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Catfishes from the North-Western Part of Lake Tanganyika: Contribution to a Reference Library of DNA Barcodes

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Abstract: In spite of the global barcoding effort, there is still a lack of genetic data on African freshwater fishes. We aimed to contribute to bridging this gap by providing molecular data on commercially important catfish species from the north-western part of Lake Tanganyika. We collected 215 catfish specimens and sequenced the standard vertebrate barcoding gene (COI) for 41 specimens. Additionally, we sequenced 20 specimens for the mitochondrial Cyt-b gene to make the link to previously published datasets. We identified 11 species using morphology, compared DNA sequences with those available on GenBank, and employed Automatic Barcode Gap Discovery (ABGD) and phylogenetic approaches. The dataset includes the first molecular data (COI and Cyt-b) for *Chrysichthys acsiorum*, as well as the first-ever COI sequences for *Dinotopterus cunningtoni* and *Malapterurus tanganyikaensis*. Our findings extend the known distribution of *C. acsiorum* by approximately 100 km. Additionally, we demonstrated the difficulty in delineating species of *Chrysichthys* and *Synodontis* from Lake Tanganyika with molecular tools. For *Chrysichthys*, automated methods, such as ABGD, failed to delineate species. However, barcoding does seem promising as all the individual species are resolved as clades. Within *Synodontis*, the study found a strong similarity between *S. grandiops* and *S. multipunctatus*, highlighting a need for revision. Our findings emphasize the necessity for integrative taxonomy in the study of catfishes from Lake Tanganyika.

Keywords: COI; Cyt-b; taxonomy; *Synodontis*; *Chrysichthys*; *Chrysichthys acsiorum*; Congo; DRC; Siluriformes; ABGD

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1. Introduction

Lake Tanganyika, one of the Great Lakes of East Africa [1], boasts an impressive level of endemism among various groups of animals, such as cichlid fishes [2], gastropods [3], and monogenean flatworms [4]. The high level of species richness and endemism in cichlids (241 species in the basin, only two of which are not endemics) led researchers to treat the ichthyofauna of Lake Tanganyika as consisting of two groups: cichlids and no-cichlids [2,5].

Among non-cichlid fishes, Mastacembelidae [6] and several families of catfishes (Siluriformes) also exhibit significant levels of endemism. Lake Tanganyika contains 35 species of catfishes, belonging to six families (Auchenoglanididae, Bagridae, Clariidae, Claroteidae, Malapteruridae, and Mochokidae), of which 31 occur in the north-western part of the lake [7]. Outside of the lake, but within the watershed, representatives of two other families can be found, namely Amphiliidae and Schilbeidae [8]. Given the inconsistencies in the classification of catfish species, genera, and families, we will, in this study, follow the nomenclature of Fricke, Eschmeyer, and van der Laan [9] for the names of different families and Fermon et al. [7] for lower taxonomic ranks. Among these families, three demonstrate important levels of endemism in the lake: Claroteidae [10], Clariidae [11], and Mochokidae [12]. Within the aforementioned families, several genera, such as *Chrysichthys* Bleeker, 1858 [10] and *Synodontis* Cuvier, 1816 [13,14], contain taxonomic problems, which merit investigation with molecular tools, including DNA barcoding. This technique uses the genetic information contained in a short and standardized section of DNA to identify specimens and delineate species. This methodology may also provide information regarding phylogenetic relationships among species, shed light on the presence of species-rich assemblages of 'cryptic' species, and aid in the discovery of new species [15–17]. Furthermore, it facilitates the validation of diagnostic features through comparison with public databases such as GenBank [18] or the Barcode of Life Data System (BOLD) [19].

To date, relatively few studies have included molecular data of catfishes from Lake Tanganyika. For Mochokidae, in particular for *Synodontis*, the following genetic markers have been studied: Cyt-b [12,14,20,21], COI [21], D-loop and ND6 [14], tRNA-pro and RAG2 [21], and RADseq [22]. Molecular data are also available for Claroteidae and Auchenoglanididae for which several markers were amplified: RADseq [22], COI, Cyt-b, S7, RAG2, and Plagl2 [10]. Additionally, some sequence data are available for a few Tanganyikan representatives of Clariidae (Cyt-b, 18S-ITS1-5.8S-ITS2-28S) [11,23], Malapteruridae (RAG1, RAG2) [24], and Bagridae (12S) [25].

There is hardly any molecular data available on catfish species from the Congolese part of Lake Tanganyika, which contains almost the entire western part of the lake. Hence, this study aims to fill this gap by providing molecular data that can serve for further in-depth research on catfish species from Lake Tanganyika. We specifically focused on species of economic importance by sampling at fish markets. For a representative set of specimens, we amplified and sequenced the classic DNA barcode gene [26]: Cytochrome *c* oxidase subunit I (COI). In addition, we sequenced the Cytochrome *b* (Cyt-b) gene for a subset of specimens in order to bridge our newly generated COI sequences with previously published sequences from phylogenetic studies [10,13,27].

2. Materials and Methods

2.1. Data Collection

We collected specimens from seven different sampling sites (Figure 1). Fish specimens were acquired from local fishermen at various landing sites between 27 December 2022 and 22 January 2023. For each specimen, a fin clip was preserved in 100% ethanol for genetic analyses. Fish vouchers were fixed in 10% formalin and transported to the Department of Biology at the 'Centre de Recherche en Hydrobiologie d'Uvira' (CRH-Uvira, Democratic Republic of the Congo) for morphological identification at the species level following the regional taxonomic key of Fermon et al. [7]. These identifications were checked in the morphological laboratory using different identification keys [28–30]. All collected specimens were deposited at the Royal Belgian Institute of Natural Sciences (Belgium). Specimens were registered in the vertebrate collection under collection number IG 34574 (see Supplementary Materials File S1). In certain cases, fin clips were collected without

voucher specimens (see Supplementary Materials File S1). This study was conducted under Nagoya permit N°004/IANCCB-RDC/SG-EDD/BTB/2023.

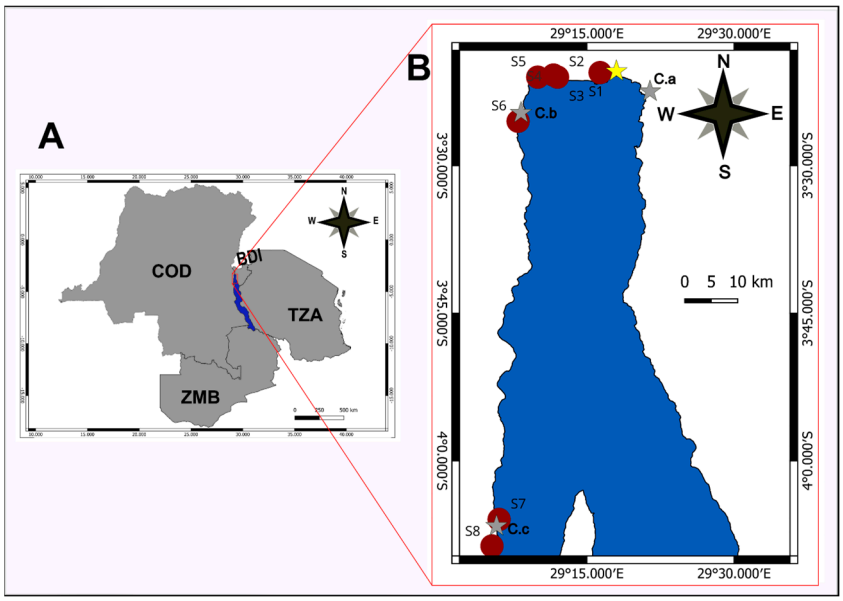

Figure 1. (**A**) Lake Tanganyika and its four surrounding countries. COD = Democratic Republic of Congo; TZA = Tanzania; BDI = Burundi, and ZMB = Zambia. (**B**) Sampling sites (in brown dots and detailed in Table 1) in the north-western part of Lake Tanganyika and the cities (in grey stars) found in this area; C.a: Bujumbura, C.b: Uvira, and C.c: Baraka. The yellow star represents the village of Kajaga in Burundi.

Table 1. List of morphologically identified specimens per species. A species name highlighted in bold indicates that no genetic information was available on GenBank prior to this study. With N: number of specimens collected with between parentheses the number of specimens for which no voucher was kept, COI, Cyt-b: number of sequences obtained in this study for these markers; COI_GB, Cyt-b_GB: whether any sequences of these genes were available on GenBank for the species.

Species	N	COI	Cyt-b	COI_GB	Cyt-b_GB
Auchenoglanis occidentalis	6 (2)	3	2	Yes	Yes
Bagrus docmak	15	3	1	Yes	Yes
Chrysichthys acsiorum	17 (1)	4	3	No	No
Chrysichthys brachynema	6 (2)	3	0	Yes	Yes
Chrysichthys grandis	4	1	1	Yes	Yes
Chrysichthys sianenna	109	2	2	Yes	Yes
Clarias gariepinus	15 (2)	13	3	Yes	Yes
Clarias werneri	8	1	1	Yes	Yes
Dinotopterus cunningtoni	1	1	1	No	Yes
Malapterurus tanganyikaensis	13	2	2	No	Yes
Synodontis grandiops	21	8	4	Yes	Yes

2.2. DNA Extractions, Sanger Sequencing and Data Acquisition

We performed genomic DNA extractions on a representative subset of our collection, consisting of 48 samples, using the NucleoSpin Tissue Kit (Macherey–Nagel), following the manufacturer's instructions. We then conducted PCR amplification targeting the mitochondrial COI gene using a combination of M13-tailed primers VF2_t1, FishF2_t1, FishR2_t1, and FR1d_t1 [31], following a protocol adapted from Decru et al. [32]. For each PCR mix, the composition was as follows: 2.5 µL of PCR buffer (Qiagen; 10×; Hilden, Germany), 2.5 µL of dNTP (2 mM), 1.25 µL of the primer cocktail (2 µM), and 0.2 *Taq* DNA

Polymerase (Qiagen, 5 units per µL). To these, we added 16.75 µL of Ultrapure Sterile Water and 2.0 µL of the DNA extracts. We conducted the amplification on a Biometra TOne Thermal Cycler (Analytik Jena, Jena, Germany) using the following program temperature profile: 3 min at 94 °C, followed by 31 cycles of 40 s at 94 °C, 40 s at 52 °C, and 1 min at 72 °C, plus a final extension of 10 min at 72 °C. The protocol used 31 cycles instead of 35 in order to reduce the amount of aspecific amplification. The resulting PCR products were controlled by agarose gel (1.2%) electrophoresis. Before bidirectional Sanger sequencing (forward and reverse), we conducted enzymatic purification of the PCR product using ExoSAP (Fermentas; Thermo Fisher Scientific, Waltham, MA, USA).

We also conducted PCR amplification using the primer pair L15267 and H16461 [33] on a subset of representative specimens (n = 21) targeting the mitochondrial Cyt-b gene. The PCR composition per reaction was as follows: 2.5 µL of PCR buffer (Qiagen; 10×), 2.5 µL of dNTP (2 mM), 2.5 µL of each primer (2 µM), and 0.2 Taq DNA Polymerase (Qiagen, 5 units per µL). To these, we added 13 µL of UltraPure Sterile Water and 2.0 µL of the DNA extracts. We conducted the amplification using the following program temperature profile: 1 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 30 s at 54 °C, and 1 min 30 s at 72 °C, plus a final extension of 10 min at 72 °C. The sequencing was performed by an external company (Macrogen Europe, Amsterdam, The Netherlands).

The chromatograms were edited using Geneious Prime® version 2024.0.5 (https://www.geneious.com). We conducted automatic trim for low-quality base calls on sequence ends (error probability threshold: 0.01). We assembled corresponding forward and reverse sequences into consensus sequences, and visually controlled and edited the sequences when needed. Ambiguous calls were coded following the nomenclature of the International Union of Pure and Applied Chemistry (IUPAC). Primer sequences were also trimmed from the consensus sequences. All the COI and Cyt-b sequences were deposited in a publicly accessible database GenBank.

2.3. Molecular Data Analysis

To validate the different morphologically derived identifications, we first compared our different COI sequences with those available on GenBank using the Basic Local Alignment Search Tool algorithm (BLAST) [34]. For each sequence, we checked whether the best match was with a con- or a heterospecific. Additionally, we calculated the nucleotide similarity between our sequences and conspecific sequences found on GenBank. In order to do this, we downloaded every sequence that was accessible (on the 25 April 2024), and that was conspecific with species from our dataset, and used Fabox [35] to generate distinct haplotypes. The intraspecific and interspecific distances, based on the alignment of COI sequences (see Supplementary Materials File S2), were calculated using MEGA software version 11 [36]. Following model selection in MEGA XI using the Bayesian Information Criterion, we opted for the highest-ranked model available in this software to calculate pairwise distances. Therefore, the Tamura-Nei [37] model was chosen.

To complement the aforementioned analyses and confirm the number of different taxa we found based on morphological grounds, we also estimated the suggested number of species by analysing COI sequences using the Automatic Barcode Gap Discovery (ABGD) method for primary species delimitation [38]. We then compared the ABGD species delimitation with another species delimitation computed using the Bayesian Poisson Tree Processes (bPTP) method to assess the consistency and robustness of the proposed species boundaries [39]. The bPTP analysis was conducted without the outgroup to achieve a more robust species delineation. Default parameters were used, except for the number of Markov chain Monte Carlo iterations, which was increased to 100,000. We complemented this with an additional phylogenetic analysis by constructing a maximum likelihood tree using

RAxML-NG [40] to check if sequences are taxonomically sorted and if the result corresponds to the partition delineated with the ABGD method. We used the Asian species *Rita rita* (Hamilton, 1822) [41] as an outgroup, as this species was shown to be early divergent in Siluroidei. The best substitution model (TIM1uf) [42] was selected using jModelTest [43], and branch support (BS) was computed using 1000 bootstrap trees. The COI sequences have been deposited in GenBank, and the accession numbers are PQ37296-PQ37336 (see Supplementary Materials File S3).

We also selected one or two representative specimens per identified species and COI lineage and investigated them further using the Cyt-b marker (the Cyt-b sequences have been deposited in GenBank, with accession numbers PQ661643-PQ661662). We integrated the Cyt-b sequences we obtained with those from previous studies that focused on specific groups of catfishes and that included a lot of sequences of representatives of Lake Tanganyika. For this, we downloaded data from these studies on Claroteidae [10], on *Synodontis* [13] and on African and Asian members of Clariidae [27]. For Claroteidae [10], we restricted our analysis to the sequences belonging to *Chrysichthys.* We added our sequences to this data to create three different datasets which were analysed to provide additional insights into the taxonomic assignment of our specimens and investigate how the new sequences might provide insight into the evolutionary history of these different groups of catfishes. For all the three datasets, we used the same outgroups as in the referenced studies: *Auchenoglanis occidentalis* (Valenciennes, 1840) for that of *Chrysichthys* [10], *Microsynodontis* sp. for that of *Synodontis* [12], and *Clarotes laticeps* (Rüppell, 1829) for the clariid dataset [27]. The sequences for each dataset were aligned using Muscle v5 with default settings [44] and the resulting alignments were trimmed to remove missing data. Phylogenetic reconstructions were conducted using RAxML-NG as detailed for the COI dataset. We selected and used the substitution model GTR+I for the dataset of *Chrysichthys* and the GTR+I+G model [45] for the two datasets about *Synodontis* and African clariids.

3. Results

3.1. Morphological Identification

Morphological identifications conducted on 215 specimens collected from the northwestern part of Lake Tanganyika revealed a total of 11 species. These included one species of Auchenoglanididae: *A. occidentalis*, the sole representative of this family in Lake Tanganyika; one species of Bagridae: *Bagrus docmak* (Fabricius, 1775), also the only species of this family in the lake; four species of Claroteidae: *Chrysichthys acsiorum* Hardman, 2008; *C. brachynema* Boulenger, 1990; *C. grandis* Boulenger, 1971 and *C. sianenna* Boulenger, 1906; three species of Clariidae: *Clarias gariepinus* (Burchell, 1822); *C. werneri* Boulenger, 1906; and *Dinotopterus cunningtoni* Boulenger, 1906; and one representative of both Mochokidae: *Synodontis grandiops* Wright and Page, 2006 and Malapteruridae: *Malapterurus tanganyikaensis* Roberts, 2000. Voucher specimens were kept for 207 out of 215 specimens (Supplementary Materials File S2).

3.2. Molecular Data

We successfully amplified and sequenced the COI gene for 41 out of 48 specimens and the Cyt-b gene for 20 specimens (Table 2). Among the eleven species encountered, eight already had sequences of COI on GenBank, whereas Cyt-b data were available for all but one species (see Table 1). The similarities among conspecifics between the COI sequences obtained in this study and those present on GenBank ranged from 97.7% to 100% (Table 2). For the eight species for which COI sequences were available on GenBank, six exhibited higher rates of similarity with conspecific sequences than with heterospecific sequences. One species, *C. grandis*, showed nearly identical similarity percentages with both conspecific

(99.9%, accession number HG803479) and heterospecific (*C. plathycephalus* Worthington and Ricardo 1937, 99.3% accession number HG803473) sequences. For one species, *S. grandiops*, we found the highest similarity with sequences of unspecified '*Synodontis* sp.' (accession number LC535211). It is important to note that LC535211 was derived from larval fishes as reported in Takahashi and Koblmüller [46]. Hence, the authors were unable to ascertain whether these larvae belonged to *S. multipunctatus* Boulenger, 1898, or *S. grandiops*. The second highest similarity was found with a sequence of *S. multipunctatus* (98.9–99.54%) with accession number HF565910 and voucher ID BMNH:2006.3.6.9 from [17]. The distance between COI sequences is represented in Table 3. The minimum intraspecific distance was 0%, whereas the maximum was 1%, observed among four sequences of *C. acsiorum*, two sequences of *M. tanganyikaensis*, and eight sequences of *S. grandiops*. The minimum interspecific distance, estimated at 1%, was observed between *C. acsiorum* and *C. grandis*. The maximum interspecific distance in our dataset, at 23%, was noted between *A. occidentalis* and *C. gariepinus*.

Table 2. Sequences of the specimens that were morphologically identified in this study and for which conspecific COI sequences are present on GenBank: P.S_COI: Number of COI sequences from the present study; GB: number of COI sequences present on GenBank (excluding those of the present study); Ht: number of haplotypes obtained using sequences from GenBank and sequences from the present study, gen.sim; (%): highest genetic similarities with sequences from GenBank; C/H: highest genetic similarities conspecific (C) or heterospecific (H) sequences with '?' indicating uncertainty of the identification; gen.sim.C (%): range of similarities (in %) with conspecific sequences available on GenBank.

Species	P.S_COI	GB	Ht	gen.sim. (%)	C/H	gen.sim.C (%)
Auchenoglanis occidentalis	3	28	17	98.9–99.6	C	90.6–98.9
Bagrus docmak	3	6	4	99.9–100	C	93.0–98.4
Chrysichthys brachynema	3	2	4	97.7–98.2	C	95.3–97.8
Chrysichthys grandis	1	1	2	98.5–99.9	C/H	99.9
Chrysichthys sianenna	2	3	4	100	C	97.9–100
Clarias gariepinus	13	162	111	9.8–99.9	C	99.9
Clarias werneri	1	1	2	98.8	C	98.8
Synodontis grandiops	8	1	8	99.4–100	?	94.2–95

Table 3. Distances in % between 41 COI sequences generated in this study using the Tamura–Nei model. Intraspecific distances are indicated between parentheses in the first column, interspecific pairwise distances are indicated below the diagonal. Abbreviations represent the studied species, with S_1: *Auchenoglanis occidentalis*, S_2: *Bagrus docmak*, S_3: *Chrysichthys acsiorum*, S_4: *Chrysichthys brachynema*, S_5: *Chrysichthys grandis*, S_6: *Chrysichthys sianenna*, S_7: *Clarias gariepinus*, S_8: *Clarias werneri*, S_9: *Dinotopterus cunningtoni*, S_{10}: *Malapterurus tanganyikaensis*, and S_{11}: *Synodontis grandiops*. All sequences comprised 652 nucleotides.

	S_1	**S_2**	**S_3**	**S_4**	**S_5**	**S_6**	**S_7**	**S_8**	**S_9**	**S_{10}**	**S_{11}**
S_1 (0)	-										
S_2 (0)	18–19	-									
S_3 (0–1)	18	19	-								
S_4 (0)	18–19	16	14–15	-							
S_5 (-)	18	19	1	14–15	-						
S_6 (0)	18	18	4	13–15	3	-					

Table 3. *Cont.*

	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}	S_{11}
S_7 (0)	22–23	20	21	18–19	19–20	20	-				
S_8 (-)	19–20	18	21–22	17–18	22	21–22	10	-			
S_9 (-)	21	20	20	19–20	20	20	7	8	-		
S_{10} (1)	17–18	16	19	17–19	19	18	21	19–20	20–21	-	
S_{11} (0–1)	18–19	21	19–20	16–18	19–20	19–20	19–20	19–21	20–21	20–21	-

3.2.1. ABGB and bPTP Methods

The ABGD method yielded results different from our morphological identifications. The barcode gap occurred at the inflection point of the sigmoid curve for ranked pairwise distances (p-dist = 0.12, Figure 2A), closely matching values (p-dist ≈ 0.1) that are commonly used to delineate species, see [38]. The ABGD method indicated that the COI dataset contained ten groups (see Supplementary Materials File S2) following the recursive method, potentially representing nine distinct species (ten with the outgroup). The vast majority of these groups were homogenous, meaning they consisted of individuals morphologically assigned to the same species. Specifically, we identified the following seven homogenous groups: *S. grandiops*, *C. gariepinus*, *M. tanganyikaensis*, *B. docmak*, *A. occidentalis*, *C. werneri* and *D. cunningtonni*. The sequences belonging to *Chrysichthys* were grouped into two distinct and heterospecific groups as follows: The first group comprised seven individuals, including four *C. acsiorum* (collection numbers RBINS-VZ-PISCES 29160, 29050, 29057 and one specimen without voucher with field number T73), two *C. sianenna* (RBINS-VZ-PISCES 29060 and one specimen without voucher with field number T45), and one *C. grandis* (RBINS-VZ-PISCES 29056). Within this group, the similarity rates among *C. acsiorum* sequences were higher, ranging from 98.86% to 99.85% of similarity, than those with heterospecifics. Sequences of *C. sianenna* had high similarity (99.69%) among themselves. High levels of similarity, ranging from 98.32% to 99.16% were observed between sequences of *C. acsiorum* and *C. grandis* while the comparison between *C. acsiorum* and *C. sianenna* indicated a lower resemblance, varying between 95.42% and 96.29%. The second group, a conspecific group, consisted of three individuals belonging to *C. brachynema*. Comparing their sequences revealed a similarity ranging from 98.08% to 98.27%. None of the potential partitions suggested by ABGD contained 11 putative species, i.e., the true number of species in our dataset. The bPTP method offered two different outputs, depending on whether a Bayesian framework (highest posterior probability-supported delimitation) or a maximum likelihood framework was used. The Bayesian bPTP delineation dramatically over-evaluated the number of species, with up to 25 species. In that regard, most of the species are however poorly supported with only eight of the putative species having posterior probability superior to 0.7. The maximum likelihood species delimitation provided support for 12 different species and was hence more aligned with the morphological and ABGD delimitation. The bPTP recognised more species in *Chrysichthys* than ABGD, delineating the three morphological species *C. sianenna*, *C. grandis* and *C. acsiorum* (Figure 3). The bPTP method, however, designated *C. acsiorum* as two distinct species, with specimen T73 identified as a separate species.

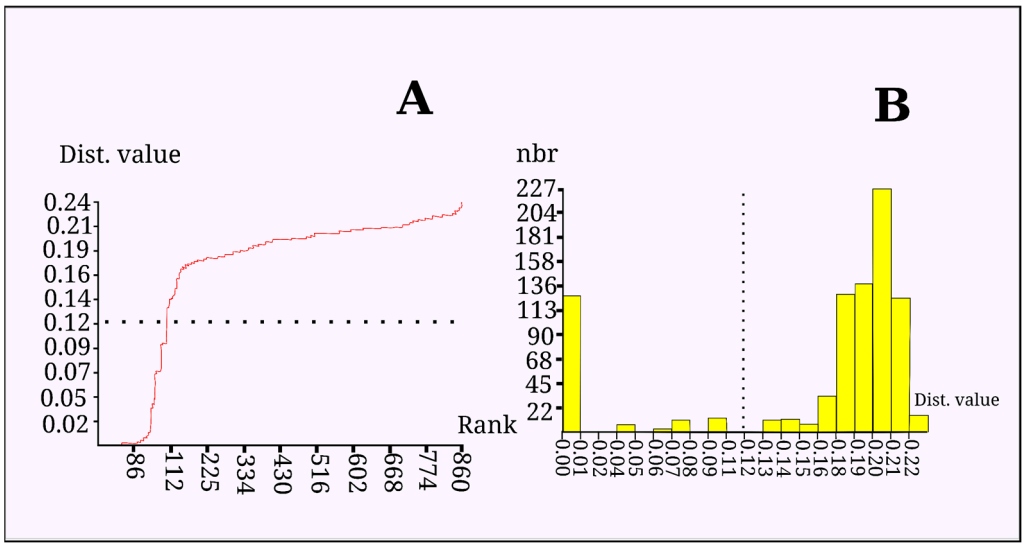

Figure 2. ABGD method results on our dataset of 41 sequences of COI. (**A**) ranked pairwise differences. (**B**) distribution of pairwise differences. The suggested barcode gap, represented by the p-distance, corresponding to the difference between intraspecific and interspecific genetic distances, is identified as the inflection point on the sigmoid curve of ranked pairwise genetic distances (dotted black line). This gap is also illustrated in panel (**B**).

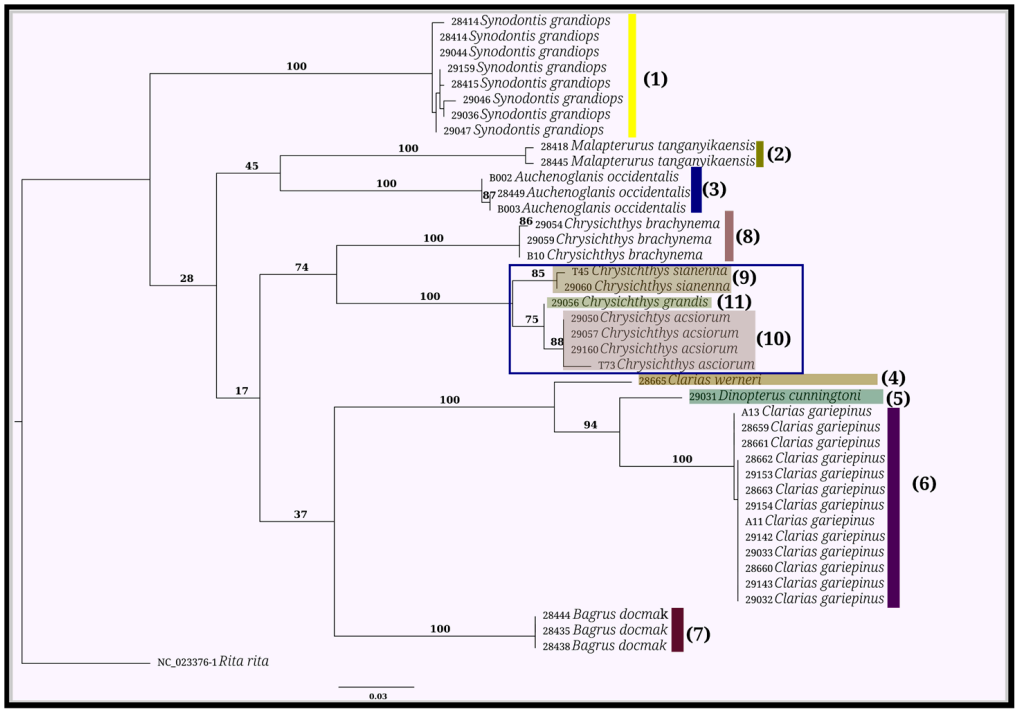

Figure 3. Maximum likelihood tree constructed from COI sequences obtained in this study. Specimen names are preceded by their registration number or by a field number for specimens for which no voucher specimen was available. The outgroup sequence stems from [41]. The numbers represent the different clades and subclades as mentioned in Section 3.2.2. All clades were resolved as separate groups by the ABGD analysis, except for subclades 9–11 which were resolved as a single group. The colors delineate different groups. The scale bar represents the number of expected substitutions per site.

3.2.2. Maximum Likelihood Phylogenetic Inference on COI Data

The phylogeny obtained from the 41 COI sequences reveals a set of eleven well-supported clades by BS values (Figure 3): (1) *S. grandiops*, (2) *M. tanganyikaensis*, (3) *A. occidentalis*, (4) *C. werneri*, (5) *D. cunningtoni*, (6) *C. gariepinus*, and (7) *B. docmak*. The clade containing specimens belonging to *Chrysichthys* was subdivided into two distinct clades, each supported by BS values of 100%. The first clade (8) encompassed sequences of *C. brachynema*. The second clade was divided into three, moderately supported, subclades: (9) a subclade encompassing sequences of *C. sianenna*, (10) one encompassing sequences of *C. acsiorum*, and (11) the sequence of *C. grandis*. This tree aligned with the ABGD results. It also matched well with the morphological identifications as all conspecific specimens were sorted monophyletically although subclades 9, 10, and 11 had only moderate BS support.

3.2.3. Maximum Likelihood Phylogenetic Inference of Cytochrome *b* Data

We generated an ML-based phylogenetic reconstruction by combining the novel Cyt-b sequences from specimens of *Chrysichthys* from this study with those of Peart et al. [10]. Two clades were clearly distinguished in the phylogenetic tree. The first clade, containing almost all the species identified in this study, was supported by a high BS value, and included *C. sianenna*, *C. platycephalus*, *C. grandis*, *C. acsiorum*, and sequences of an unidentified *Chrysichthys* sp. 1. The second clade, supported by a low BS value, included the remaining species involved in the study of Peart et al. [10], including *C. brachynema* (Figure 4).

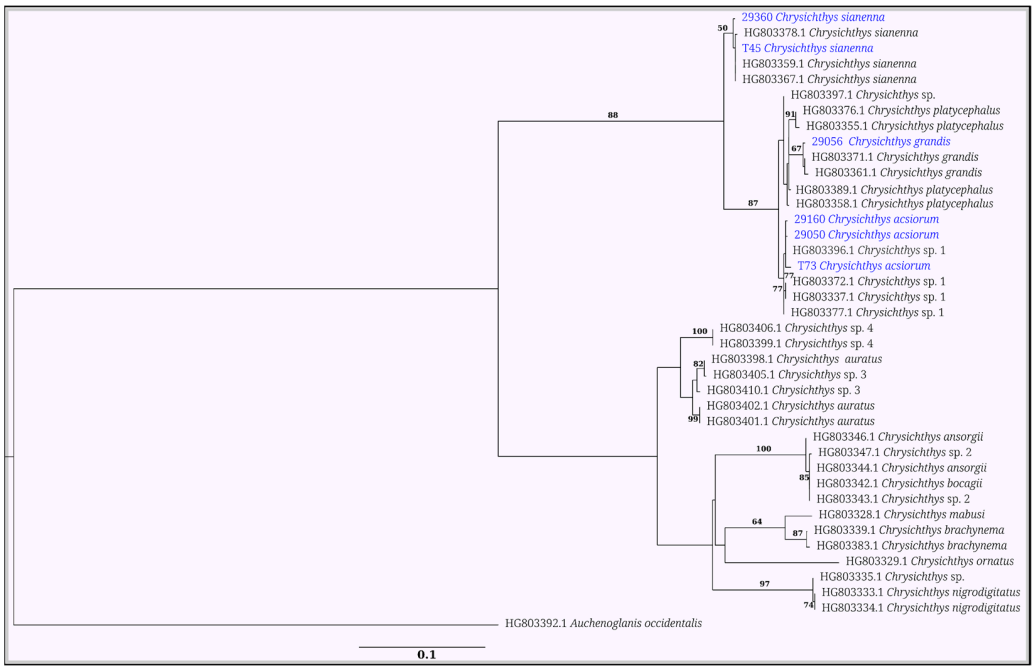

Figure 4. Maximum likelihood tree constructed using Cyt-b sequences of *Chrysichthys* spp. Sequences generated in this study are shown in blue. The remaining sequences were obtained from Peart et al. [10]. The indicated bootstrap values are those equal to or greater than 50. The scale bar represents the number of expected substitutions per site.

The phylogeny reveals that *C. sianenna* and *C. grandis* sequences are phylogenetically sorted with conspecifics whereas *C. acsiorum* clusters with specimens identified as *Chrysichthys* sp. 1. GenBank BLAST results consistently showed a high similarity between *C. acsiorum* and *Chrysichthys* sp. 1 (ranging from 99.2% to 99.8%). *Chrysichthys plathycephalus* was not resolved as a monophyletic group although BS values in this clade were low.

We also constructed a phylogenetic tree combining Cyt-b sequences of *Synodontis* from this study with those from Pinton et al. [13] and Day et al. [20] (Figure 5). In this phylogeny, *S. grandiops* was rendered paraphyletic by a sequence of *S. multipunctatus*, stemming from the same specimen (BMNH2006.3.6.9) as above. This observation concurs with the similarity that ranged from 99.77% to 100% between our sequences assigned morphologically to *S. grandiops* and *S. multipunctatus* from GenBank.

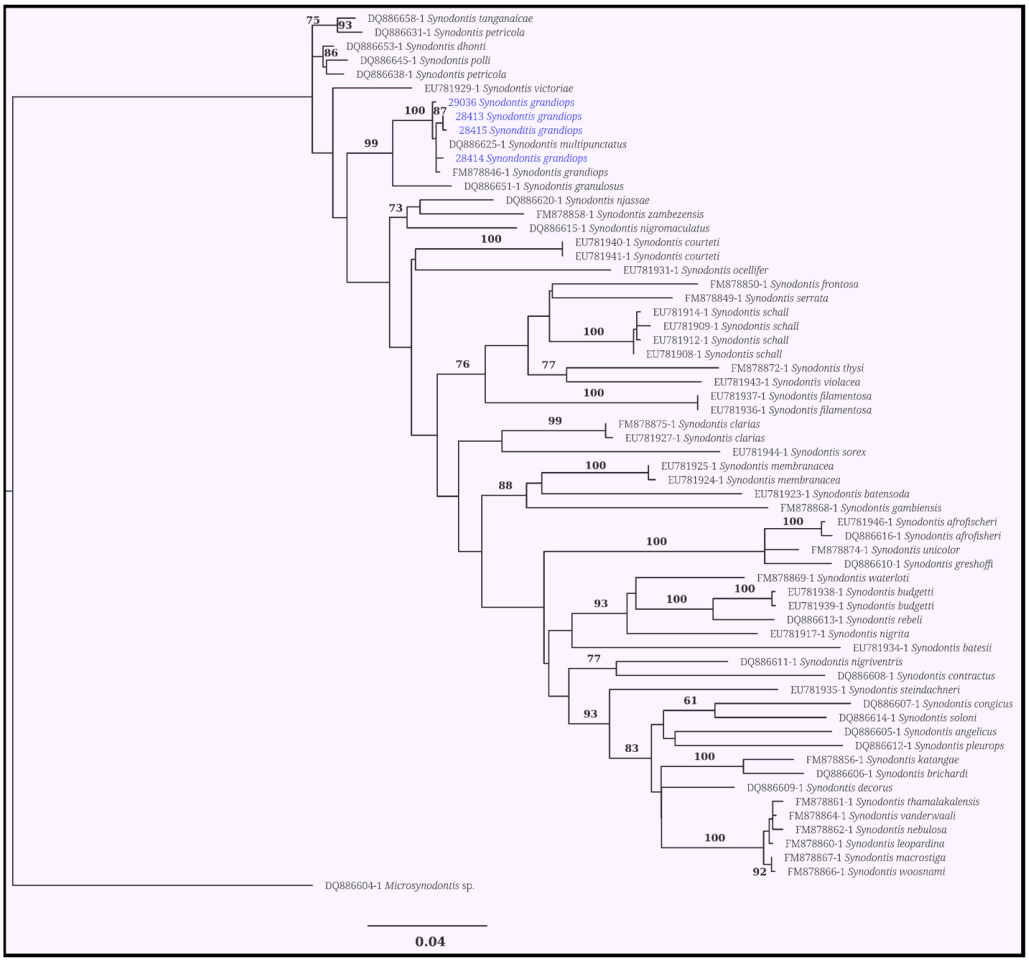

Figure 5. Maximum likelihood tree constructed using Cyt-b sequences of *Synodontis* spp. Sequences generated in this study are shown in blue. The remaining sequences were obtained from Pinton et al. [13]. The indicated bootstrap values are those equal to or greater than 50. The scale bar represents the number of expected substitutions per site.

In the phylogeny reconstruction of Clariidae (Figure 6), the sequence of *D. cunningtoni* from this study is sorted with a conspecific sequence from Agnèse and Teugels [27]. The sequence of *C. werneri* is clearly isolated from the other species. All our sequences of *C. gariepinus* form a monophyletic group that has *C. anguillaris* as a sister group, albeit with limited bootstrap support. The sequence of *C. gariepinus* included in the dataset of Agnèse and Teugels [27] is more distantly related to its conspecific sequences from our dataset as it falls within a different clade, together with sequences of species of *Bathyclarias*.

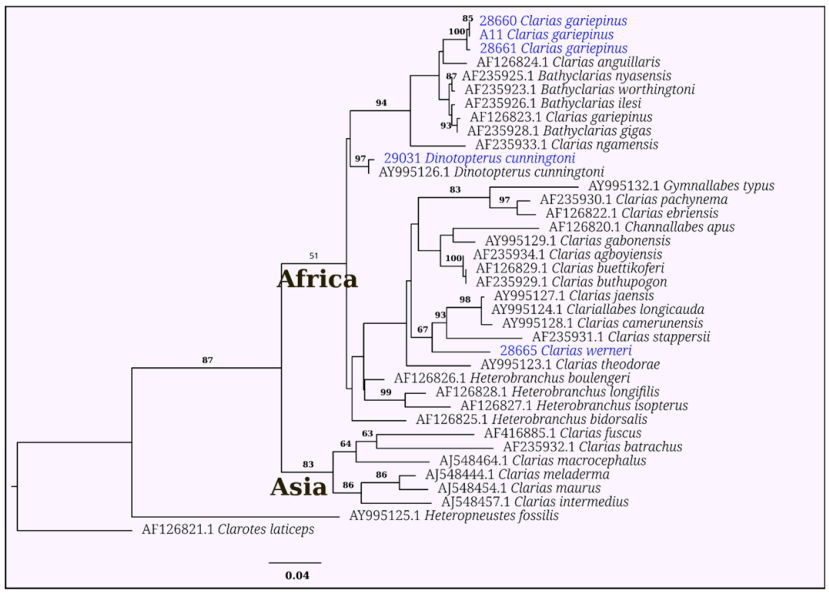

Figure 6. Maximum likelihood tree constructed using Cyt-b sequences of different species of Clariidae. Sequences generated in this study are shown in blue. The remaining sequences were obtained from the study by Agnèse and Teugels [27]. The indicated bootstrap values are those equal to or greater than 50. The scale bar represents the number of expected substitutions per site. Clades 'Asia' and 'Africa' as in Agnèse and Teugels [27].

4. Discussion

We used morphological and genetic approaches to identify catfish specimens obtained from fishermen at the north-western shores of Lake Tanganyika. We identified 11 catfish species using a morphological approach. Genetic methods validated our morphological identifications although ABGD underestimated the species diversity of the sample. The ABGD method suggested our dataset contained nine species differing from our morphological assessment of 11 species. This discrepancy arose because the ABGD method proposed two partitions for *Chrysichthys* rather than the four different species present in the dataset. This is no surprise as ABGD may lower the predicted number of groups by combining closely related species, such as those found in adaptive radiations (when the value for the parameter p is set close to 0.1) [38]. In contrast, the bPTP approach revealed 12 species. This agrees with what is reported in the literature where tree-based methods such as bPTP are mentioned as being prone to oversplitting. However, although bPTP may suggest more species than are actually present in the specific case of *C. acsiorum*, it has the advantage of more accurately delimiting other species within *Chrysichthys*. We will discuss these discrepancies below.

Chrysichthys

Members of *Chrysichthys* can be distinguished from other genera of Claroteidae in Lake Tanganyika (*Bathybagrus*, *Lophiobagrus*, and *Phyllonenus*) by their nasal barbels, forked causal fin, 37–44 vertebrae, fully ossified mesocoracoid arch, and free orbital rim [47]. Species of *Chrysichthys* in Lake Tanganyika can be categorized into two groups: *C. brachynema* (with *C. myriodon* Boulenger, 1900 as a junior synonym) with a well-developed postcleithral process, and *C. grandis*, *C. graueri*, *C. platycephalus*, *C. sianenna*, and *C. stappersii* that lack this character. Diagnostic features that separate the latter group from the former include differences in head and body proportions, palatal tooth plates, barbel lengths, and arrangement, gill raker number and form, and the limits of the lower and upper jaws [47]. It should be noted that *Chrysichthys* is rendered paraphyletic by the other Lake Tanganyika genera [10].

Additionally, Mo (1991) [48] placed all species in the latter group into *Bathybagrus* Bailey and Stewart 1984.

Due to inconsistencies in the revision of Mo (1991) [48], two databases that are authoritative in the field of fish taxonomy hold differing views on the classification of *Chrysichthys* from Lake Tanganyika. FishBase continues [8] to use Mo's classification, whereas the Catalog of Fishes [9] adopts that of Bailey and Stewart. Herein we followed the latter as it is also used by Fermon et al. [7]. It should be noted, however, that the COI sequences of *C. acsiorum*, *C. grandis* and *C. sianenna* revealed similarities with *Bathybagrus tetranema* (Bailey and Stewart 1984) between 96.2% and 97.7%, whereas similarities between *B. tetranema* and *C. brachynema* fall between 86.3% et 86.6% (see GenBank HG803444, HG803454 and HG803463 [10]). This raises questions about the current generic assignment of the Lake Tanganyika representatives of Claroteidae.

The phylogenetic tree constructed with COI sequences reveals two major groups. These groups correspond exactly to two partitions obtained by the ABGD method, namely: the first with the three individuals of *C. brachynema* and the second with the seven individuals of the other species of *Chrysichthys*. The subdivision into two groups for *Chrysichthys* of Lake Tanganyika is also supported by Cyt-b data. These results are therefore consistent with those obtained by Peart et al. [10].

This study provides the first available sequence data for *C. acsiorum*. This species was described based on museum specimens collected from Lake Tanganyika [28]. Since its description, it has been reported only once in the literature, quite recently [49]. The holotype and paratypes of the species originate from the same locality, Kajaga in Burundi; a locality not far from the region studied here (Figure 1). Regarding its geographical distribution, Hardman et al. [28] note that *C. acsiorum* appears to be limited to the village of Kajaga. In the current study, some specimens of *C. acsiorum* have been collected at Mwadiga, about 108 km further south revealing that the species is not restricted to Burundi.

In the study by Peart et al. [10] on Claroteidae of Lake Tanganyika, *C. acsiorum* was not included. However, the phylogenetic tree obtained with Cyt-b revealed that *C. acsiorum* grouped with *Chrysichthys* sp. 1 that was sampled in Kigoma (as HG 803396) and Mpulungu (HG 803377) [10]. Hence, we assume that the sequences of *Chrysichthys* sp. 1 from Peart et al. [10] may indeed represent *C. acsiorum*. More recently, Peart et al. [49] confirmed that the specimen listed as *Chrysichthys* sp. 1 in [10] from Kigima (HG 803396) is registered as *C. acsiorum* in the collections of the Cornell University Museum of Vertebrates (collection number CUMV95203). They did, however, not follow this identification in their study [49] as they found some discrepancies in measurements taken on the specimen and in the description of the species. Our morphological identifications did not reveal such differences. Hence, it remains to be examined whether the differences observed by Peart et al. [49] can be explained by geographical or ontogenetic variation. If this were the case, the description of *C. acsiorum* and the key for Lake Tanganyika representatives of *Chrysichthys* as presented in Hardman [28] would need to be changed. This would not necessarily be needed for the key of Fermon et al. [7], as this key is designed to identify specimens collected from the north-western shores of Lake Tanganyika. If the identity of the aforementioned specimens is confirmed as *Chrysichthys acsiorum*, then this species would be present in all sub-basins of Lake Tanganyika. In spite of their economic importance, the species of *Chrysichthys* of Lake Tanganyika continue to pose taxonomic challenges. Based on the results of the phylogenetic analysis of Claroteidae of Lake Tanganyika, Peart et al. [10], suggest that a revision of this family in this ancient lake is necessary. This is an opinion we share. It should be noted, however, that the genus formed a radiation in the lake and that hence, standard barcoding genes might be inadequate to delineate species [10,50]. Due to hybridization, which is an important factor in adaptive radiations [51], the evolutionary history of maternally

inherited mitochondrial genes might be different from the speciation process. Additionally, explosive speciation might simply not have allowed for enough time for mutations to accumulate. Both factors can explain the failure of ABGD to delineate the species in the clade of *Chrysichthys* that radiated (See Figure 3, clades 9–11).

Clariidae

Both COI and Cyt-b enabled us to unambiguously differentiate the three species of Clariidae collected here. Agnèse and Teugels [27] utilized Cyt-b, which allowed them to distinguish African from Asian representatives of Clariidae and to subdivide the African species into two groups: A and B. Group A comprised *Bathybagrus* spp., *Heterobranchus* spp., *D. cunningtoni*, *C. ngamensis*, *C. anguillaris*, and *C. gariepinus*. They also observed that *Heterobranchus* spp. and *D. cunningtoni* formed a monophylum that was sister to the monophyletic group consisting of *C. ngamensis*, *C. anguillaris*, *C. gariepinus*, and *Bathybagrus* spp. Only the monophyly of the latter group is supported by our findings. However, the low BS values obtained here do not allow us to draw any conclusions regarding the relationship between *Dinotopterus* and *Heterobranchus*. The grouping of the sequence of *C. gariepinus* from the dataset of Agnèse and Teugels [27] with species of *Bathyclarias* can be explained by the hypothesis that species of *Bathyclarias* derived from a population of *C. gariepinus* in Lake Malawi [52] and hence are daughter species of this wide-spread species [29].

Synodontis

The species of *Synodontis* from Lake Tanganyika have already been the subject of several morphological [29] and genetic studies [12,14,20]. One species in particular, *S. multipunctatus*, has attracted scientific attention due to its peculiar reproductive strategy, which involves parasitizing the broods of cichlid fishes [53]. Brood parasitism is observed in birds [54] and insects [55], but uniquely among fish in *S. multipunctatus*, and, potentially *S. grandiops*, with which it is often confused [12]. As the specimens of *S. grandiops* studied here have very similar or identical COI sequences to those obtained from catfish fry in parasitized cichlid broods [46], we added proof of *S. grandiops* being a brood parasite. However, confusion exists on the delineation of these species, which is reflected in our results. Both markers used revealed that the specimens identified as *S. grandiops* were 99.1% similar to what has been identified as *S. multipunctatus*, a finding also observed elsewhere [20,49]. In the key of Fermon et al. [7], the two species are mentioned to differ in morphometrics with *S. grandiops* having a larger eye (64.2% to 81.0% of snout length vs. 44.9% to 62.0%) and more branched pectoral fin rays (7 vs. 8) and in obtaining a much smaller maximum total length (150 mm vs. 280 mm) than *S. multipunctatus* [29]. It was recently reported that the currently known morphological delimitation of species of *Synodontis* from Lake Tanganyika does not correspond accurately to the genetic delimitation [49].

5. Conclusions

By integrating morphological and genetic methodologies, we identified 11 economically important catfish species from the north-western part of Lake Tanganyika. However, the ABGD method suggests the presence of only nine species, highlighting discrepancies with our morphological assessment, particularly within those species of *Chrysichthys* that are known to belong to radiation. Additionally, for species of *Synodontis*, a comparison with GenBank revealed potentially heterospecific matches. These findings underscore the existence of taxonomic problems in catfishes from Lake Tanganyika. We believe that these could be solved using an integrative taxonomic approach in species delimitation, as genetic methods may either amalgamate closely related species or fragment those with substantial intraspecific diversity. A yet unexplored avenue in revisions of catfishes is the inclusion of

data from monogenean gill parasites, as exemplified in a study on some tropheine cichlids from Lake Tanganyika [56]. We suggest adding data on these gill parasites in revisions of *Chrysichthys* and *Synodontis* of Lake Tanganyika, as these may provide relevant insights into fish systematics, considering their often-high host specificity.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/d17010008/s1. File S1: RBINS_specimens.xlsx Caption: List of fish specimens collected for this study. Specimens are deposited in the collection of the Royal Belgian Institute of Natural Sciences (RBINS). File S2: COI_alignment.fasta Caption: Edited alignment of Cytochrome *c* oxidase subunit I (COI) sequences. Positions with missing data were removed from the alignment. The file is in FASTA format. File S3: Accession_GenBank.xlsx Caption: Accession numbers for the genetic data generated in this study (COI and Cyt-*b*).

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