchi K, et al. Evolutionary history of Otophysi (Teleostei), a major clade of the modern freshwater fishes: Pangaean origin and Mesozoic radiation. *BMC Evolutionary Biology*, 2011;11:177.

Novel TJ, Eytan RI, Dornburg A, et al. Resolution of ray-finned fish phylogeny and timing of diversification. *Proceedings of the National Academy of Sciences,* 2012;109: 13698–13703.

Peart CR, Dasmahapatra KK, Day JJ. Contrasting geographic structure in evolutionarily divergent Lake Tanganyika catfishes. *Ecology and Evolution* 2018;8:2688-2697.

Peart CR, Bills R, Wilkinson M. et al. Nocturnal claroteine catfishes reveal dual colonisation but a single radiation in Lake Tanganyika. *Molecular Phylogenetics and Evolution* 2014;73:119–128.

Poyato-Ariza FJ. A revision of Rubiesichthys gregalis WENZ 1984 (Ostariophysi, Gonorynchiformes), from the Early Cretaceous of Spain. In: Arratia G, Viohl G. (eds.), *Mesozoic Fishes: Systematics and Paleoecology.* Munich: Dr. Friedrich Pfeil, 1996;319–328.

Poyato-Ariza FJ, Grande T, Diogo R. Gonorynchiform interrelationships: Historic Overview, analysis, and revised systematics of the group. In: Grande T, Poyato-Ariza FJ, Diogo R. (eds.), *Gonorynchiformes and Ostariophysan Relationships: A Comprehensive Review.* Enfield: Science Publishers, 2010; 227–338.

Smith GR. Phylogeny and biogeography of the Catostomidae, freshwater fishes of North America and Asia. In: Mayden R. (ed.), *Systematics, Historical Ecology, and North American Freshwater Fishes.* Stanford: Stanford University Press, 1992; 778–826.

Wilson M. Calibration of Eocene varves at Horsefly, British Columbia, Canada, and temporal distribution of specimens of the Eocene fish *Amyzon aggregatum*. *Kaupia Darmstaedter Beitraege Zur Naturgeschichte* 1993;2:27–38.

Wright JJ, Page LM. Taxonomic revision of Lake Tanganyikan *Synodontis* (Siluriformes: Mochokidae). *Bulletin of the Florida Museum of Natural History* 2006;46:99–154.

Table S3. List of specimens from Lake Tanganyika used in the morphological and stable isotope analyses in this study.

Species	Morphological Analysis	Stable Isotope Analysis
Claroteine		
Bathybarus tetranema	RMCA83-04-P-1_2, RMCA83-04-P-1_2, RMCA94-031-P-0026, RMCA95-098-P-0044-0050, RMCA95-098-P-0044-0050, RMCA95-098-P-0044-0050, RMCA95-098-P-0044-0050, RMCA95-098-P-0041-0043, RMCA95-098-P-0041-0043, RMCA95-098-P-0041-0043	C287, C289, C364
Chrysichthys acsiorum	AMNH236052, AMNH217411, AMNH217411, AMNH217411	
Chrysichthys grandis	CU90324, RMCA14347, RMCA94-069-P-0216-0218	C291
Chrysichthys graueri	CU95203 (JPF 1627), CU95203 (JPF 1626), RMCA96-083-P-0685-0687, RMCA128678, RMCA95-098-P-0066	C292
Chrysichthys platycephalus	CU95204 (JPF 1624), CU95204 (JPF 1625), RMCA92-081-P-0167, RMCA92-081-P-0169, RMCA63791-63792, RMCA63791-63792, RMCA44994-44996, RMCA44994-44996, RMCA95-098-P-0067-0070, RMCA95-098-P-0067-0070, RMCA83-19-P-3, RMCA91-034-P-0620, C33, C14, C56, CU88726 (213), CU88726 (203)	C105, C107, C108, C110, C131, C156, C158, C187, C189, C240, C262, C290, C293, C294, C315, C335, C345, C362, C363, C65
Chrysichthys sianenna	RMCA92-081-P-0146, RMCA92-081-P-0104, RMCA92-081-P-1659, RMCA92-081-P-1785-1800, RMCA92-081-P-1660-1667, RMCA92-081-P-1660-1667, RMCA92-081-P-1660-1667, RMCA92-081-P-1660-1667, RMCA92-081-P-1660-1667, RMCA92-081-P-1660-1667, AMNH217384, AMNH97210	C199, C201, C241, C242, C288, C314, C354, C75
Chrysichthys stappersii	RMCA90189, RMCA14236	
Lophiobagrus aquilus	C228, C311, C238, C73, C236, RMCA94-031-P-0034, RMCA83-04-P-3-7, RMCA83-04-P-3-7, C112, C66	C228, C311, C238, C73, C236, C113, C119, C211, C213, C309, C322, C326
Lophiobagrus asperispinis	RMCA14359A, RMCA14359B, RMCA92-081-P-1677, BMNH 1920-5-25-75	
Lophiobagrus brevispinis	RMCA131093, RMCA81-16-P-1-13, RMCA81-16-P-1-13, RMCA81-16-P-1-13, RMCA81-16-P-1-13, RMCA81-16-P-1-13, RMCA131093, BMNH 1983-2-8:7-10, BMNH 1983-2-8:7-10, BMNH 1983-2-8:7-10	C114, C117, C123, C125, C127, C130, C192, C214, C219, C221, C222, C232, C249, C252, C253, C254, C296, C77, C83, C85, C94, C98
Lophiobagrus cyclurus (B)	C398, C379, C408, C365, C378, C384, C383, C375, C381	
Lophiobagrus cyclurus (K)	C15, C40, C38, C9, C41, C53, C36, C4, C38, C40, C389, C10	
Lophiobagrus cyclurus (Z)	C155, C139, C149, C243, C268, C133, C173, C264, C134, C267	C155, C139, C149, C243, C268, C137, C138, C146, C148, C150, C154, C159, C210, C233, C239, C244, C245, C260, C261, C265
Phyllonemus aff. Brichardi	C111	C111, C71, C76
Phyllonemus filinemus	RMCA92-081-P-0141, RMCA92-081-P-1678, C22, C21, C6, C19, C2, C42, C26, C49	

Species	Morphological Analysis	Stable Isotope Analysis
Claroteine		
Phyllonemus sp. B	C23, C24, C44, C17, C18, C16, C20, C29, C25, C28, C45	
Phyllonemus sp. C	C61, C92, C190, C95, C115, C96	C61, C92, C190, C95, C115, C96, C118, C91, C97, C99
Phyllonemus typus	C144, C324, C145, C136, C188, C147, RMCA90250-90252, RMCA90249	C144, C324, C145, C136, C188, C147, C132, C140, C193, C194, C195, C323
Synodontis		
Clade 1	CU88758	
Clade 2	BMNH 2006.3.6.16 (5208), BMNH 2006.3.6.15(5126), BMNH 2006.3.6.18 (5046), BMNH 2006.3.6.17 (5213), S9, S14, S17, S8, S15, S12, S10, S18, S13	
Clade 3	S183, S153, S164, S166, S154, S199, S165, S167, S158, S167, BMNH 2005-9-26-3 (5148), S179	S183, S153, S164, S166, S154, S199, S165, S167, S158, S167, S182, S192, S193, S66, S73, S85
Clade 4	S78, S106, S94, S59, S81, S80, S60, S83, S103, BMNH 2005-9-26-18 (5052), BMNH 2005-9-26-2 (5100), S150	S78, S106, S94, S59, S81, S80, S60, S83, S103, S147, S67, S68, S74, S77, S79, S82, S84, S87, S91, S92, S96
Clade 5	S214, S243, S162, S159, BMNH 2006-3-6-30 (5149), BMNH 2006-3-6-29 (5145), BMNH 2006-3-6-31 (5146), BMNH 2006-3-6-32 (5147), BMNH 2007-8-29-28-30(5152), S149, S150, S149	S214, S243, S162, S159, S145, S169, S61, S75, S86, S89, S90
Clade 6	S173, S198, S172, S213, S211, S163, S197, S196, S173, S170, S161, S171, BMNH 2005-9-26-1 (5124), BMNH 2007-8-29-28-30(5153)	S173, S198, S172, S213, S211, S163, S197, S196, S173, S170, S161, S171, S151, S181, S188, S189, S194, S202
Synodontis dhonti	14344	
Synodontis grandiops	BMNH 1982-4-13-4785, BMNH 1982-4-13-4784, BMNH 1982-4-13-4789-4791 (3), BMNH 1982-4-13-4789-4791 (4), BMNH 1982-4-13-4789-4791 (1), BMNH 1982-4-13-4786, BMNH 1955-12-20-1837, BMNH 1955-12-20-1833, BMNH 1982-4-13-4787-4788 (2), BMNH 1982-4-13-4787-4788 (1)	
Synodontis granulosus	82-12-P-13-16, 82-12-P-13-16, 82-12-P-13-16, 82-12-P-13-16, 94-069-P-0289, A1-094-P-0052, 100902, 14157, BMNH 1906-9-6-40, BMNH 1936-6-15-1199-1201	
Synodontis multipunctatus	BMNH 2005-9-26-19-23, BMNH 2005-9-26-19-23, S3, S35, S2, S288, S285, S257, S282, S270, S275, S254	S160, S174, S175, S176, S177, S178, S180, S187, S205, S206, S207, S208, S221, S249

AMHN, American Museum of Natural History; BMNH, Natural History Museum, London; CUMV [CU], Cornell University Museum of Vertebrates; RMCA Royal Museum of Central Africa. Field numbers (starting with the letter C or S) and those in parenthesis denote Day lab specimens.

Table S4a. List of genetic samples and associated Genbank numbers used in the Ostariophysian and 'Big Africa' phylogenies. Novel sequences generated for this study are in bold.

Species	RAG1 Exon 3	ENC1	Plagl2	RAG2	CO1	*Cytb*
Acanthodoras cataphractus	DQ492466					
Acrochordonichthys rugosus	DQ492444					
Ageneiosus ucayalensis	DQ492463					
Ailia coila	DQ492452					
Akysis sp.	DQ492445					
Alosa pseudoharengus		**Novel**	**Novel**			
Amblyceps sp.	DQ492451					
Ameiurus natalis	**Novel**	**Novel**	**Novel**			
Ameiurus nebulosus	DQ492510					
Amphilius cf. jacksonii	**Novel**	**Novel**	**Novel**	DQ492378		
Amphilius uranoscopus	**Novel**	**Novel**	**Novel**	Novel		
Anaspidoglanis macrostoma	DQ492499			DQ492386		
Anduzedoras oxyrhynchus	**Novel**	**Novel**	**Novel**			
Apteronotus albifrons	**Novel**	**Novel**	**Novel**			
Arius felis	**Novel**	**Novel**	**Novel**			
Astroblepus sp. 1	DQ492438					
Astroblepus sp. 2	DQ492439					
Astyanax mexicanus		**Novel**	**Novel**			
Atopochilus savorgnani	DQ492493			DQ492380		
Auchenoglanis occidentalis	**Novel**	**Novel**		HG803251	HG803487	HG803403
Bagarius yarrelli	DQ492446					
Bagre marinus	DQ492524					
Bagrichthys macropterus	**Novel**	**Novel**	**Novel**			
Bagrus docmak	**Novel**	**Novel**	**Novel**			
Bagrus ubangensis	**Novel**	**Novel**	**Novel**			
Barbatula barbatula	**Novel**		**Novel**			
Batasio tigrinus	DQ492460					
Bathybagrus tetranema	DQ492502		HG803287	HG803215	HG803444	HG803360
Batrochoglanis raninus	DQ492473					
Belonoglanis sp.	**Novel**		**Novel**	**Novel**		
Belonoglanis tenuis	DQ492489			DQ492376		
Brachyplatystoma filamentosum	**Novel**	**Novel**	**Novel**			
Brycon pesu	**Novel**	**Novel**	**Novel**			
Bullockia maldonadoi	DQ492434					
Callichthys callichthys	**Novel**	**Novel**	**Novel**			
Carpiodes carpio	**Novel**		**Novel**			
Catostomus commersoni	**Novel**	**Novel**	**Novel**			
Centromochlus heckelii	DQ492465					

Species	**RAG1 Exon 3**	**ENC1**	**Plagl2**	**RAG2**	**CO1**	***Cytb***
Cephalocassis borneensis	DQ492525					
Cetopsis candiru	DQ492533					
Cetopsis coecutiens	**Novel**		**Novel**			
Chaca chaca	DQ492469					
Chaca sp.	DQ492470					
Chalceus macrolepidotus	**Novel**	**Novel**	**Novel**			
Chanos chanos	**Novel**	**Novel**	**Novel**			
Chiloglanis niloticus	**Novel**	**Novel**	**Novel**	HF565738	HF565846	HF565994
Chrysichthys auratus	**Novel**	**Novel**	HG803321	HG803250	HG803486	HG803402
Chrysichthys brachynema	**Novel**		HG803308	HG803235	HG803467	HG803383
Chrysichthys mabusi	**Novel**		HG803260	HG803188	HG803412	HG803328
Chrysichthys platycephalus	**Novel**	**Novel**	HG803282	HG803210	HG803439	HG803355
Chrysichthys sianenna	**Novel**	**Novel**	HG803286	HG803214	HG803443	HG803359
Chrysichthys sp.	**Novel**		HG803288	HG803216	HG803445	HG803361
Chrysichthys sp.	**Novel**		HG803304	HG803231	HG803461	HG803377
Citharinus congicus	**Novel**		Novel			
Clarias batrachus	DQ492521					
Clarias gabonensis	DQ492519					
Clarotes laticeps	**Novel**		HG803324	HG803255	HG803491	HG803407
Conorhynchos conirostris	DQ492477					
Corydoras aurofrenatus	**Novel**	**Novel**	**Novel**			
Corydoras cf. trilineatus	DQ492437					
Cranoglanis bouderius	**Novel**	**Novel**	**Novel**			
Cromeria nilotica	**Novel**	**Novel**	**Novel**			
Danio rerio	**Novel**	**Novel**	**Novel**			
Denticeps clupeoides	**Novel**		**Novel**			
Diplomystes nahuelbutaensis	**Novel**	**Novel**	**Novel**			
Distichodus notospilus	DQ492425					
Eigenmannia macrops	**Novel**		**Novel**			
Electrophorus electricus	**Novel**	**Novel**	**Novel**			
Erethistes sp. 1	DQ492449					
Erethistes sp. 2	DQ492450					
Erimyzon oblongus	**Novel**	**Novel**	**Novel**			
Euchilichthys dybowskii	DQ492494			DQ492381		
Farlowella cf. nattereri	DQ492441					
Galeichthys ater	**Novel**	**Novel**	**Novel**			
Galeichthys peruvianus	**Novel**	**Novel**	**Novel**			
Glyptothorax cf. trilineatus	DQ492447					
Goeldiella eques	DQ492480					

Species	RAG1 Exon 3	ENC1	Plagl2	RAG2	CO1	*Cytb*
Gogangra viridescens	DQ492448					
Gogo arcuatus	**Novel**	**Novel**	**Novel**			
Gonorynchus abbreviatus	**Novel**	**Novel**	**Novel**			
Gonorynchus greyi	**Novel**	**Novel**	**Novel**			
Grasseichthys gabonensis	**Novel**	**Novel**	**Novel**			
Gymnorhamphichthys petiti	**Novel**		**Novel**			
Gymnotus sp.	**Novel**	**Novel**	**Novel**			
Helicophagus waandersii	DQ492515					
Helogenes marmoratus	DQ492534					
Hemibagrus wyckiodes	**Novel**	**Novel**	**Novel**			
Hemisilurus moolenburghi	**Novel**	**Novel**	**Novel**			
Henonemus punctatus	DQ492432					
Heterobagrus bocourti	**Novel**	**Novel**	**Novel**			
Heterobranchus longifilis	DQ492520					
Heteropneustes fossilis	DQ492522					
Hoplias sp.	**Novel**					
Hoplomyzon sexpapilostoma	DQ492536					
Horabagrus brachysoma	DQ492454					
Hydrolycus scomberoides	**Novel**		**Novel**			
Hypentelium nigricans	**Novel**	**Novel**	**Novel**			
Hypophthalmus edentatus	DQ492474					
Ictalurus punctatus	**Novel**	**Novel**	**Novel**			
Ictiobus bubalus	**Novel**	**Novel**	**Novel**			
Imparfinis cf. cochabambae	DQ492481					
Imparfinis cf. stictonotus	DQ492483					
Imparfinis stictonotus	DQ492482					
Ketengus sp.	DQ492526					
Kneria paucisquamata	**Novel**	**Novel**	**Novel**			
Kneria ruaha	**Novel**	**Novel**	**Novel**			
Kryptopterus minor	DQ492486					
Lacantunia enigmatica	EF078914			EF078916		
Laides hexanema	DQ492453					
Lamontichthys stibaros	DQ492440					
Leiocassis poecilopterus	**Novel**	**Novel**	**Novel**			
Leporinus copelandii	**Novel**	**Novel**	**Novel**			
Leptodoras linnelli	**Novel**	**Novel**	**Novel**			
Liobagrus aequilabris	**Novel**		**Novel**			
Lophiobagrus aquilus	**Novel**	**Novel**	HG803292	HG803220	HG803449	HG803365

Species	RAG1 Exon 3	ENC1	Plagl2	RAG2	CO1	*Cytb*
Lophiobagrus brevispinis	DQ492504		HG803291	HG803219	HG803448	HG803364
Lophiobagrus cyclurus	**Novel**		HG803295	HG803223	HG803452	HG803368
Lophiobagrus cyclurus			HG803307	HG803234	HG803464	HG803380
Lophiobagrus cyclurus	**Novel**		HG803312	HG803239	HG803471	HG803387
Loricaria simillima	**Novel**		**Novel**			
Malapterurus beninensis	**Novel**	**Novel**	**Novel**	**Novel**		
Malapterurus shirensis	**Novel**	**Novel**	**Novel**	**Novel**		
Malapterurus sp.	**Novel**	**Novel**				
Malapterurus tanganyikaensis	DQ492498			DQ492385		
Micromyzon akamai	DQ492537					
Microsynodontis sp.	DQ492496			DQ492383		
Mochokus niloticus	**Novel**	**Novel**	**Novel**	HF565739	HF565847	HF565995
Moxostoma macrolepidotum		**Novel**	**Novel**			
Mystus bimaculatus	**Novel**	**Novel**	**Novel**			
Nematogenys inermis	**Novel**	**Novel**	**Novel**			
Neolebias philippei			**Novel**			
Neosilurus ater	DQ492529					
Notemigonus crysoleucas	**Novel**	**Novel**	**Novel**			
Noturus insignis	DQ492513					
Noturus stigmosus	**Novel**	**Novel**	**Novel**			
Ochmacanthus alternus	DQ492433					
Olyra longicaudata	DQ492459					
Opsariichthyus uncirostris	**Novel**	**Novel**	**Novel**			
Pangasianodon hypophthalmus	**Novel**	**Novel**	**Novel**			
Pangasius larnaudii	DQ492516					
Parailia congica	**Novel**	**Novel**	HG803269	HG803196	HG803421	
Parailia sp.	DQ492509			DQ492396		
Parakneria slekii	**Novel**	**Novel**	**Novel**			
Parakneria vilhenae	**Novel**	**Novel**	**Novel**			
Parauchenoglanis balayi	DQ492500			DQ492387		
Parauchenoglanis fasciatus	**Novel**	**Novel**		HG803252	HG803488	HG803404
Parauchenoglanis ngamensis		**Novel**	HG803262	HG803190		HG803330
Pareutropius debauwi	**Novel**	**Novel**	HG803270	HG803197	HG803422	HG803338
Phalacronotus apogon	DQ492485					
Phenacogrammus interruptus	**Novel**		**Novel**			
Phractocephalus hemi	**Novel**	**Novel**	**Novel**			
Phractolaemus ansorgii	**Novel**	**Novel**	**Novel**			
Phractura lindica		**Novel**	**Novel**	**Novel**		
Phractura longicauda	DQ492490			DQ492377		

Species	**RAG1 Exon 3**	**ENC1**	**Plagl2**	**RAG2**	**CO1**	***Cytb***
Phyllonemus aff. brichardi	**Novel**		HG803278	HG803205	HG803434	HG803350
Phyllonemus filinemus	**Novel**	**Novel**	HG803310	HG803237	HG803469	HG803385
Phyllonemus sp. A			HG803263	HG803191	HG803414	HG803331
Phyllonemus sp. B			HG803311	HG803238	HG803470	HG803386
Phyllonemus sp. C	**Novel**		HG803283	HG803211	HG803440	HG803356
Phyllonemus typus	DQ492503		HG803281	HG803209	HG803438	HG803354
Pimelodella cristata	DQ492478					
Pimelodus ornatus	DQ492475					
Plotosus lineatus	**Novel**	**Novel**	**Novel**			
Porochilus rendahli	DQ492530					
Pseudeutropius brachypopterus	DQ492455					
Pseudopimelodus bufonius	DQ492471					
Pseudopimelodus mangurus	DQ492472					
Pterobunocephalus sp.	DQ492535	**Novel**	**Novel**			
Pterocryptis anomala	DQ492487					
Pterygoplichthys multiradiatus	DQ492443					
Pygocentrus nattereri	**Novel**	**Novel**	**Novel**			
Pylodictis olivaris	**Novel**	**Novel**	**Novel**			
Rhamdia sp.	DQ492479					
Rhamphichthys sp.	**Novel**	**Novel**	**Novel**			
Rheoglanis dendrophorus	**Novel**	**Novel**	**Novel**	DQ492393		
Rita rita	DQ492518					
Schilbe intermedius	**Novel**	**Novel**	HG803277	HG803203	HG803432	HG803348
Scoloplax distolothrix	DQ492435					
Semotilus atromaculatus	**Novel**	**Novel**	**Novel**			
Sternopygus macrurus	**Novel**	**Novel**	**Novel**			
Sternopygus sp.	DQ492426					
Synodontis aff. ilebrevis	**Novel**	**Novel**	**Novel**		HF565878	DQ886644
Synodontis aff. schall	**Novel**	**Novel**	**Novel**	HF565817	HF565952	HF566067
Synodontis aff. tanganyicae	**Novel**	**Novel**	**Novel**	HF565831	HF565975	DQ886658
Synodontis afrofischeri	**Novel**	**Novel**	**Novel**	HF565744	HF565852	DQ886618
Synodontis angelica	**Novel**	**Novel**	**Novel**	**Novel**	HF565856	DQ886605
Synodontis batesii	**Novel**			HF565752	HF565862	HF566005
Synodontis grandiops		**Novel**	**Novel**		HF565890	FM878846
Synodontis granulosa	**Novel**	**Novel**	**Novel**	HF565777	HF565892	HF565777
Synodontis greshoffi	**Novel**	**Novel**	**Novel**		HF565894	HF566025
Synodontis irsacae	**Novel**	**Novel**	**Novel**	HF565767	HF565879	DQ886653
Synodontis lucipinnis	**Novel**	**Novel**	**Novel**	HF565787	HF565904	DQ886631
Synodontis membranaceus		**Novel**	**Novel**	HF565790	HF565908	HF566035
Synodontis multipuntata	**Novel**	**Novel**		HF565791	HF565910	DQ886625

Species	**RAG1 Exon 3**	**ENC1**	**Plagl2**	**RAG2**	**CO1**	***Cytb***
Synodontis petricola	**Novel**	**Novel**	**Novel**			
Synodontis polli	**Novel**	**Novel**	**Novel**	HF565809	HF565941	DQ886645
Synodontis sorex	**Novel**	**Novel**	**Novel**	HF565823	HF565960	HF566074
Synodontis velifer	**Novel**	**Novel**	**Novel**	HF565836	HF565982	HF566089
Synodontis victoriae	**Novel**	**Novel**	**Novel**	HF565837	HF565984	DQ886657
Synodontis wamiensis	**Novel**	**Novel**	**Novel**	HF565839	HF565986	HF566092
Synodontis zambezensis	**Novel**	**Novel**	**Novel**	HF565844	HF565991	FM878858
Trachelyopterus galeatus	DQ492464					
Tribolodon brandti	**Novel**	**Novel**	**Novel**			
Trichomycterus guianense	DQ492431					
Wallago sp.	DQ492488					
Zaireichthys brevis	**Novel**	**Novel**	**Novel**	**Novel**		
Zaireichthys sp.	**Novel**	**Novel**		**Novel**		
Zaireichthys sp.	**Novel**			**Novel**		

Table S5b. List of novel genetic samples and associated Genbank numbers used in the Lake Tanganyikan *Synodontis* Cytochrome *b* phylogeny. Light grey shading denotes the outgroup.

Species	**Clade name**	**Field and Genbank numbers**
S. zambezensis		FM878858
S. victoriae		DQ886657, EU781929, EU781930
S. granulous		DQ886650, DQ886651
S. multipunctatus		S4, S36, S258, DQ886621, S270, S174, S207, S208, S253, DQ886624, DQ886625, DQ886628, DQ886629, DQ886623, S177, S178, S255, S259, S257, S1, S206, S260, DQ886630, FM878846, S7, S37, S256, S254, S2, S6, S35, S250, 5164, DQ886627, S275, S282, S288, S3
S. cf. tanganyicae	Clade 1	DQ886658
S. petricola	Clade 2	S16, DQ886633, DQ886631, DQ886632, DQ886634, S15, S12, S9, S10, S8, S13, S14, S17, S18
S. dhonti	Clade 3	S66, S73, S154, S164, S153, S85, S179, DQ886653, S182, S192, S193, DQ886652, S158, S165, S167, S199, S166, S183
S. polli	Clade 4	S67, S74, S77, S82, S84, S87, S91, S96, S106, S78, S80, S81, S60, S94, S79, S92, DQ886645, DQ886646, S59, S83, S68, S147, S103
S. aff. petricola	Clade 5	DQ886640, DQ886641, S149, S150, S243, S159, S162, S214, DQ886637, DQ886635, S169, DQ886638, DQ886639, S145, S61, S75, S86, S89, S90
S. aff. petricola	Clade 6	DQ886644, S194, S198, S170, S172, S188, S213, S151, S181, S189, S202, DQ886643, S173, S171, S196, S197, S211, S161, S163

Table S3. Comparison of evolutionary models based on the PC data for the Claroteinae. Models were assessed using Akaike Information Criterion (AIC) values and Akaike Weights (AW). Bold text denotes the best model.

Model	**Brownian motion**		**Ornstein-Uhlenbeck**		**Early burst**	
PC axis	**AICc**	**Akaike Weight**	**AICc**	**Akaike Weight**	**AICc**	**Akaike Weight**
PC1	55.938	**0.676**	59.120	0.138	58.519	0.186
PC2	56.544	**0.688**	59.312	0.172	59.726	0.140
PC3	50.856	**0.632**	52.798	0.239	54.038	0.129
PC4	38.065	**0.658**	40.368	0.208	41.247	0.134

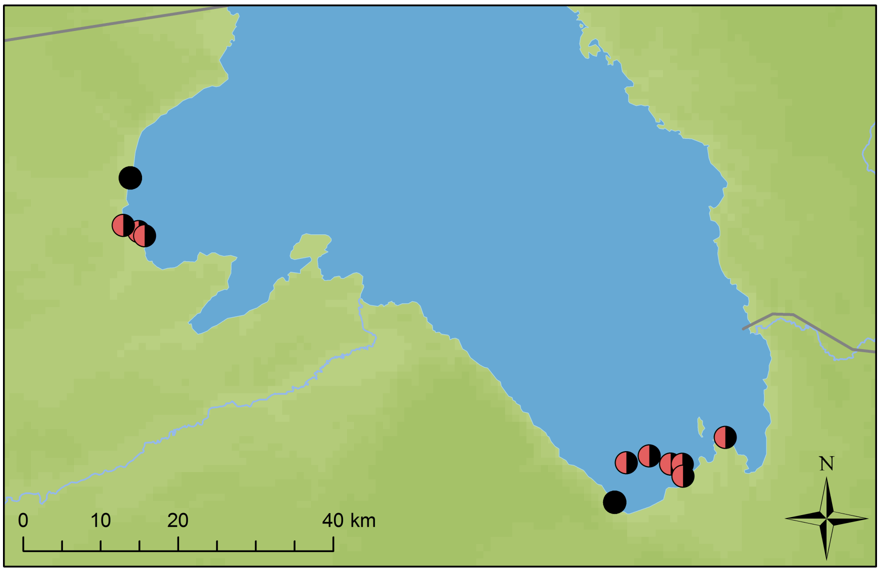


Figure S1. Sampling sites in Zambia used for isotopes. Sites where only claroteine samples were collected are shown in black, sites where samples from both radiations were collected are shown as half red half black.

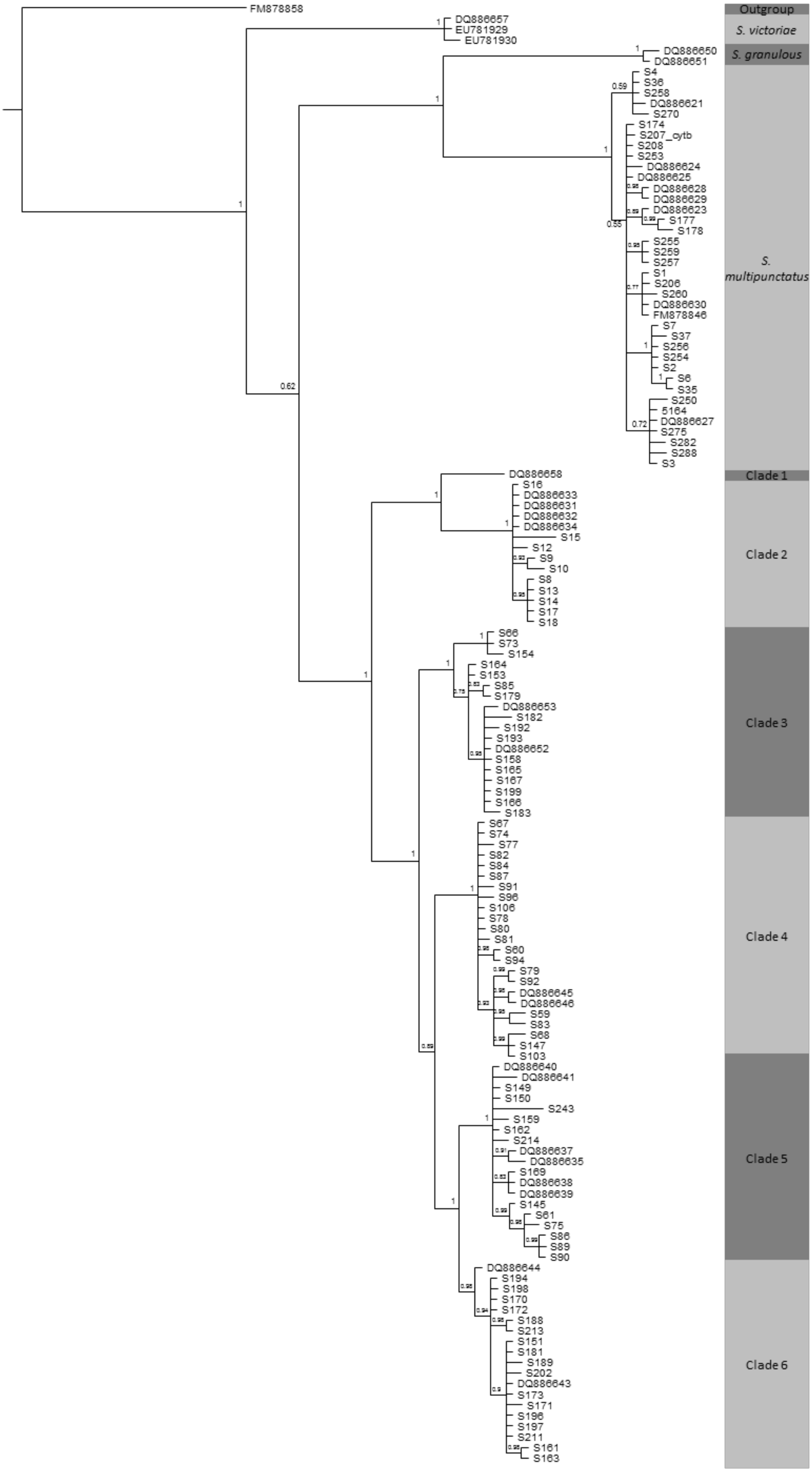


Figure S2. Bayesian *Cytochrome b* tree showing the clade designations for *Synodontis* samples used in this study. Genbank accession numbers include the following named taxa in each of the clades: Clade 1 = *S. cf. tanganyicae*; Clade 2 = *S. petricola*; Clade 3 = *S. dhonti*; Clade 4 = *S. polli*; Clade 5 = *S. aff. petricola*; Clade 6 = *S. aff. petricola*.

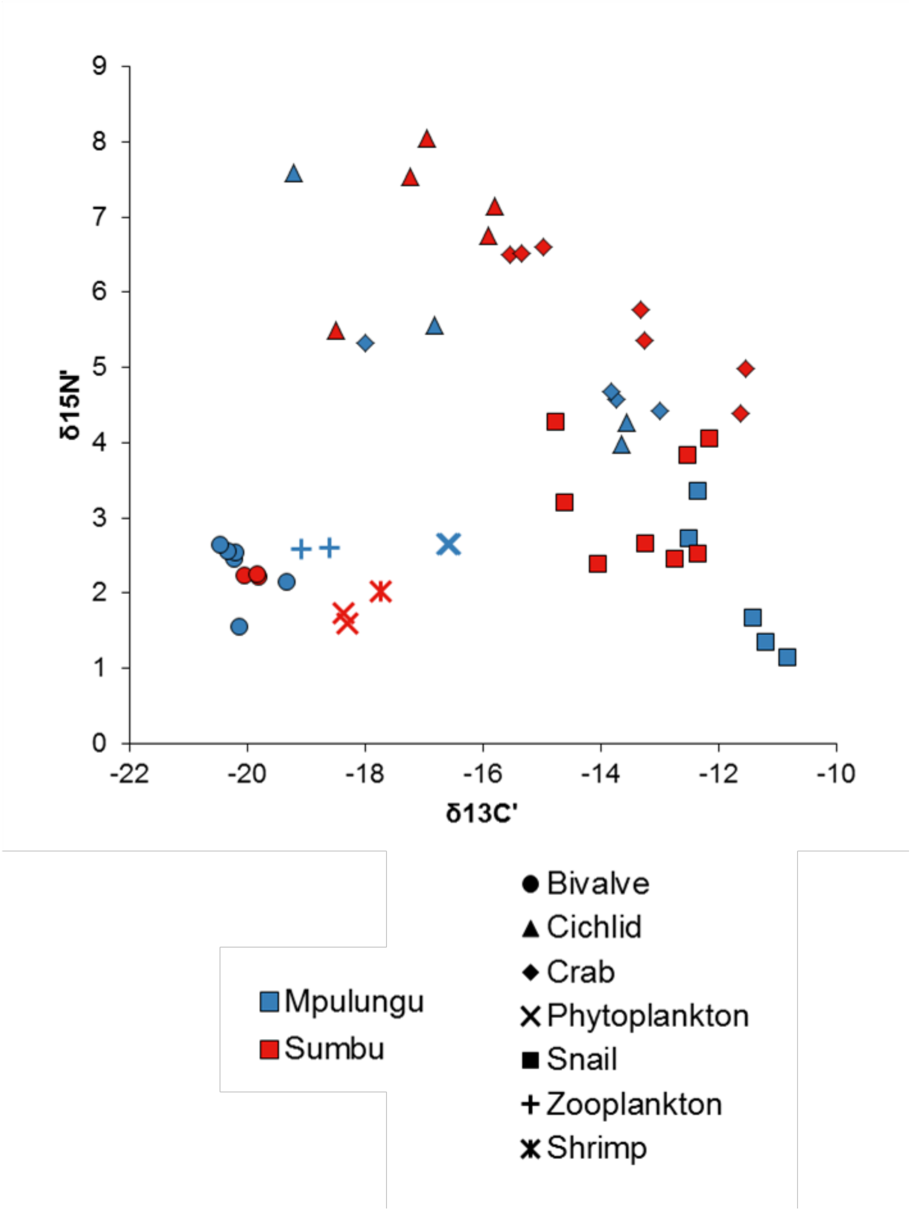
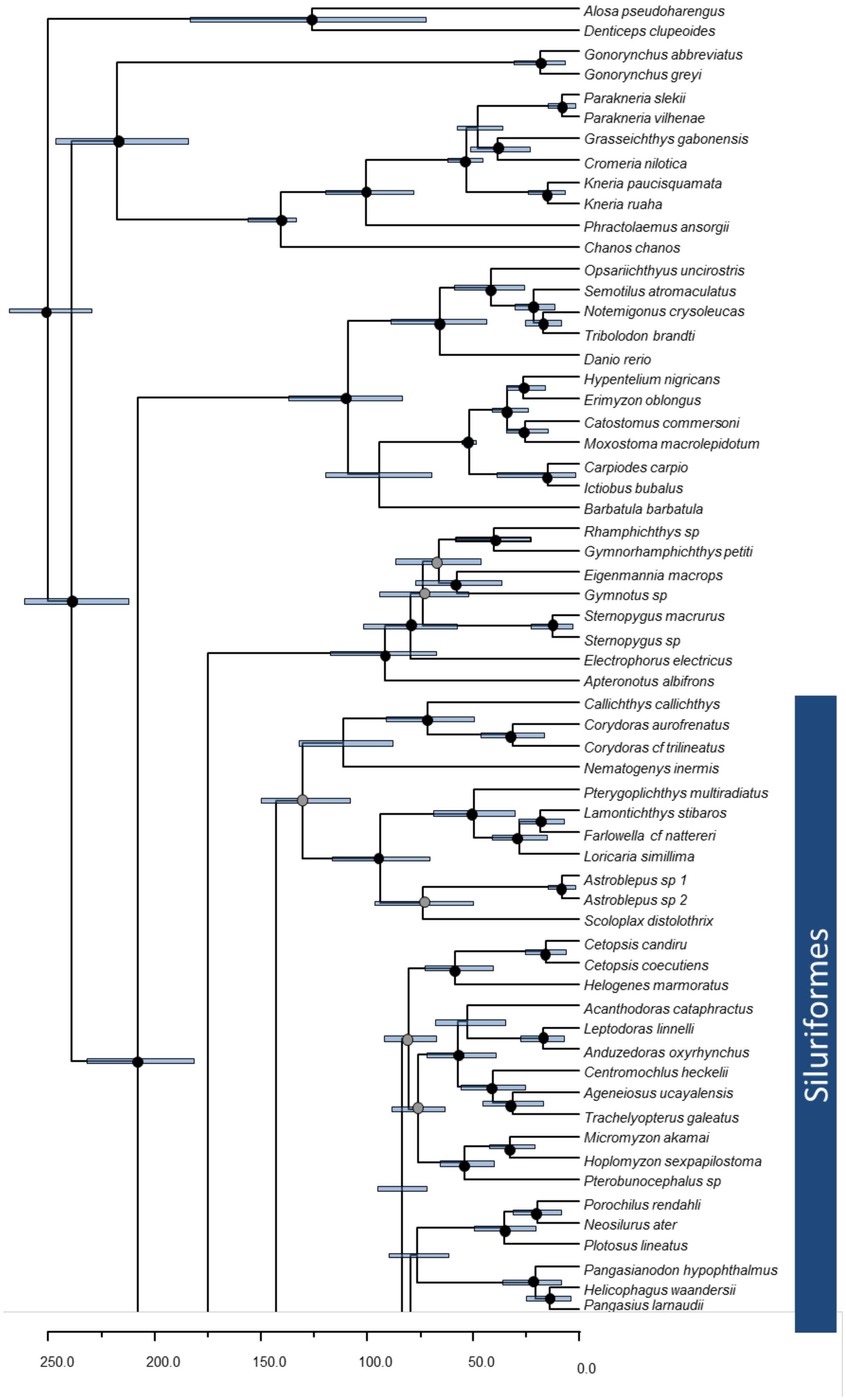
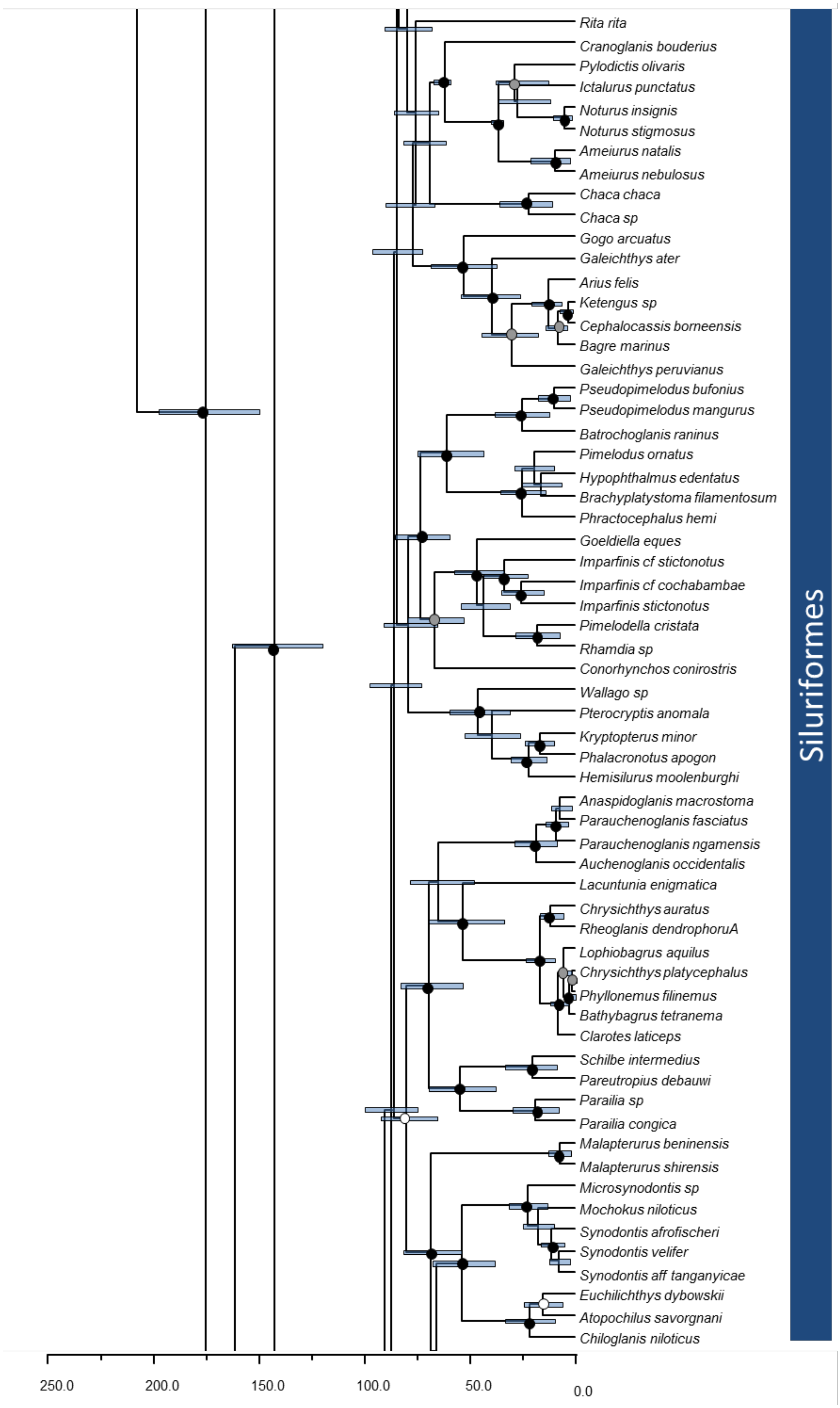


Figure S3 Scatter chart showing δ13C vs. δ15N for baseline samples from Mpulungu (blue) and Sumbu (red). The baseline organisms are shown using different shaped markers outlined in the key.


Alosa pseudoharengus
Denticeps clupeoides
Gonorynchus abbreviatus
Gonorynchus greyi
Parakneria slekii
Parakneria vilhenae
Grasseichthys gabonensis
Cromeria nilotica
Kneria paucisquamata
Kneria ruaha
Phractolaemus ansorgii
Chanos chanos
Opsariichthyus uncirostris
Semotilus atromaculatus
Notemigonus crysoleucas
Tribolodon brandti
Danio rerio
Hypentelium nigricans
Erimyzon oblongus
Catostomus commersoni
Moxostoma macrolepidotum
Carpiodes carpio
Ictiobus bubalus
Barbatula barbatula
Rhamphichthys sp
Gymnorhamphichthys petiti
Eigenmannia macrops
Gymnotus sp
Sternopygus macrurus
Sternopygus sp
Electrophorus electricus
Apteronotus albifrons
Callichthys callichthys
Corydoras aurofrenatus
Corydoras cf trilineatus
Nematogenys inermis
Pterygoplichthys multiradiatus
Lamontichthys stibaros
Farlowella cf nattereri
Loricaria simillima
Astroblepus sp 1
Astroblepus sp 2
Scoloplax distolothrix
Cetopsis candiru
Cetopsis coecutiens
Helogenes marmoratus
Acanthodoras cataphractus
Leptodoras linnelli
Anduzedoras oxyrhynchus
Centromochlus heckelii
Ageneiosus ucayalensis
Trachelyopterus galeatus
Micromyzon akamai
Hoplomyzon sexpapilostoma
Pterobunocephalus sp
Porochilus rendahli
Neosilurus ater
Plotosus lineatus
Pangasianodon hypophthalmus
Helicophagus waandersii
Pangasius larnaudii
Siluriformes
250.0
200.0
150.0
100.0
50.0
0.0


Rita rita
Cranoglanis bouderius
Pylodictis olivaris
Ictalurus punctatus
Noturus insignis
Noturus stigmosus
Ameiurus natalis
Ameiurus nebulosus
Chaca chaca
Chaca sp
Gogo arcuatus
Galeichthys ater
Arius felis
Ketengus sp
Cephalocassis borneensis
Bagre marinus
Galeichthys peruvianus
Pseudopimelodus bufonius
Pseudopimelodus mangurus
Batrochoglanis raninus
Pimelodus ornatus
Hypophthalmus edentatus
Brachyplatystoma filamentosum
Phractocephalus hemi
Goeldiella eques
Imparfinis cf stictonotus
Imparfinis cf cochabambae
Imparfinis stictonotus
Pimelodella cristata
Rhamdia sp
Conorhynchos conirostris
Wallago sp
Pterocryptis anomala
Kryptopterus minor
Phalacronotus apogon
Hemisilurus moolenburghi
Anaspidoglanis macrostoma
Parauchenoglanis fasciatus
Parauchenoglanis ngamensis
Auchenoglanis occidentalis
Lacuntunia enigmatica
Chrysichthys auratus
Rheoglanis dendrophoruA
Lophiobagrus aquilus
Chrysichthys platycephalus
Phyllonemus filinemus
Bathybagrus tetranema
Clarotes laticeps
Schilbe intermedius
Pareutropius debauwi
Parailia sp
Parailia congica
Malapterurus beninensis
Malapterurus shirensis
Microsynodontis sp
Mochokus niloticus
Synodontis afrofischeri
Synodontis velifer
Synodontis aff tanganyicae
Euchilichthys dybowskii
Atopochilus savorgnani
Chiloglanis niloticus
Siluriformes
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100.0
50.0
0.0

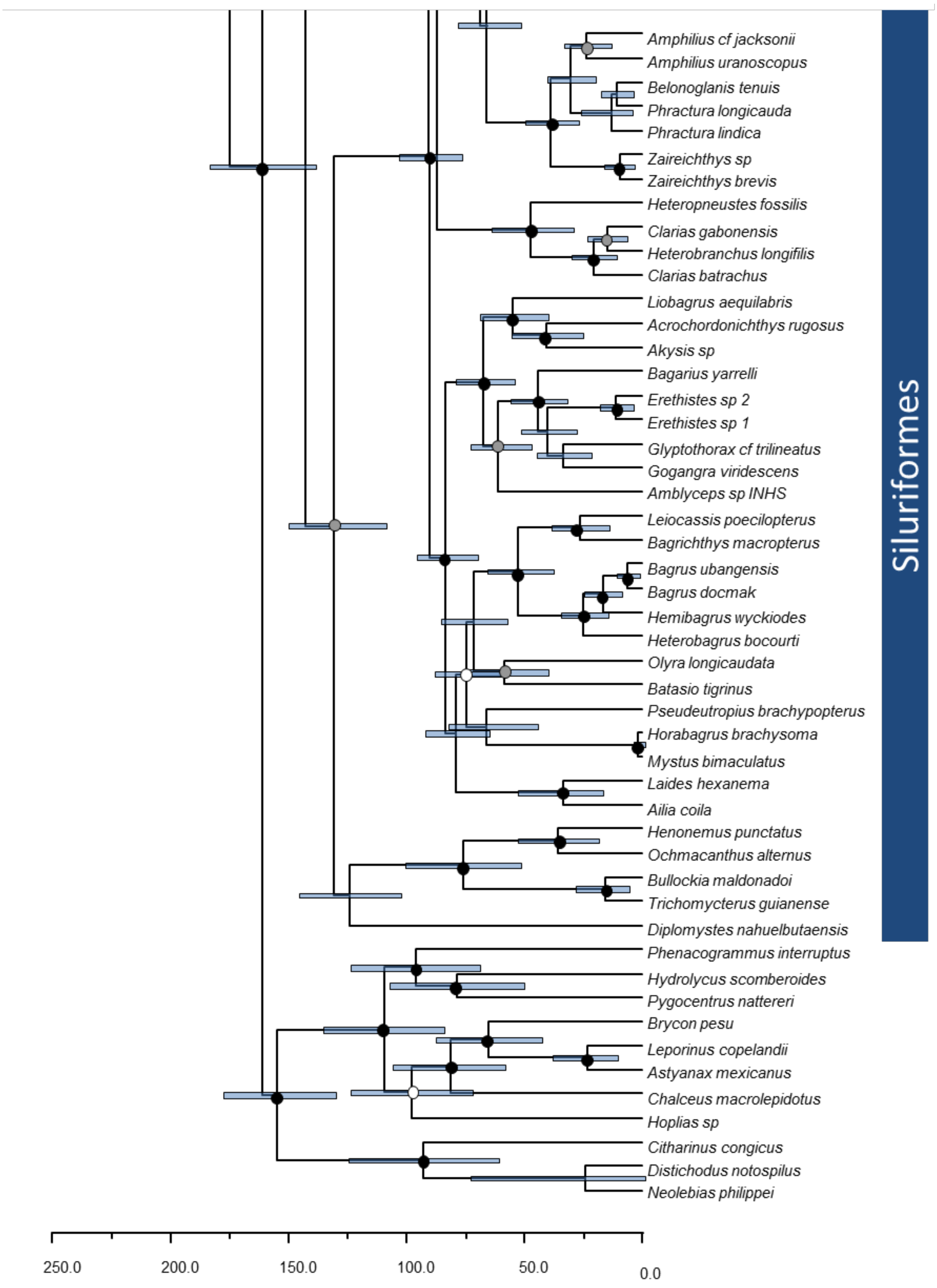

Figure S4. BEAST tree for the superorder Ostariophysi. The order Siluriformes (catfishes) is highlighted. Scale bar is Millions of years (Ma). Node bars show 95% confidence intervals around node ages. Black circles on nodes represent a posterior probability of 1, grey circles a posterior probability greater than 0.95, and white circles greater than 0.9.