

Phylogeny and biogeography of African freshwater lates perches (Teleostei, Latidae)

Christian Arnold Schögg

(01414237)

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Priv.-Doz. Mag. Dr.rer.nat. Stephan Koblmüller

Karl-Franzens Universität Graz

Institut für Biologie

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Summary

Lates perches or Nile perches are a historically controversial clade within the order of Perciformes. Due to morphological similarities to the subfamily Centropominae (“snooks”), they were originally handled as Latinae, a subfamily in its own rights within the family Centropomidae. More recent phylogenetic analyses however, led to the elevation of this former subfamily, resulting in a separate family called Latidae. Comparative analyses of Indo-Asian *Lates* species and „Snooks" though, revealed close genetic relationships, which again led to a systematic reevaluation. Thus, the lates perches were regarded as a subfamily Latinae within the family Centropomidae again. The question of whether the two genera *Lates* and *Psammoperca* now form their own family or yet belong to the family of Centropomidae, is still controversial. Lates perches have a very large distribution area and occur in fresh and brackish water on three continents (Africa, Asia, Australia) and the Pacific and Indian Ocean.

Whereas previous work based on the analysis of genetic data focused mainly on Asian (and Australian) species including the closely related Centropomidae, this thesis is focused on the phylogenetic analysis of the African species of the genus *Lates*. In general, my work builds on the molecular genetic approach of previous studies, albeit a phylogenetic view of the entire taxon of the lates perches is emphasized. From Africa, as of 2019, seven species of lates perches are described. These include also four endemic species found only in Lake Tanganyika (*Lates angustifrons*, *Lates mariae*, *Lates microlepis* and *Lates stappersii*). In this work, as for the first time, the four Tanganyika species have been analyzed in a molecular phylogenetic context. Furthermore, four local lineages of *Lates niloticus*, a widespread species in African rivers and lakes have been investigated. The results of the phylogenetic analyzes have brought a new perspective on the phylogenetic position of the African *Lates* species.

The *Lates* Radiation in Africa is relatively a very young and also the settlement of *Lates* in Africa happened less than 10 million years ago compared to other genera or families.

It also turned out that the African *Lates* species split into two sister clades, a clade in the catchment area of the Congo including Lake Tanganyika and a clade in the northern sub-Saharan region.

Zusammenfassung

Die Riesenbarsche oder auch Nilbarsche genannt, sind eine historisch umstrittene Klade innerhalb der Ordnung der Perciformes (Barschartige). So wurden sie ursprünglich aufgrund von morphologischen Gemeinsamkeiten/Ähnlichkeiten als Unterfamilie Latinae zusammen mit den „Snooks“ (Centropominae) der gemeinsamen Familie der Centropomidae zugewiesen. Aktuellere Studien kamen jedoch zu dem Ergebnis, dass die Riesenbarsche eine eigene Familie (Latidae) darstellen. Genetische Analysen der Indo-asiatischen Arten der Riesenbarsche wurden 2011 mit denen von „Snooks“ verglichen und ergaben wiederum enge genetische Übereinstimmungen, was zur derzeitigen systematischen Einordnung führte. Somit werden die Riesenbarsche wiederum als Unterfamilie Latinae in der Familie der Centropomidae betrachtet. Die Frage, ob die beiden Gattungen *Lates* und *Psammoperca* nun eine eigenständige Familie bilden oder doch zur Familie der Centropomidae zählen, ist bis heute umstritten. Riesenbarsche haben ein sehr großes Verbreitungsgebiet und kommen im Süß- und Brackwasser auf drei Kontinenten (Afrika, Asien, Australien) und marin im Pazifischen und Indischen Ozean vor.

Meine Arbeit beschäftigt sich mit der phylogenetischen Analyse der afrikanischen Arten von Nilbarschen. Die zum damaligen Zeitpunkt beschriebenen asiatischen (und australischen) Arten wurden bereits 2011 gemeinsam mit den eng verwandten Vertretern der Centropomidae molekulargenetisch analysiert. Meine Arbeit baut auf dieser molekulargenetischen Methodik auf, wodurch nun eine phylogenetische Betrachtung des gesamten Taxons der Riesenbarsche ermöglicht wurde. Aus Afrika waren bis 2019, sieben Arten von Riesenbarschen beschrieben. Dazu gehören die vier im Tanganyikasee endemisch vorkommenden Arten *Lates angustifrons*, *Lates mariae*, *Lates microlepis*, und *Lates stappersii*, die im Zuge dieser Arbeit erstmals mit modernen molekulargenetischen Methoden analysiert und phylogenetisch erfasst wurden. Weiters wurden vier lokale Artlinien der in afrikanischen Flüssen und Seen weitverbreiteten Art *Lates niloticus* untersucht.

Die Ergebnisse der phylogenetischen Analysen haben eine neue Perspektive auf die phylogenetische Position der afrikanischen *Lates* Arten gebracht.

Die Radiation der Gattung *Lates* in Afrika ist relativ jung, und auch die Ansiedlung von *Lates* in Afrika fand im Vergleich zu anderen Gattungen oder Familien vor weniger als 10 Millionen Jahren statt.

Es stellte sich auch heraus, dass sich die afrikanischen *Lates* Arten in zwei Schwestergruppen aufteilten, eine Gruppe im Einzugsgebiet des Kongo einschließlich des Tanganjikasees und eine Gruppe in der nördlichen Region südlich der Sahara.

Introduction

The genus *Lates* (Latidae) includes species that occur in marine, brackish and freshwater habitats. They span a large geographic range covering three continents (Africa, Asia and Australia) and two oceans (Indian Ocean, Pacific Ocean) (<https://www.fishbase.de/Nomenclature/ScientificNameSearchList.php>). Therefore, the different species of *Lates* also have a strikingly different distribution pattern with respect to their habitats. Four species of *Lates* occur in coastal areas and estuaries of the Indo-Pacific region, but cannot be found along the African coast. The remaining seven *Lates* species live in large African freshwater systems, especially in large rivers and some of the large African Rift Valley Lakes. All species of the genus *Lates* are carnivorous, preying mainly on other fish like cichlids and cyprinids, but also aquatic and semi-aquatic vertebrates and invertebrates (Mkumbo et al. 1992; Coulter 1976), (Supplementary figure 4). Due to their large maximum body size and their active hunting behavior, most *Lates* species are also the top predators in their respective habitats. With their body coloring patterns are these predators, especially the juvenile *Lates* perches ideal camouflaged. (Supplementary figures 2 and 3). Especially the African *Lates* species and the Barramundi (*Lates calcarifer*) are very important food fish for the local population (Supplementary figure 5). In several locations even large fishing industries have developed. A well-known example poses Lake Victoria, where the Nile perch (*Lates niloticus*) was introduced in 1962 (Goudswaard et al. 2008) and a large fishing industry was built specifically around the Nile perch. Nowadays these fish are being exported to many different parts of the world. In supermarkets in Europe and other parts of the world, the Nile perch is available as a food fish commonly known as "Victoria Perch" (Turon et al. 2006). However, the introduction of Nile perch into Lake Victoria, triggered a massive cascade of ecological consequences. The native fish species composition of Lake Victoria, which consisted largely of endemic cichlids, was not adapted to the appearance of a large predator and many species were massively decimated, leading even to the extinction of some endemic species (Pringle 2005). However, Lake Tanganyika poses a crass counterexample to the ecological disaster of Lake Victoria. Here, the natural fish community has developed in the presence of the top predators *Lates* perches over a long period of time.

Currently, seven species are described within the genus *Lates* in Africa, spanning a range of different distribution areas (Figure 1). *Lates niloticus* has a very wide distribution range,

which extends from the Ethiopian highlands on the Blue Nile to the catchment area of the Congo and the Niger to the West African coast of Senegal. Also, in the Rift Valley Lakes Lake Albert, Lake Turkana, Lake Chamo, Lake Abaja and Lake Chad the presence of *Lates niloticus* is confirmed (<https://www.iucnredlist.org/search?query=Latidae&searchType=species>). A possible occurrence in Lake Chew Bahir is doubted. In addition, *Lates niloticus* was introduced by humans in Lake Victoria, Lake Kyoga and Lake Nabugabo (Ogutu-Ohwayo 1990). The remaining six species occur in three large Rift Valley lakes. In Lake Tanganyika, the four endemic species *Lates angustifrons*, *Lates mariae*, *Lates microlepis* and *Lates stappersii* occur. In Lake Albert it is assumed that in addition to *Lates niloticus* also a second species, the endemic *Lates macrophthalmus* (Worthington 1929) is present. In Lake Turkana also a second *Lates* species occurs sympatrically with *Lates niloticus*, which is the endemic *Lates longispinis* (Worthington 1932). *Lates macrophthalmus* and *Lates longispinis* were described as distinct species in 1929 and 1932 respectively, by the British ecologist Edgar Barton Worthington (Worthington 1929, Worthington 1932).

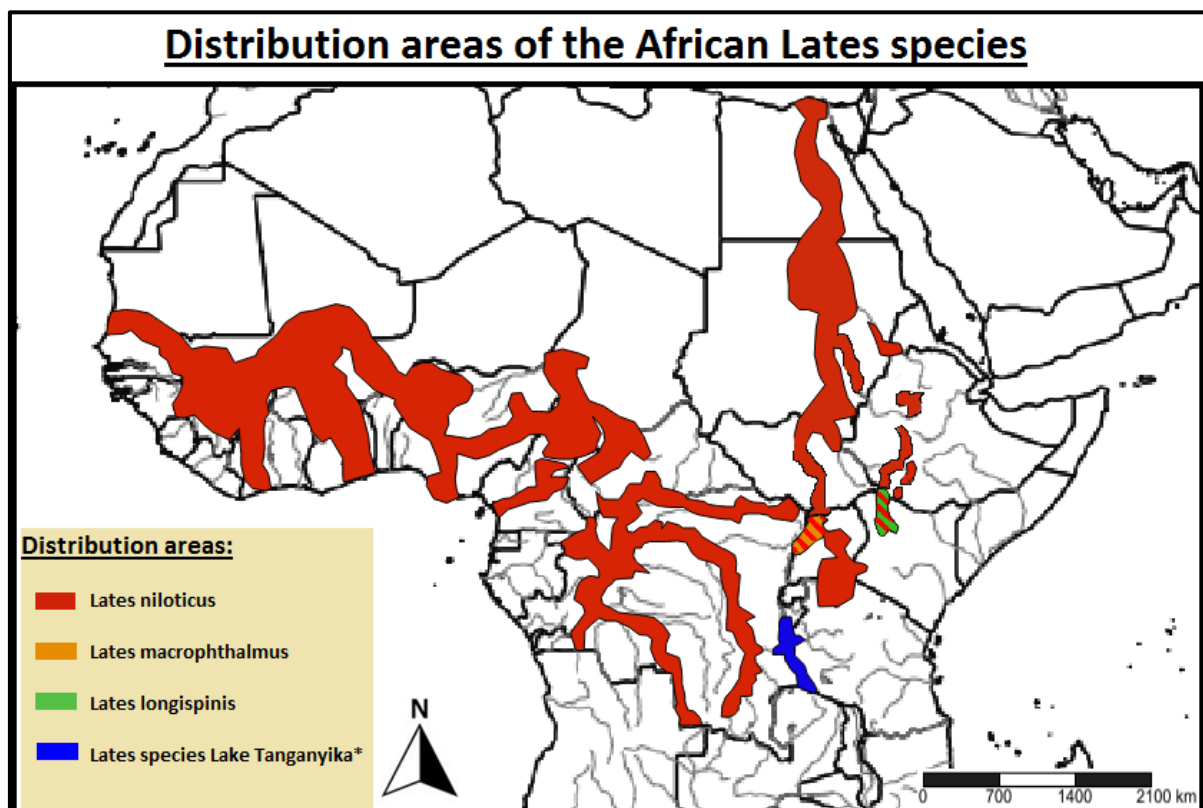


Figure 1: Map of distribution areas of African Lates species. * *Lates* species of Lake Tanganyika include *Lates angustifrons*, *Lates mariae*, *Lates microlepis* and *Lates stappersii*.

As a result of further investigations, it was noted that despite the smaller body size of these two species, they were adapted to deep-water habitats (Campbell et al. 2005) in the two lakes, similarly to *Lates mariae* in Lake Tanganyika. However, the species status of these two species is still questionable, as no clear morphological and genetic findings do confirm their species status. Furthermore, hardly any literature confirming the validity is available either. Thus, it is more likely that *Lates macrophthalmus* and *Lates longispinis* are deep-water morphs of *Lates niloticus* (Van Steenberge, unpublished data). This study also attempted to sample specimens of *Lates macrophthalmus* and *Lates longispinis* by Co-author and assistant of this study Clemens Lorber at Lake Albert and Lake Turkana. Also, based on the few known external characteristics, three specimens identified as *Lates macrophthalmus* and two specimens identified as *Lates longispinis* were analyzed. Unfortunately, the phylogenetic analyzes revealed that all five specimens were *Lates niloticus*. African lates perches have been reclassified and taxonomically examined many times since the first description of the species *Lates niloticus* over 250 years ago (Linnaeus 1758), (Namulawa et al. 2013). So far, the interspecific relationships within the genus *Lates* are still not fully understood. While some detailed morphological studies leading to phylogenetic hypotheses have been carried out (Greenwood et al 1976; Otero 2004), only few studies based on molecular genetic data exist (Hauser et al 1998). Specifically, despite their grave importance as top predators for the ecosystem of Lake Tanganyika (Coulter 1976) -the second largest available freshwater reservoir on earth (Schubert et al. 2006)- on the one hand, and as a food source and commodity for more than 10 million people living around the lake (<https://www.africangreatlakesinform.org/article/lake-tanganyika>, visited on 27.07.2019) on the other hand, the four endemic *Lates* species from Lake Tanganyika were never analyzed with molecular genetic methods before. Based on morphological data, the four endemic species of Lake Tanganyika are assumed to pose a monophylum within African lates perches (Greenwood et al 1976). In order to understand the relationships and today's distribution pattern of the African *Lates* species, it is important to know how and when the African continent was populated by ancient lates perches. Two facts are relevant in this aspect, firstly, the age of the African species and secondly, the historical distribution of the ancient *Lates* species at the time of the species emergence. The approximate age of the African species can be interpreted by the results of the phylogenetic analysis. A closer look at the ancient distribution pattern of the genus *Lates* in Africa is of course no longer possible today.

However, it is possible to approximately date back their origin due to occasional findings of fossils (Figure 2). The comparison of fossil finds with a similar age leads to an information network, which helps to draw conclusions about the colonization of Africa by lates perches. In order to fully explain recent distribution patterns, information from ancient geography and hydrology is also needed.

What we already know today is that the spread of *Lates* in Africa is closely related to the formation of the African Rift Valley and its associated processes. Above all, the historical course of the large African rivers and lakes in and around the African Rift Valley provides the important insights to understand today's distribution pattern of lates perches in Africa. The formation of the African Rift Valley led to constant changes in the catchment area of the major rivers Nile, Congo and Niger (Goudie 2005). Up and down processes formed large valleys and basins and changed them again constantly (Ring et al. 2014). Large regions of central Africa have been drained into different river basins over the course of millions of years. This favored the spread of Fish communities and also lates perches along the African continent south of the Sahara (Salzburger et al. 2014).

There are two common hypotheses about the relationships of the African *Lates* species (Figure 3). Greenwood provides the four Lake Tanganyika species (*Lates angustifrons*, *Lates mariae*, *Lates microlepis* and *Lates stappersii*) as a monophyletic sister group to all other *Lates* species in Africa and Asia (Greenwood 1976). Otero contradicts Greenwood's hypothesis that the Lake Tanganyika species are of monophyletic origin. Only *Lates mariae* and *Lates microlepis* are of monophyletic origin and *Lates angustifrons* and *Lates stappersii* are the two sister groups thereof (Otero 2004).

Phylogenetic hypothesis based on morphological characters:

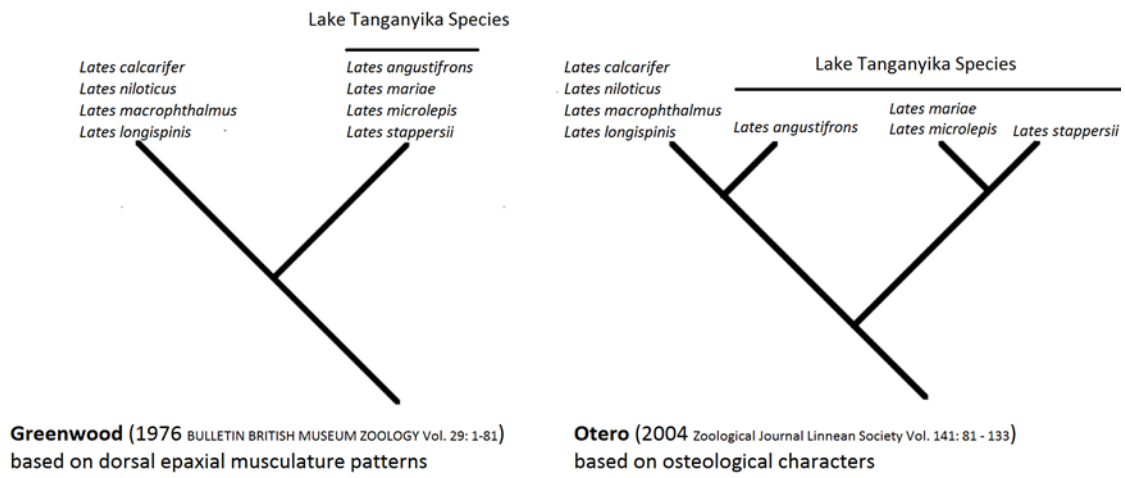


Figure 2: Phylogenetic hypothesis about the genus *Lates*.

Both hypotheses are based on morphological analyzes. Greenwood's analysis is based on dorsal epaxial musculature patterns and the analysis of Otero on osteological characters.

Fossil finds of genera Lates and Eolates in Africa, Europe and the Middle East represented by geological epochs:

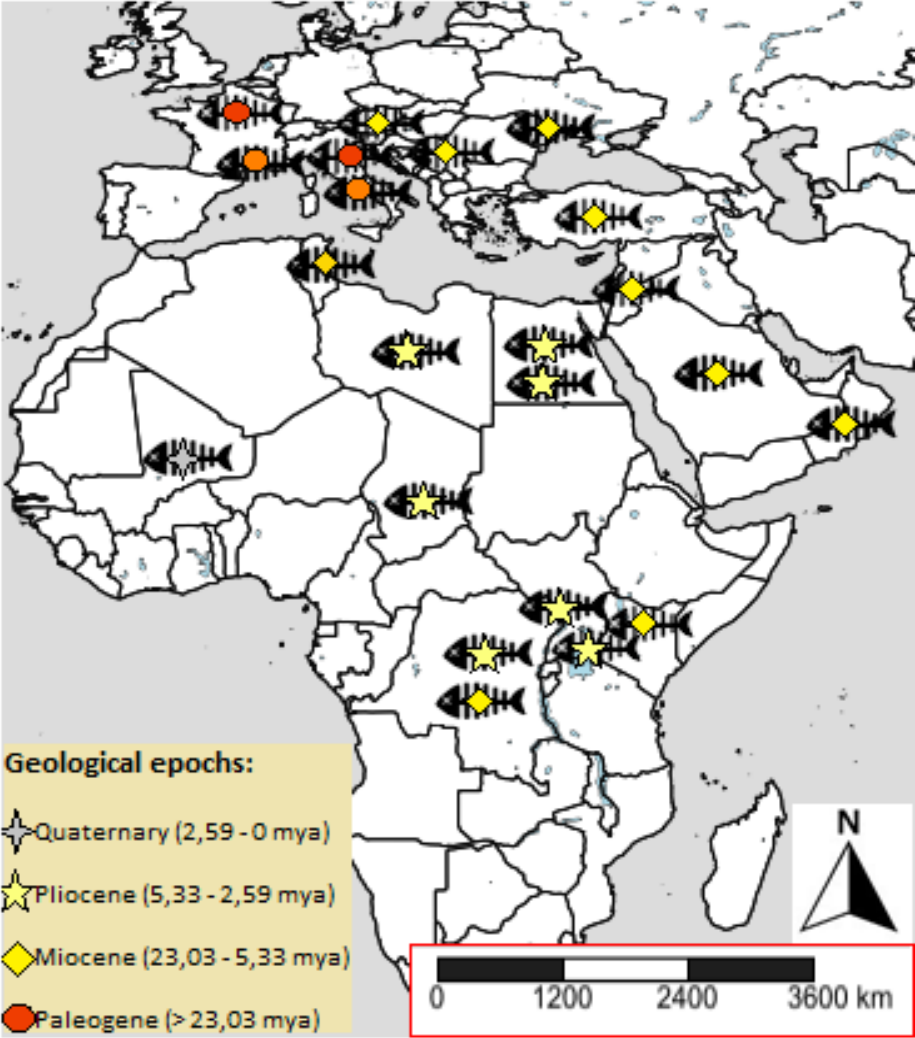


Figure 3: Fossil finds of the genera *Lates* and *Eolates* in countries of Africa, Europe and the Middle East. Color coded symbols indicate different geological epochs. Data: Otero 2004

Aims of this work

This work should provide new insights into diversity, biogeography and evolution of African lates perches.

The main aim of this work is to examine the systematic and phylogenetic relationship of African lates perches and compare it with their Indo-Asian relatives (Li et al. 2011) to gain new insights into the taxonomy of the family of Latidae (Mooi & Gill 1995).

Another aim of this study is the genetic analysis of the species *Lates niloticus* with its extensive distribution scheme in Africa (Figure 1) based on samples from several geographically isolated regions.

A main focus of the study is concentrated on the position of the four endemic species of Lake Tanganyika in the family Latidae. These have never before been studied by molecular genetic methods and analyzed phylogenetically. In addition, the results should be able to ascertain which of the two hypotheses regarding the phylogenetic relationships of the African *Lates* species is correct, or whether both are to be falsified (Figure 2). One of the most important questions in this Case to be answered in this study is whether the African lates perches are of monophyletic descent.

Furthermore, this work should help to improve the understanding of the colonization of the African continent by the genus *Lates*, as well as their current distribution.

The purpose of this study was to finally answer the last phylogenetic questions about Lake Tanganyika Species within the family Latidae, thus closing a circle that began more than 250 years ago with Carl Linnaeus and a giant freshwater fish discovered in Africa.

Materials and methods

a. Taxon sampling

A total of 177 specimens of *Lates* from Congo, Zambia, Tanzania, Burundi, Uganda, Kenya and Nigeria were sampled for this study (Supplementary tables 2 and 3). Most of the samples from Lake Tanganyika were sampled during a two-week field trip in 2018 in Zambia at the south coast of Lake Tanganyika near the small town Mupulungu. Samples were also obtained from Lake Turkana in Kenya near the city of Lodwar and some samples from Lake Albert in Uganda near the city of Masindi (Figure 4). Samples were also used from previous studies on Nile perch parasites and museum records (Supplementary Table 1). These samples were kindly provided by the Royal Museum for Central Africa, Leuven (Belgium), the University of Leuven (Belgium) and the Masaryk University Brno (Czech Republic). Some fishes were also bought from the aquarium trade (Supplementary Table 1). In total 105 new samples were obtained for this study, the remaining samples were borrowed.

The DNA samples were collected as fin clips taken from the fish and fixed in Eppendorf tubes with a volume of 2 milliliters in 95% ethanol for further processing. There are three methods for obtaining suitable tissue for the sampling of the respective species (Supplementary figure 7). The most commonly practiced method is to visit the local fish markets for sampling of the fishermen's prey. Another method is active fishing with fishing rod and lure for sampling live fish before releasing. And finally, snorkeling/scuba diving with nets or gillnets to actively catch juvenile Nile perch and sample them. All three methods have their advantages and disadvantages. Some species, such as *Lates stappersii*, could only be sampled at the fish market because they cannot be caught in their habitat with a fishing rod or a small net. Fish samples from the fish markets often were of poor quality, which unfortunately made many of these samples useless in later DNA extraction. Fishing with rod and lure was very difficult and brought little success at the time of our field trip (March/April 2018), because it was just the end of the rainy season and the lake and its tributaries had high water levels. The advantage of active fishing, however, is that you can get fresh samples, which contain enough usable DNA for extraction. The third option for getting the samples, catching by snorkeling is only possible with juvenile fish, because adults stay in great depths and are hardly accessible. The problem here is that only the species *Lates microlepis* lives in the

immediate coastline (depth max 10m) during their juvenile stage. Due to these prevailing conditions, all three sampling methods had to be used to prepare enough samples for the study.

Furthermore, I have also tried to find samples of *Lates longispinnis* in Lake Turkana and *Lates macrophthalmus* in Lake Albert. The problem, however, is that in both cases, not even local fishermen could safely confirm the existence of these two species. Thus, possible specimens of these two species could only be considered and sampled based on comparative images (Sampling codes: Lalo, *Lates longispinis*; Lamc, *Lates macrophthalmus*; Lasp, *Lates* sp. = indefinite individual).

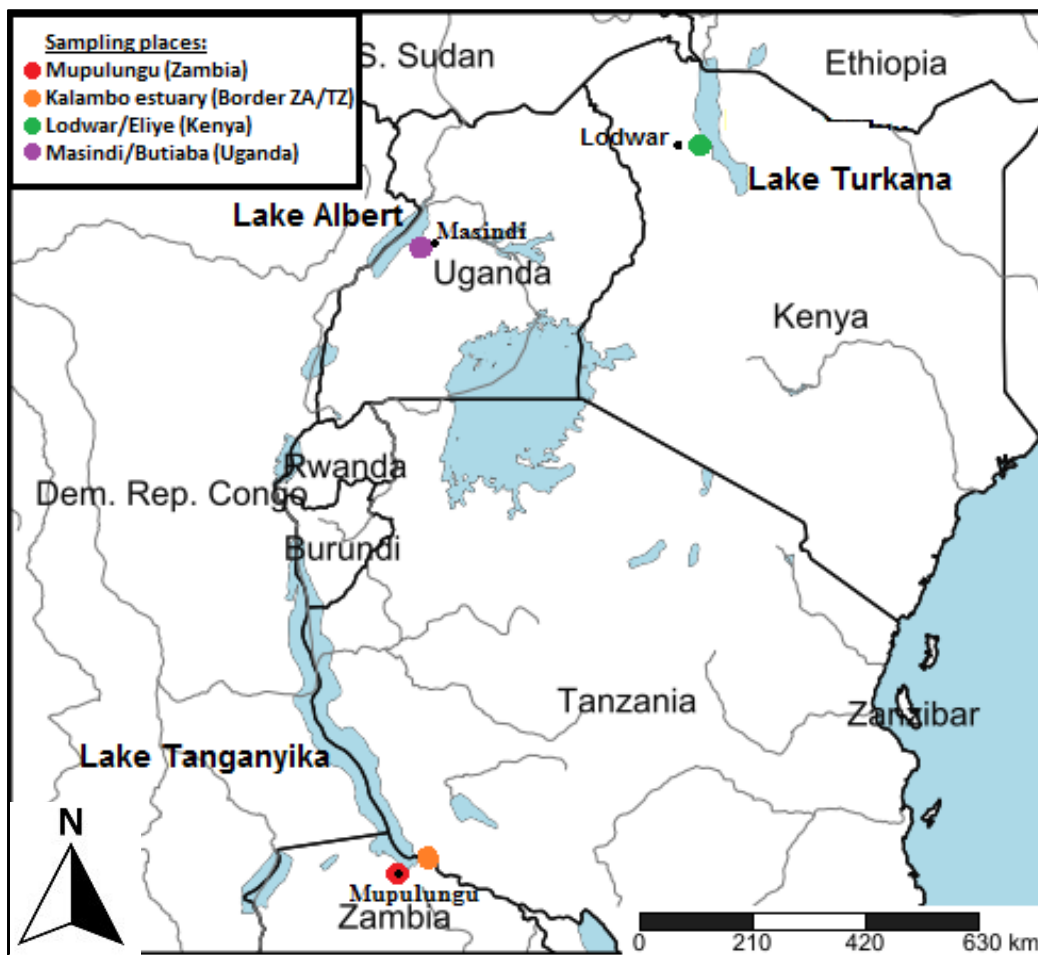


Figure 4: Sampling Places on the African Rift Valley lakes Lake Tanganyika, Lake Turkana, Lake Albert

b. DNA extraction, polymerase chain reaction and sequencing

DNA extraction from tissues is the first step in the laboratory in the amplification of DNA by PCR and the sequencing of these PCR results. I used two extraction methods. The first method is based on an extraction kit. The DNA samples were here extracted from the ethanol-conserved fin tissues using the DNeasy Blood and Tissue Kit (Qiagen). This efficient method allows high yields of DNA from tissue samples. I compared this more expensive extraction method with another based on the use of Chelex-100 resin (Richlen and Barber 2005). This chelating resin was also used to extract DNA from the fin clip samples for PCR amplification. This extraction process is faster, easier and cheaper than the former. However, the extraction results are also of noticeably lesser quality than the extraction kit. When the fin clips were taken from live or freshly killed fish, the difference in quality was less obvious. But especially with samples taken from fish markets, which often lacked appropriate cooling, no DNA could be obtained with the Chelex method. Therefore, most of the samples had to be extracted with the extraction kit.

The polymerase chain reaction (PCR) was used to amplify the target genes to generate enough copies of the particular DNA segment for a successful sequencing reaction. (Table 1). For some markers a nested PCR was needed to obtain useful results. This nested PCR was used to amplify genes if the first round of PCR did not produce a clean product. For this, the products of the first round of PCR (PCR 1) were diluted 10-100-times and used as a template for a second PCR with a set of new primers (PCR 2). All PCRs were performed in a total volume of 10,0 ml per sample, including 0.1mL SupraTherm (GeneCraft) Taq DNA Polymerase (1U), 1,0 mL 10 x PCR buffer, 0,35 mL dNTP mixture (0,25 μ M), 0.25 mL of the respective forward and reverse primers (0,25 μ M), 2,0 mL DNA template, and 6,05 mL double distilled water (Double-distilled water is double-microfiltered, demineralized water in a particularly pure form). PCR products were also visualized on agarose gels in an electrophoresis to check the quality and size of the amplification

Last step before sequencing reaction is to purify the PCR products with ExoSAP-IT (ThermoFisher). The sequencing reaction was performed according to the Sanger method. The total volume per sample was 8 mL, including 0,4 mL Bigdye (ThermoFisher), 0,25 mL forward or reverse primers, 1,6 mL 5x sequencing buffer, 2,75 mL double distilled water and

3 mL PCR-products. After sequencing reaction, the DNA fragments were purified with Sephadex™ G-50 (GE Healthcare) and visualized on an ABI 3130xl capillary sequencer (Applied Biosystems).

In this study, eleven core protein coding genes and one mitochondrial gene were analyzed as phylogenetic markers. All genes used in this study were developed and optimized by Chenhong Li (Li et al., 2008; Li and Orti, 2007; Li et al., 2007; Li et al., 2010; Li et al., 2011) and in the comparative study used for the phylogeny of snooks and Indo-Asian *Lates* perches (Li et al., 2011). Recombination-activating gene 1 (rag1) from the mentioned study (Li et al. 2006) was omitted in this work.

In a first step, a so-called "screening" was performed on all extracted 177 specimens in order to produce a sample set for the phylogenetic analyzes from these results. For this purpose, DNA barcodes were generated based on mtDNA CO1 gene (Hebert et al., 2003; Ward et al. 2005). Subsequently, following 11 nDNA markers and 1 mtDNA marker (Table 1) were sequenced for 31 individuals of African *Lates* species.

In addition, sequences from Indo-Asian *Lates* and *Psammoperca* Species (Li et al., 2011) were also downloaded from GenBank for further analysis and comparison.

Table 1: Primer compositions, annealing temperatures, numbers of cycles, best substitution model and length of amplified fragment (bp). Table was changed after Li et al. 2011. * Gene markers are named following annotations in ENSEMBLE. 16S rRNA; Ficd, FIC domain containing; kbtbd4, Kelch repeat and BTB (POZ) domain containing 4; kiaa-1234 Leucine-rich repeat and WD repeat-containing protein, KIAA1239-like; myh6, myosin, heavy polypeptide 6; plagl2, pleiomorphic adenoma gene-like 2; RIPK4, receptor interacting serine-threonine kinase 4; sidkey, si:dkey-174m14.3; SLC10A3, zgc:85947; sreb2, Super conserved receptor expressed in brain 2; Zic1, zic family member 1; znf536, novel zinc finger protein Fragment; Li et al. 2011** Abbreviations: TN93: Tamura Nei; HKY85: Hasegawa-Kishino-ano; K80: Kimura 2 *** COI, Cytochrome c oxidase I: Screening step/barcoding; Ward et al. 2005

Gene *	Primer sequences	Annealing [°C]	# cycles	Models **	bp	PCR steps
16S						
16s_135F	5' GCAATAGAVAWAGTACC GCAAGG 3'	52	45	TN93	815	
16S_1072R	5' CCTTYGCACGGTYARAATAC 3'					
ficd						
ficd_F166	5' GTSGTCCARGCGGAYCACCTCTA 3'	52	45	HKY85	664	
ficd_R965	5' GTGCATTTGGCKATRAATCGRA 3'					
kbtbd4						
kbtbd4_F79	5' TGTGAYGAGGACGAYGCSATCAG 3'	56	45	K80	582	
kbtbd4_R861	5' TCAGGCCAGWACRAACTGCCAGT 3'					
KIAA 1239						
KIAA1239_F273	5' GAGGCTCGAAARCTNTGGTGGCT 3'	56	45	K80	908	
KIAA1239_R2079	5' GTCCACAGAARGCRTACATYCCATC 3'					
myh6						
myh6_F459	5' CATMTTYTCCATCTCAGATAATGC 3'	50	45	TN93	716	
myh6_R1325	5' ATTCTCACCACTCCAGTTGAA 3'					
plagl2						
plagl2_F9	5' CCACACACTCYCCACACAGAA 3'	55	45	K80	670	1st
plagl2_R930	5' TTCTCAAGCAGGTATGAGGTAGA 3'					
plagl2_F51	5' AAAAGATGTTTACC GMAAAGA 3'	58				2nd
plagl2_R920	5' GGTATGAGGTAGATCCSAGCTG 3'					
Ripk4						
RIPK4_F57	5' GCCAAGTTGATGAAGATCC TVCAG 3'	58	45	HKY85	571	
RIPK4_R880	5' CCCTCTTCTATCAGCATYTRACTGT 3'					
Sidkey						
sidkey_F116	5' CGGATGARGYCTGCAGCAG 3'	50	45	HKY85	1011	1st
sidkey_R1360	5' GCTGGGCTTKGT CAGACTGT 3'					
sidkey_F247	5' GACCTSTACAGCAGY GACAC 3'	56				2nd
sidkey_R1355	5' GCCTTKGTCAGACTGCCTT 3'					
SLC10A						
SLC10A3_F486	5' CCTCATYTYGTCAACAAGTGTGC 3'	57	45	HKY85	645	1st
SLC10A3_R1273	5' GCATCTCCRAVGTGCTGCT 3'					
SLC10A3_F520	5' AAGGTGGAGGTGGAGGTGCT 3'	59				2nd
SLC10A3_R1256	5' CTSAGGGCCACGATGAAGG 3'					
sreb2						
sreb2_F10	5' ATGGCGAACTAYAGCCATGC 3'	52 - 54	45	HKY85	881	
sreb2_R1094	5' CTGGATTTTCTGCAGTASAGGAG 3'					
zic1						
zic1_F9	5' GGACGCAGGACCGCARTAYC 3'	56	45	HKY85	659	1st
zic1_R967	5' CTGTGTGTCTCTTTTGTGRATYTT 3'					
zic1_F16	5' GGACCGCAGTATCCACAYMT 3'	58				2nd
zic1_R963	5' GTGTGTCTTTTGTGAATTTTYAGRT 3'					
znf 536						
znf536_F1	5' ATGGAGGACTCYAGTTTGTG 3'	62	45	TN93	994	
znf536_R1532	5 AGGAGCGATCGYTTYTCATTTTC 3'					
COI***						
FishF1	5' TCAACCAACCACAAAGACATTGGCAC 3'	50	40	***	700	
FischR1	5' TAGACTTCTGGGTGCCAAAGAATCA 3'					

c. Data handling and phylogenetic analyses

Raw sequences of each gene were corrected manually and aligned using MUSCLE (Edgar 2004) in the MEGA 7 software - Molecular Evolutionary Genetics Analysis (Kumar et al. 2016). Separate alignments were generated for each gene individually.

First, a Maximum Likelihood (ML) analysis was conducted for each gene using the best fitting substitution model (Table 1) and 1000 Bootstrap (BS) replicates as statistical branch support on the web-based PhyML (<http://www.atgc-montpellier.fr/phyml/>). The substitution models were calculated for each gene based on the Bayesian Information Criterion (BIC). The aim of these analyzes is to generate single genealogical trees for all 12 genes.

In a second step the genetic data including all genes was concatenated in a combined dataset of mtDNA and nDNA markers (Table 1) for generating a time calibrated Bayesian Inference tree. The time calibrated tree including all 12 genes was generated based on Bayesian Inference (BI) with the program BEAST v.2.5.2 (Bouckaert et al. 2014), which was run for 500 million generations with a sampling frequency of 5000 generations and a burnin phase of 25%. Data for this tree was partitioned per gene using the best fit substitution models for each gene suggested by the model test and a molecular clock (model "relaxed uncorrelated lognormal clock") for each gene (Table 1). We employed a lognormally distributed prior on the root age based on a minimum age of the oldest *Eolates* fossil of 48,6 million years (Otero 2004). This calibration point is the splitting between Psammopercinae and Latinae. All prior information for this calibration point (M = 15.0, S = 1.0, offset 48.6 million years) was set in BEAUTi.

Furthermore, mean net distances between clades were calculated for each Gene in MEGA7 using the best fitting substitution model, proposed by the model test (Table 2) (Substitution tables 4).

Results

In this study, DNA-barcodes were generated for 117 out of 177 samples in the so-called "screening step". Based on these genetic barcodes, which rely on interspecific variation to discriminate between species, a final set of samples representing all species and sampling locations was created. For this purpose, the mitochondrial cytochrome oxidase subunit 1 (COI) gene was sequenced.

Based on these results, a total of 31 good-quality samples from the African *Lates* species (*Lates niloticus*, *Lates angustifrons*, *Lates mariae*, *Lates microlepis*, *Lates macrophthalmus* and *Lates longispinis*) and additionally from *Lates niloticus* samples from four distribution areas in Africa (Nile basin, Congo basin, West Africa, Turkana Omo basin / East Africa) were analyzed for 11 nDNA genes and one mtDNA gene (Table1). Samples assigned to *Lates macrophthalmus* and *Lates longispinis* (Lasp44, Lasp45, Lalo2) could only be assigned to images using morphological differentiation criteria. These samples could not be distinguished from *Lates niloticus* according to the genetic analyzes, which is why they were also regarded as *Lates niloticus* in the results. Of the 31 samples that were subjected to the genetic analysis, two samples were finally counted to *Lates mariae*, six samples to *Lates microlepis*, four samples to *Lates stappersii*, four samples to *Lates angustifrons* and 15 samples to *Lates niloticus*. Of the 15 samples from *Lates niloticus*, five were assigned to one Nile lineage, two to a West African lineage, four to a Congo lineage and four to an East African lineage. Individual alignments were created for all genes, containing all 31 samples ranging from 550 bp to 1100 bp. For the mtDNA 16S gene a relatively high mutation rate within the *Lates* species, similar to the mtDNA COI gene, could be observed. For all 11 nDNA genes, much lower mutation rates were detected.

The single gene analyses showed a very heterogeneous picture. No general topology was recovered by all individual genealogical trees in the ML analysis (Supplementary figures 1). These divergent results from the maximum likelihood-based single-tree analyzes were also reflected by the on average low genetic distances (Supplementary Tables 4).

Molecular genetic results though could support the assumption that there are four major lineages of *Lates niloticus* and that there are genetic differences between the four lineages.

Time calibrated Bayesian Inference tree shows a recent speciation of the African *Lates* species. These separated from their Asian relatives (*Lates japonicus* and *Lates calcarifer*) about 9-10 million years ago.

The African *Lates* species divide into two sister clades. One sister clade in the catchment area of the Congo, including the Lake Tanganyika species, and a second clade containing samples from the vast area of West Africa to the Ethiopian Highlands, including the Nile river catchment. The split of these two clades occurred around 3 million years ago. According to the results of the species tree analysis, the Congo clade predates the second clade. The splitting of clades, lineages and species is always 100% supported in the species with the exception of the splitting of the African clade into *Lates japonicus* (79%), which confirms the result as best as possible.

The splitting of clades, lineages and species is always 100% supported in the species with the exception of the splitting of the African clade off the Asian clade (*Lates japonicus*, 79%), which confirms the result as best as possible.

The results of the species tree analysis can replace both hypotheses of a monophyletic descent of *Lates niloticus* and *Lates calcarifer* (Greenwood 1976) and the hypothesis of a polyphyletic descent of Lake Tanganyika species (Otero 2004) (Figure 3). *Lates niloticus* shares a lineage with all other African *Lates* species. The Tanganyika species pose a monophyletic lineage and are genetically relatively closely related to *Lates niloticus* from the Congo basin, from which they have diverged about 2.5 million years ago.

The West African lineage shows greater genetic differences to both Nile basin lineage and Congo basin lineage. But still it is clearly associated to the *niloticus* clade. However, the Congo lineage of *Lates niloticus* is more closely related to the Tanganyika species (Figure 5). The two very closely related lineages of *Lates niloticus*, the Nile basin lineage and Turkana/Omo basin lineage show only very slight genetic differences., since the Turkana/Omo basin was populated from the Nile and only about 1.9 - 1,3 million years ago, when the two basins still had a connection, happened (Johnson et al., 2009) (Figure 6).

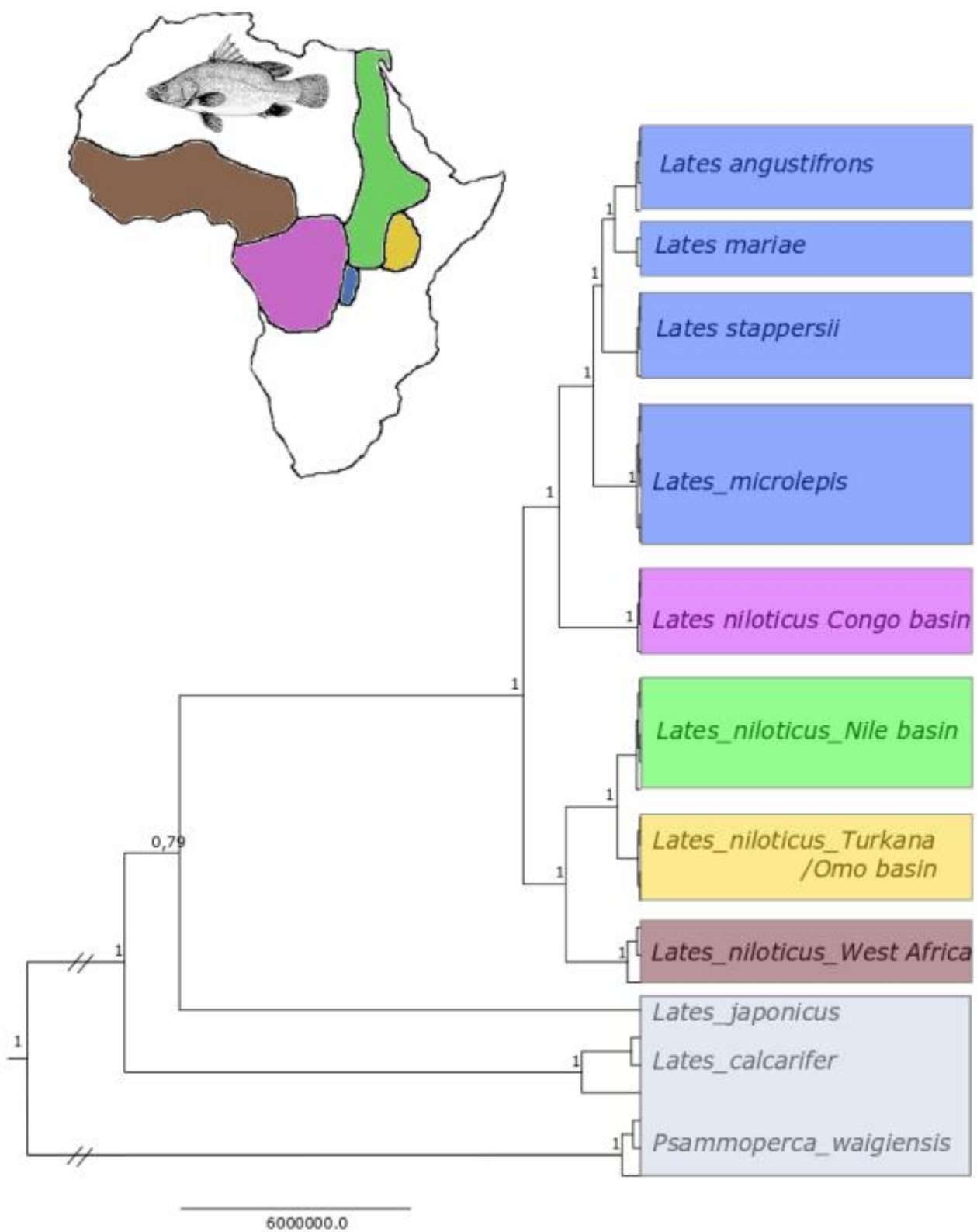


Figure 5: Species tree based on 11 nDNA and one mDNA markers of the African Lates species. Colors represent the geographic origin; node labels indicate posterior probabilities.

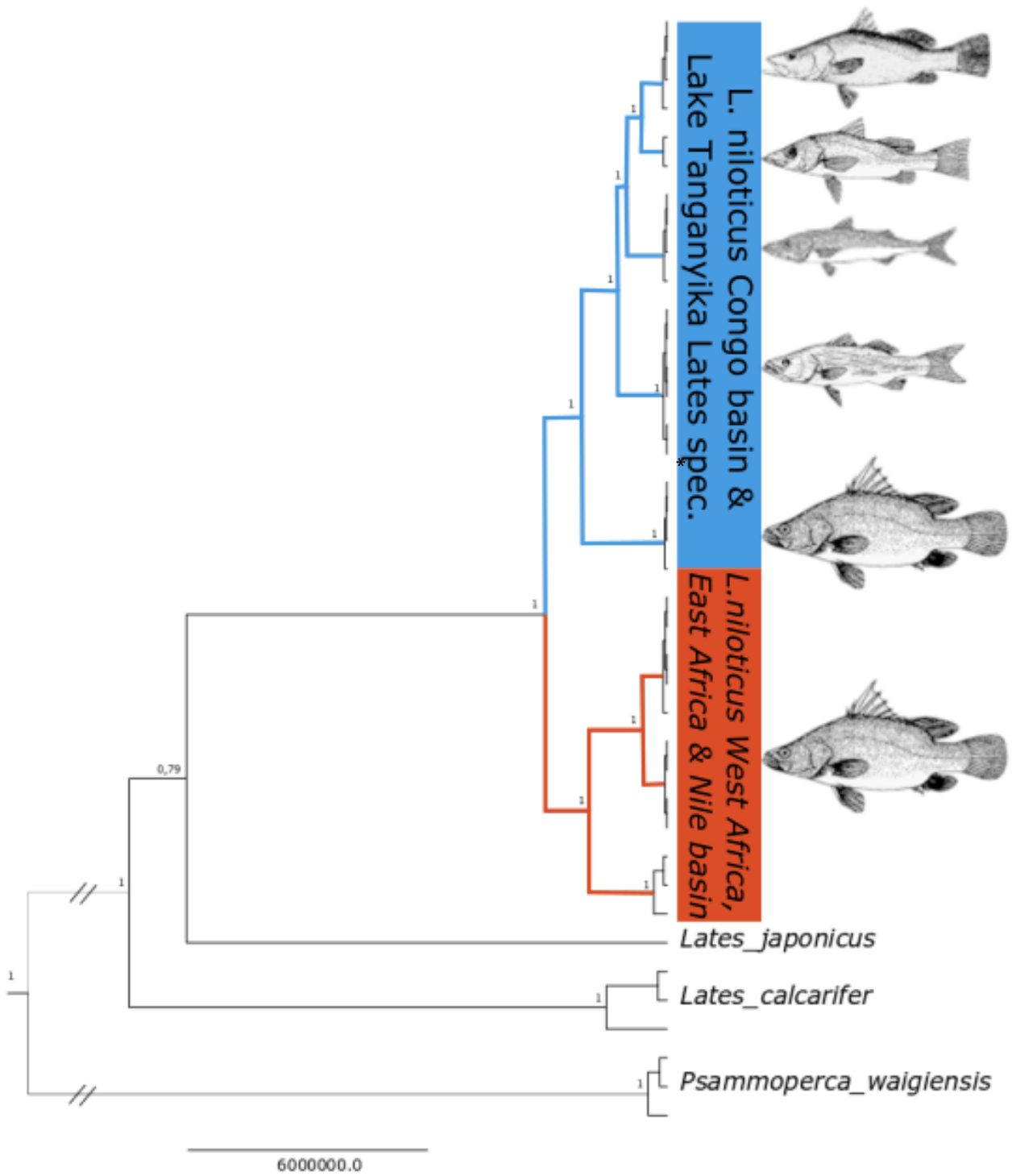


Figure 6: Species tree highlighting the geographic distribution of *L. niloticus* samples. The separation into two major clades is indicated by different colors.

Comparison of the mean net distances of the respective sequences for each gene revealed that both the Tanganyika species (*Lates angustifrons*, *Lates mariae*, *Lates microlepis* and *Lates stappersii*) and the *Lates niloticus* lineages (West Africa, Congo basin, Nile basin and East Africa - Turkana / Omo basin) are still genetically closely related.

The genetic distances between the African *Lates* species (0,0 % to 4,0 %) are very low in most genes except the for the mDNA16S gene and the nDNA *ficd*, *sreb2* and *ZNF* genes (Table 2, Supplementary Tables 4). Single-tree analysis (maximum likelihood) of the mitochondrial 16S showed a very similar picture to the species tree analysis of all genes combined (Bayesian inference). Therefore, the 16S gene also serves as a representative in genetic distances for an overall representation of the combined 12 genes.

	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,16										
Lates_calcarifer	0,18	0,08									
Lates_niloticus_West_Africa	0,21	0,12	0,10								
Lates_mariae	0,20	0,12	0,10	0,03							
Lates_microlepis	0,20	0,12	0,10	0,03	0,02						
Lates_stappersii	0,20	0,12	0,10	0,02	0,01	0,02					
Lates_angustifrons	0,20	0,12	0,10	0,03	0,00	0,02	0,01				
Lates_niloticus_Congo	0,20	0,11	0,10	0,03	0,02	0,03	0,03	0,03			
Lates_niloticus_Nile	0,20	0,10	0,10	0,03	0,04	0,04	0,03	0,04	0,04		
Lates_niloticus_Turkana/East_Africa	0,20	0,10	0,10	0,03	0,04	0,04	0,03	0,04	0,04	0,00	

Table 2: The following table shows the net mean distances calculated from 16S sequences between the four Tanganyika species (*Lates angustifrons*, *Lates mariae*, *Lates microlepis*, *Lates stappersii*) and the four lineages of *Lates niloticus* (West Africa, Congo basin, Nile basin and East Africa - Turkana / Omo basin).

Discussion

DNA Barcoding has proven a useful tool to genetically discriminate the different species of *Lates* collected for this study (Hebert et al. 2003). This was especially important for the four Lake Tanganyika species, as their morphological determination was particularly difficult in juvenile individuals (Supplementary figure 8). In some cases, morphology-based identification of *Lates mariae* and *Lates microlepis* samples was not reflected by the genetic barcode, thus rendering some samples with misleading labels (juvenile Lami 1 (*Lates microlepis*) turned out to be *Lates mariae* based on the COI data).

The phylogenetic analysis of one mDNA and 11 nDNA markers revealed that the Lake Tanganyika species are not as expected alone as sister clade to *Lates niloticus* (Greenwood 1976). The Congo Lineage of *Lates niloticus* is the sister clade Lake Tanganyika species (Figure 5), which of course makes sense geographically. Here further investigation of the Congo line with morphological analyzes would be very important to find out if there are consistent morphological differences between the lines of *Lates niloticus* and, in the positive case, possibly to raise the Congo line to its own species status. The genetic results at least suggest this very closely. This was mainly supported by the time calibrated Bayesian Inference tree analysis, but stands in contrast to most single gene analyzes (Supplementary figures 1), with the exception of SLC10A3, ZNF, KBTBD4, and especially 16S (Pamilo and Nei 1988; Doyle 1992). Due the fact that changes in mtDNA are four times more likely than in nDNA, the effect of mtDNA on the time calibrated Bayesian Inference tree analysis is very high (Moore 1995). A high level of nodal support supports the results. The estimated time of divergence, based on the BEAST analysis, allows for certain links to hydrogeological data on Earth-time events (Goudie 2004) and is also supported by fossil stocks (Otero 2004).

Of the Lake Tanganyika species *Lates microlepis* is the most basal, which is quite surprising at first glance. Due to morphological similarities to *Lates Stappersii*, these two species would have been closer to each other, theoretically. The genetic proximity of *Lates angustifrons* and *Lates mariae* makes perfect sense. *Lates mariae* looks like a Deepwater adapted form of *Lates angustifrons* (Figure 5; Supplementary figure 6).

Of course, this result can also be geographically proven, as the Lake Tanganyika always had a constant outflow into the Congo basin. The Nile lineage and the Turkana / Omo Lineage are genetically and geographically very close to each other, while the West African Lineage is already slightly more distant from the Nile lineage.

It can be assumed, that the species found in Lake Tanganyika derived from a population in the Congo river. Due to the disruption of catchment areas, these populations got separated, disabling gene flow and leading to the picture we observe today.

The split of the genus *Lates* in Africa in these two clades occurred around 3 million years ago at a time when the central African highlands and their waters were subject to constant geomorphological change and their discharges into the major drainage systems of central Africa changed several times (Van Damme et al. 2009).

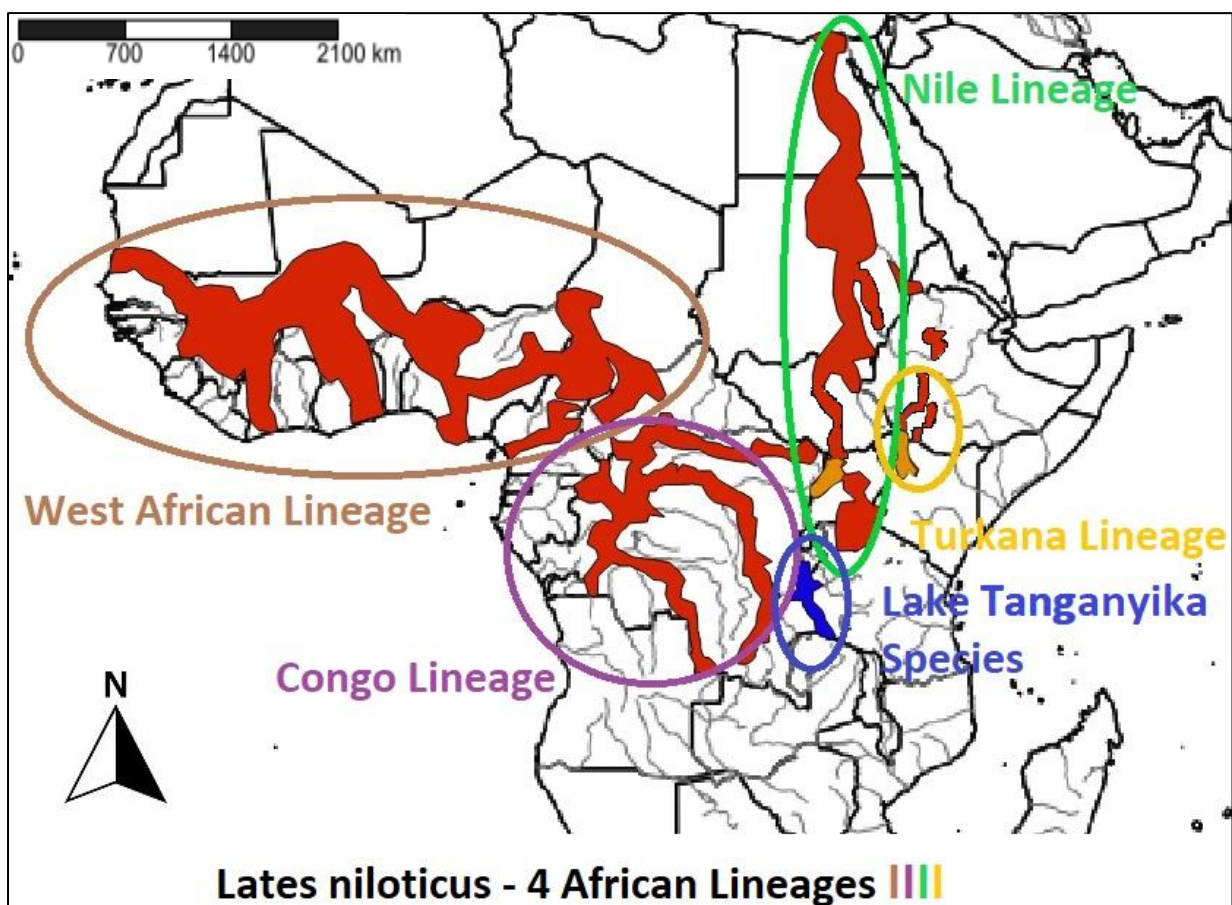


Figure 7: Distribution areas and visualization of the four lineages of *Lates niloticus*

Based on *Lates* fossils (Otero 2004), it is known that the species that originally occupied Africa have all gone extinct until today. *Lates niloticus* from the Congo basin seems to be the most primitive recent species.

Comparing the distribution pattern of the genus *Lates* (Figure 7) with other genera or families in Africa it turns out that there are similarities. For example, another predator species, *Hydrocynus* (Tigerfish, Alestidae), has a nearly identical distribution pattern throughout most of Africa (Goodier et al. 2011). Only the Zambesi is an exception, while *Lates* perches do not occur in this river, he counts to the distribution area of Tigerfishes. The genus *Hydrocynus* with its five described species is also one of the top predators in the waters in which they occur (Lewis 1974). In most of the waters of their habitat, they even co-exist with *Lates* perches.

Unfortunately, the validation of the two assumed species *Lates macrophthalmus* and *Lates longispinis* was not possible. Although the results of this study render their species state highly doubtful, additional research is recommended to fully resolve this question. Although some studies confirm the species status of the two species (Holden 1967; Harrison 1991), in principle they only refer to reports that have become largely obsolete (Worthington et al.; 1929, Worthington et al., 1932). However, at this point it is assumed that these are regionally adapted morphs of *Lates niloticus* and it is recommended that they are treated as subspecies of *Lates niloticus* (*Lates niloticus macrophthalmus*, *Lates niloticus longispinis*), until the species status has been completely confirmed or refuted by sufficient genetic data.

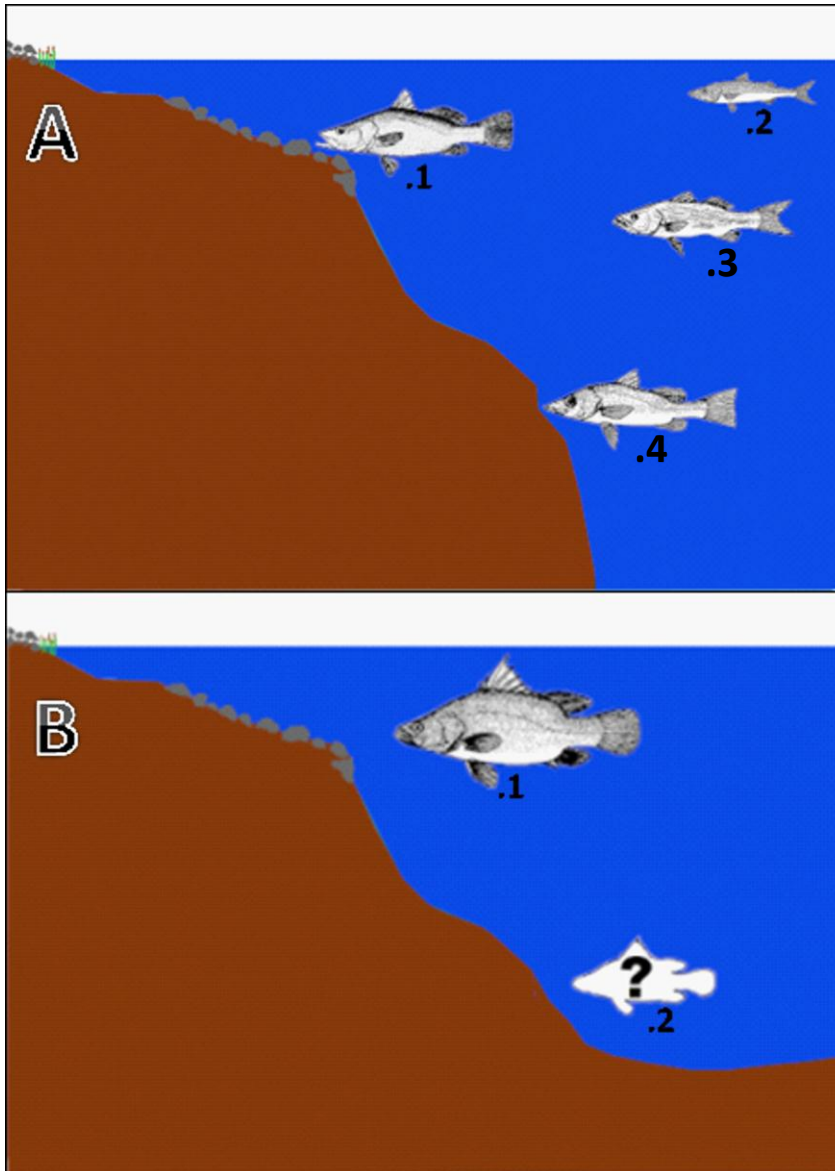


Figure 8: Theoretical scheme on the niche occupation of Lates species in the major African Rift Valley lakes. A: Lake Tanganyika with its endemic Lates species (A.1 *L. angustifrons*, A.2 *L. stappersii*, A.3 *L. microlepis*, A.4 *L. mariae*) B: Lake Albert / Lake Turkana with their possible species/morphs composition (B.1 *L. niloticus*, B.2 *L. macrophthalmus / longispinis*)

Of course, it seems very likely that there are also lates perches in the two lakes Lake Albert and Lake Turkana, which have adapted to specific habitats (for example: Deepwater habitats). However, it seems unlikely that in the two lakes have already developed own adapted species like in Lake Tanganyika. It seems much more likely that *Lates macrophthalmus* and *Lates longispinis* are morphs of *Lates niloticus* that have adapted to specific habitats (Figure 8).

Paleolake Obweruka (Figure 9) may have been an important link and keystone for the colonization and distribution of the youngest *Lates* species. Until about 2 million years ago, the lake existed and had a connection to Congo basin (Ring et al. 2014). As the lake began to

disappear, so did the connection to the Congo. Today, this region around the former Paleolake is characterized by the lakes Lake Albert and Lake Edward and has its outflow into the Nile.

The area around the recent lake Chad and especially southeast of it, could be the second hotspot for the spread of *Lates niloticus*. Today's Lake Chad is a lake without runoff, which continuously loses water surface due to dehydration, but in late Pleistocene it was still the largest lake in Africa. This Paleolake Megachad had several connections to the Niger basin during its formation phases (Goudie AS 2004). A connection to the Congo basin may also have existed before the Darfur Plume an uplift of the region between the present catchment areas of Lake Chad, Congo and Nile brought about (Goudie AS 2004). Therefore, it seems quite possible that *Lates niloticus* spread from the Congo basin, spread over the area around the present border area between Chad and the Central African Republic to West Africa. Lake Megachad also had a connection to the young Nile for a while in the early Holocene through the Bahr el Ghazal valley (Leblanc et al., 2006). However, from an Earth historical point of view, it seems far more likely that the distribution via the Paleolake Obweruka has taken place in the Nile basin.

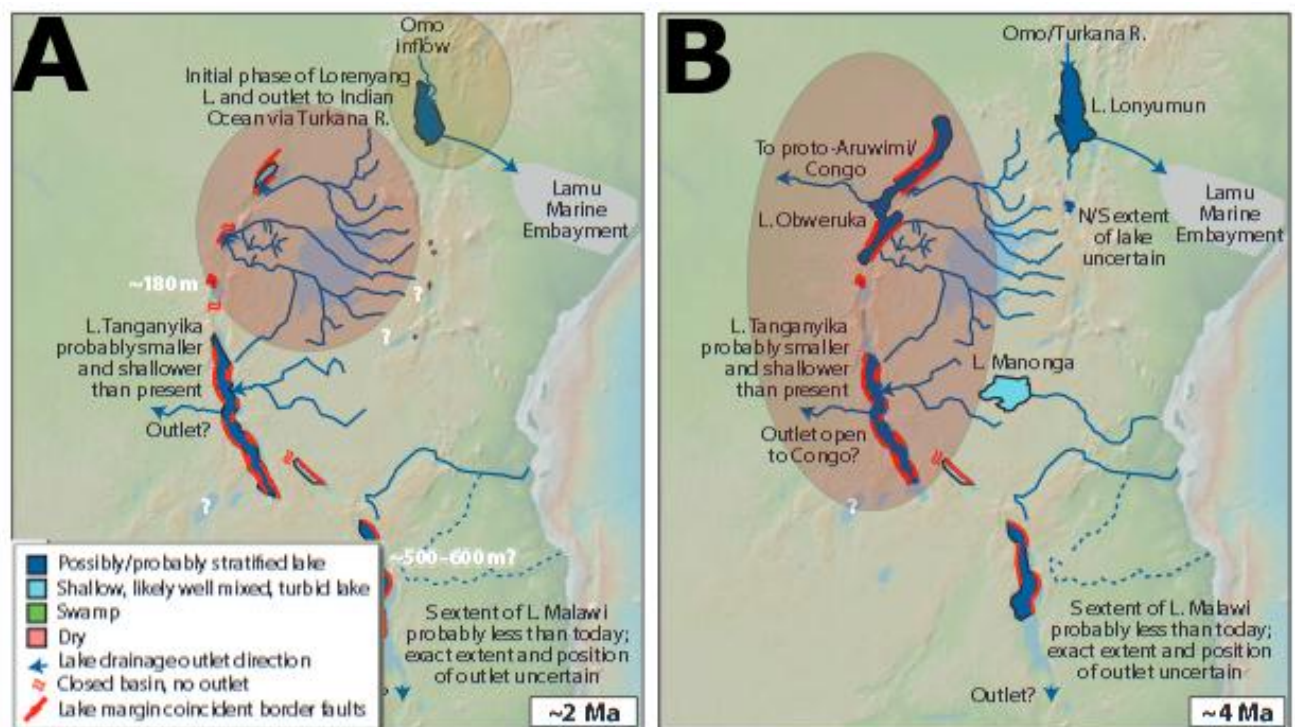


Figure 9: Paleogeographic maps of the African Rift Valley showing hydrographic configurations of the major lakes and rivers for the period (~3Ma) when the two sister clades of Lates (Congo/Tanganyika basin and West Africa/Nile basin/Turkana-Omo basin) split up (Figure 6). Map was changed after Salzburger et al. 2014

The colonization of Lake Tanganyika by *Lates* species happened relatively recently (2-1.5 million years ago) and consequently the speciation event of the four species was quite rapid in geo-historical terms. Lake Tanganyika, with its much older cichlid fish community at that time (Salzburger et al., 2014), was an ideal habitat with existing niches for top predators such as lates perches. This big ecosystem with masses of potential food sources and unoccupied ecological niches has allowed the *Lates* predators to develop a relatively large number of four species within one ecosystem.

This information, that also in Lake Tanganyika the top predators lates perches met a fully developed ecosystem when they were occupied about 1.5 million years ago and still has developed to one of the most species-rich waters of the earth, also gives hope for the situation in the Lake Victoria. Here, too, a new species community could develop from the initially suspected ecological disaster through the occupation of *Lates niloticus* over long periods of time.

It is recommended that a reappraisal of Nile Perch taxonomy be made using more modern techniques and that studies are initiated to discover how characters change during development under differing environmental conditions.

Acknowledgements

It's not often in life that you get the chance to work on something that has captivated you since childhood. Since I saw a Nile perch in a magazine for the first time as a child, I was thrilled with these unique animals and wanted to learn more about them. With this study, I was able to learn more about Lates perch than I ever dared to dream. I even got the chance to experience Lates perches up close during a field trip and to collect samples from lates at Lake Tanganyika. But all that would not have been possible on my own. That's why I want to thank some people who have made this fulfillment of my dream possible...

Representing the many people who helped me a great deal with my study, I can only name a handful of people:

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Online resources:

<https://www.africangreatlakesinform.org/article/lake-tanganyika>, visited on 27.07.2019

<https://www.simplemappr.net/>

<https://www.iucnredlist.org/>

<https://www.fishbase.de/>

<https://www.ncbi.nlm.nih.gov/genbank/>

Appendix

Supplementary table 1: Sampling information of the *Lates niloticus* samples from the Congo basin (A) and the Niger basin (B).

A: Sampling data of *Lates niloticus* samples from Congo river:

Nr Species	Date	Location		Fieldtag	Collection	Box	Tube
1 <i>Lates niloticus</i>	15/03/2010	Congo	Bought	DRC	0451	B0_09	10 B7 AB33604608
2 <i>Lates niloticus</i>	17/03/2010	Congo	10	DRC	0522	B0_09	12 D6 AB31540966
3 <i>Lates niloticus</i>	29/03/2010	Congo	Bought	DRC	18C2	B0_09	18 C2 AB35168123
4 <i>Lates niloticus</i>	28/05/2011	CongoM	PECHEUR3	DRC	8399	B1_09	17 E3 AB42878917

Each sample is numbered as in the leftmost column (Nr; in bold).

Royal Museum for Central Africa, Belgium

B: Sampling data of *Lates niloticus* samples from Niger river:

Nr Species	Date	Location		Export	SampleNr.
1 <i>Lates niloticus</i>	06/06/2018	Niger(river)	Bought NGA	Lagos	LaniN1
2 <i>Lates niloticus</i>	06/06/2018	Niger(river)	Bought NGA	Lagos	LaniN2
3 <i>Lates niloticus</i>	06/06/2018	Niger(river)	Bought NGA	Lagos	LaniN3

Samples were taken from imported fish. Fish imported from the University of Graz

Importer: Aquarium Glaser / Tropic Water, Germany

finclip	species	locality	coordinates	origin	wild/bred
T03/2/E1	Lates angustifrons	Mpulungu fishmarket			w
T03/3/E18	Lates angustifrons	Mpulungu	8°46'52" S, 31°05'30" E		w
T03/3/E8	Lates angustifrons	Mpulungu	8°46'52" S, 31°05'30" E		w
T03/4/J6	Lates angustifrons	Mpulungu fishmarket			w
T06/1/C9	Lates angustifrons	Kalambo Lodge	8°37' S, 31°37' E		w
T06/1/E2	Lates angustifrons	Kalambo Lodge	8°37' S, 31°37' E		w
T06/3/J15	Lates angustifrons	Kalambo Lodge	8°37' S, 31°37' E		w
T06/19/G10	Lates angustifrons	Chisanze			w
T03/1/G17	Lates mariae	Mpulungu	8°46'52" S, 31°05'30" E		w
T03/2/D4	Lates mariae	Mpulungu fishmarket			w
T03/2/E19	Lates mariae	Kasakalawe Lodge	8°46'52" S, 31°05'30" E		w
T03/3/I8	Lates mariae	Mbita Island	8°45'52" S, 31°05'84" E		w
T03/5/F5	Lates mariae	Nakaku	8°38'63" S, 30°52'35" E		w
T03/7/B9	Lates mariae	Mpulungu fishmarket		Lawrence Makasa	w
T04/7/C7	Lates mariae	Kalambo Lodge	8°37' S, 31°37' E		w
T04/10/D2	Lates mariae	Kalambo Lodge	8°37' S, 31°37' E		w
T04/12/J10	Lates mariae	Mpulungu fishmarket			w
T06/3/D10	Lates mariae	Mbita (Ochi's place)			w
T06/7/A3	Lates mariae	Kalambo Lodge	8°37' S, 31°37' E		w
T06/10/A5	Lates mariae	Mpulungu fishmarket			w
T07/16/G1	Lates mariae	S of Isonga	6°28'57" S, 30°09'57,2" E		w
T14/09/H4	Lates mariae	Isanga			w
T14/09/H5	Lates mariae	Isanga			w
T14/09/H6	Lates mariae	Isanga			w
T03/2/H2	Lates microlepis	Mpulungu fishmarket			w
T04/11/G4	Lates microlepis	Isanga	8°39,40' S, 31°11,60' E		w
T01/6/G5	Lates sp.	Kasakalawe	8°47'24" S, 31°04'40" E		w
T04/6/G10	Lates sp.	Kalambo Lodge	8°37' S, 31°37' E		w
T04/6/H3	Lates sp.	Kalambo Lodge	8°37' S, 31°37' E		w
T04/7/B13	Lates sp.	next to Kalambo	8°37' S, 31°12' E		w
T04/10/F1	Lates sp.	Kalambo Lodge	8°37' S, 31°37' E		w
T07/25/E10	Lates sp.	N of Kipili			w
T16/02/I05	Lates sp.	Toby's lodge			w
T07/29/C4	Lates stappersii	Mtosi	7°35'31" S, 30°37'55,5" E		w
T07/29/C5	Lates stappersii	Mtosi	7°35'31" S, 30°37'55,5" E		w
T03/3/A13	Lates stappersii	Mpulungu fishmarket			w
T03/3/A14	Lates stappersii	Mpulungu fishmarket			w

Supplementary table 2: Lates samples from the archive of the University of Graz

Supplementary table 3: Sample and reference list of all samples used for this study

Sample no.	Reference no.	Extraction	Notes	Species (determined)	Provenance	Symbol
Last_9	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_4	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_10	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_11	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Lani_1	-	x	Lani_1 u. _2 = one individual	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_2	-	x				
Lani_3	-	x	Lani_3 u. _4 = one individual	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_4	-	x				
Lani_5	-	x	Lani_5 u. _6 = one individual	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_6	-	x				
Lani_7	-	x	Lani_7 u. _8 = one individual	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_8	-	x				
Lani_9	MP 1940,1941,1942	x	no unique reference no.	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_10	MP 1940,1941,1942	x	no unique reference no.	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_11	MP 1943	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_12	MP 1944	x	Lates macrophthalmus?	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_13	MP 1945	x	Lates macrophthalmus?	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_14	MP 1946	x	Lates macrophthalmus?	Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lami_1	1-238	x		Lates microlepis	Lmicrolepis/L.Tanganyika/Burundi	Lami
Lami_2	1-238 c16	x		Lates microlepis	Lmicrolepis/L.Tanganyika/Burundi	Lami
Lami_3	4-253 c16	x		Lates microlepis	Lmicrolepis/L.Tanganyika/Burundi	Lami
Lami_4	5-254	x		Lates microlepis	Lmicrolepis/L.Tanganyika/Burundi	Lami
Lami_5	7-256 c16	x		Lates microlepis	Lmicrolepis/L.Tanganyika/Burundi	Lami
Last_219	23-235 c16	x		Lates stappersii	Lstappersii/L. Tanganyika/Burundi	Last
Last_220	24-236	x		Lates stappersii	Lstappersii/L. Tanganyika/Burundi	Last
Lasp_1	1-434 c16	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Burundi	Lasp
Lasp_2	2-235	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Burundi	Lasp
Lani_15	HP 1940	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_16	HP 1965	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_17	HP 2005	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_18	HP 2006	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_19	HP 2021	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_20	HP 2022	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_21	HP 2072	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_22	HP 2149	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_23	HP 2003	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_24	HP 2004	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_25	HP 2069	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_26	HP 2070	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Lani_27	HP 2071	x		Lates niloticus	Lniloticus/Lac Albert/DR Congo	Lani
Last_1	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_2	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_3	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_5	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_6	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_7	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_8	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_12	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_13	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_14	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_15	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_16	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_17	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_18	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_19	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_20	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_21	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Last_22	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last

Sample no.	Reference no.	Extraction	Notes	Species (determined)	Provenance	Symbol
Last_23	ioc 3	x		Lates stappersii	Lstappersii/L. Tanganyika/DR Congo	Last
Laan_1	T03/2/E1	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_2	T03/3/E8	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_3	T03/3/E18	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_4	T03/4/J6	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_5	T06/1/C9	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_6	T06/1/E2	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_7	T06/3/J15	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_8	T06/19/G10	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Lama_1	T03/2/D4	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_2	T03/2/E19	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_3	T03/3/I8	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_4	T03/5/F5	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_5	T03/7/B9	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_6	T04/7/C7	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_7	T04/10/D2	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_8	T04/12/J10	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_9	T06/3/D10	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_10	T06/7/A3	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_11	T06/10/A5	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_12	T07/16/G1	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_13	T14/09/H4	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_14	T14/09/H5	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_15	T14/09/H6	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lami_6	T03/2/H2	x		Lates microlepis	L. microlepis/L. Tanganyika/Zambia	Lami
Lami_7	T04/11/G4	x		Lates microlepis	L. microlepis/L. Tanganyika/Zambia	Lami
Lasp_3	T01/6/G5	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Lasp_4	T04/6/G10	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Lasp_5	T04/6/H3	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Lasp_6	T04/7/B13	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Lasp_7	T04/10/F1	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Lasp_8	T07/25/E10	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Lasp_9	T16/02/I5	x	Lates spec. -> Species undetermined		Lspec./L. Tanganyika/Zambia	Lasp
Last_24	T07/29/C4	x		Lates stappersii	Lstappersii/L. Tanganyika/Zambia	Last
Last_25	T07/29/C5	x		Lates stappersii	Lstappersii/L. Tanganyika/Zambia	Last
Last_26	T03/3/A13	x		Lates stappersii	Lstappersii/L. Tanganyika/Zambia	Last
Last_27	T03/3/A14	x		Lates stappersii	Lstappersii/L. Tanganyika/Zambia	Last
Lami_8	Fische Uni	x		Lates microlepis	Lmicrolepis/Lake Tanganyika	Lami
Lami_9	Fische Uni	x		Lates microlepis	Lmicrolepis/Lake Tanganyika	Lami
LaniT_1	T7/09	x		Lates niloticus	L. niloticus / Lake Turkana/Kenya	LaniT
LaniT_2	T104/09	x		Lates niloticus	L. niloticus / Lake Turkana/Kenya	LaniT
Lalo_1	188/09	x		Lates longispinis	L. Longispinis / Lake Turkana/Kenya	Lalo
LaniW_1	S41/10	x		Lates niloticus	Lniloticus/White Nile/Sudan	LaniW
LaniW_2	S107/10	x		Lates niloticus	Lniloticus/White Nile/Sudan	LaniW
Last_28	-	x		Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_29	-	x		Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Laan_9	-	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_10	-	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_11	-	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Laan_12	-	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Lama_16	-	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Laan_13	-	x		Lates angustifrons	L. angustifrons/L. Tanganyika/Zambia	Laan
Lama_17	-	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_19	-	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_20	-	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
Lama_21	-	x		Lates mariae	L. mariae/L. Tanganyika/Zambia	Lama
LaniN_1	-	x		Lates niloticus	L. niloticus/Niger river/Nigeria	LaniN
LaniN_2	-	x		Lates niloticus	L. niloticus/Niger river/Nigeria	LaniN
LaniN_3	-	x		Lates niloticus	L. niloticus/Niger river/Nigeria	LaniN

Sample no.	Reference no.	Extraction	Notes	Species (determined)	Provenance	Symbol
LaniV_1	B1			Lates niloticus	L. niloticus/Lake Victoria/Tanzania	LaniV
LaniV_2	B2			Lates niloticus	L. niloticus/Lake Victoria/Tanzania	LaniV
LaniV_3	B3	x		Lates niloticus	L. niloticus/Lake Victoria/Tanzania	LaniV
LaniV_4	B4	x		Lates niloticus	L. niloticus/Lake Victoria/Tanzania	LaniV
LaniV_5	B5		Samples from two indiv. in one Tube	Lates niloticus	L. niloticus/Lake Victoria/Tanzania	LaniV
LaniV_6				Lates niloticus	L. niloticus/Lake Victoria/Tanzania	LaniV
LaniT_3	B6			Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_4	B7	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_5	B8	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_6	B9	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_7	C1	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_8	C2	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_9	C3	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_10	C4	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_11	C5			Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_12	C6	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_13	C7	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_14	C8	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_15	C9	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_16	D1	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_17	D2	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_18	D3	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_19	D4	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_20	D5	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_21	D6	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_22	D7	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
LaniT_23	D8	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
Lalo_2	E1	x		Lates longispinis	L. longispinis/Lake Turkana/Kenya	Lalo
LaniT_24	E2	x		Lates niloticus	L. niloticus/Lake Turkana/Kenya	LaniT
Lani_28	E3	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_29	E4	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_30	E5	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_31	E6	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_32	E7	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_33	E8	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_34	E9	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_35	F1	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_36	F2	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_37	1	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_38	2	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_39	3	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_40	4	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_41	5a	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_42	5b	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_43	5c	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_44	6a			Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_45	6b	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_46	6c	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_47	7a	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_48	7b	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Lani_49	7c	x		Lates niloticus	L. niloticus/Lake Albert/Uganda	Lani
Last_29	A1			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_30	A2			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_31	A3			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_32	A4			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_33	A5			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_34	A6			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_35	A7			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_36	A8			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last
Last_37	A9			Lates stappersii	L. stappersii/L. Tanganyika/Zambia	Last

Supplementary tables 4: Genetic mean distances of the mitochondrial Genes:

ficd	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,064										
Lates_calcarifer	0,057	0,015									
Lates_niloticus_West_Africa	0,070	0,025	0,018								
Lates_mariae	0,069	0,026	0,017	0,005							
Lates_microlepis	0,069	0,026	0,017	0,005	0,000						
Lates_stappersii	0,069	0,026	0,017	0,005	0,000	0,000					
Lates_angustifrons	0,074	0,031	0,022	0,009	0,005	0,005	0,005				
Lates_niloticus_Congo	0,075	0,032	0,023	0,011	0,006	0,006	0,006	0,011			
Lates_niloticus_Nile	0,069	0,025	0,018	0,000	0,003	0,003	0,003	0,007	0,009		
Lates_niloticus_Turkana/East_Africa	0,070	0,028	0,018	0,003	0,002	0,002	0,002	0,006	0,008	0,001	

kbtbd4	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,039										
Lates_calcarifer	0,046	0,025									
Lates_niloticus_West_Africa	0,047	0,027	0,033								
Lates_mariae	0,047	0,028	0,034	0,002							
Lates_microlepis	0,045	0,026	0,031	0,007	0,005						
Lates_stappersii	0,045	0,026	0,032	0,006	0,005	0,003					
Lates_angustifrons	0,045	0,026	0,029	0,006	0,004	0,002	0,003				
Lates_niloticus_Congo	0,047	0,027	0,034	0,007	0,008	0,006	0,006	0,006			
Lates_niloticus_Nile	0,042	0,022	0,033	0,006	0,005	0,003	0,004	0,003	0,006		
Lates_niloticus_Turkana/East_Africa	0,042	0,023	0,033	0,007	0,005	0,003	0,003	0,003	0,006	0,000	

kiaa	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,043										
Lates_calcarifer	0,044	0,011									
Lates_niloticus_West_Africa	0,043	0,007	0,012								
Lates_mariae	0,046	0,006	0,008	0,002							
Lates_microlepis	0,042	0,006	0,011	0,001	0,000						
Lates_stappersii	0,041	0,005	0,011	0,001	0,000	0,000					
Lates_angustifrons	0,042	0,006	0,011	0,001	0,000	0,000	0,000				
Lates_niloticus_Congo	0,043	0,007	0,012	0,002	0,001	0,001	0,001	0,001			
Lates_niloticus_Nile	0,042	0,006	0,012	0,000	0,002	0,001	0,001	0,001	0,002		
Lates_niloticus_Turkana/East_Africa	0,043	0,007	0,012	0,000	0,002	0,001	0,001	0,001	0,002	0,000	

myh 6	Psa_wa	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis										
Lates_calcarifer	0,064									
Lates_niloticus_West_Africa	0,070	0,022								
Lates_mariae	0,069	0,022	0,000							
Lates_microlepis	0,069	0,022	0,000	0,000						
Lates_stappersii	0,074	0,027	0,004	0,004	0,004					
Lates_angustifrons	0,071	0,024	0,001	0,001	0,001	0,006				
Lates_niloticus_Congo	0,071	0,020	0,004	0,004	0,004	0,008	0,005			
Lates_niloticus_Nile	0,074	0,026	0,004	0,004	0,004	0,009	0,003	0,008		
Lates_niloticus_Turkana/East_Africa	0,070	0,022	0,000	0,000	0,000	0,004	0,001	0,004	0,003	

plagl2	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,059										
Lates_calcarifer	0,060	0,006									
Lates_niloticus_West_Africa	0,069	0,015	0,011								
Lates_mariae	0,068	0,017	0,013	0,001							
Lates_microlepis	0,069	0,018	0,014	0,003	0,001						
Lates_stappersii	0,069	0,018	0,014	0,003	0,001	0,003					
Lates_angustifrons	0,068	0,017	0,013	0,001	0,000	0,001	0,001				
Lates_niloticus_Congo	0,071	0,017	0,013	0,001	0,003	0,004	0,004	0,003			
Lates_niloticus_Nile	0,071	0,017	0,013	0,001	0,003	0,004	0,004	0,003	0,003		
Lates_niloticus_Turkana/East_Africa	0,071	0,017	0,013	0,001	0,003	0,004	0,004	0,003	0,003	0,000	

RIPK	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,074										
Lates_calcarifer	0,074	0,018									
Lates_niloticus_West_Africa	0,074	0,027	0,025								
Lates_mariae	0,076	0,021	0,020	0,005							
Lates_microlepis	0,076	0,021	0,020	0,005	0,000						
Lates_stappersii	0,076	0,021	0,020	0,005	0,000	0,000					
Lates_angustifrons	0,076	0,021	0,020	0,005	0,000	0,000	0,000				
Lates_niloticus_Congo	0,076	0,021	0,020	0,005	0,000	0,000	0,000	0,000			
Lates_niloticus_Nile	0,076	0,025	0,023	0,003	0,002	0,002	0,002	0,002	0,002		
Lates_niloticus_Turkana/East_Africa	0,078	0,023	0,021	0,003	0,002	0,002	0,002	0,002	0,002	0,000	

sidkey	Psa_wa	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis										
Lates_calcarifer	0,063									
Lates_niloticus_West_Africa	0,061	0,022								
Lates_mariae	0,060	0,025	0,007							
Lates_microlepis	0,059	0,022	0,004	0,002						
Lates_stappersii	0,060	0,022	0,005	0,003	0,001					
Lates_angustifrons	0,059	0,023	0,005	0,002	0,000	0,001				
Lates_niloticus_Congo	0,065	0,026	0,008	0,006	0,006	0,006	0,006			
Lates_niloticus_Nile	0,063	0,026	0,004	0,007	0,007	0,007	0,007	0,007		
Lates_niloticus_Turkana/East_Africa	0,060	0,024	0,005	0,006	0,006	0,006	0,006	0,007	0,005	

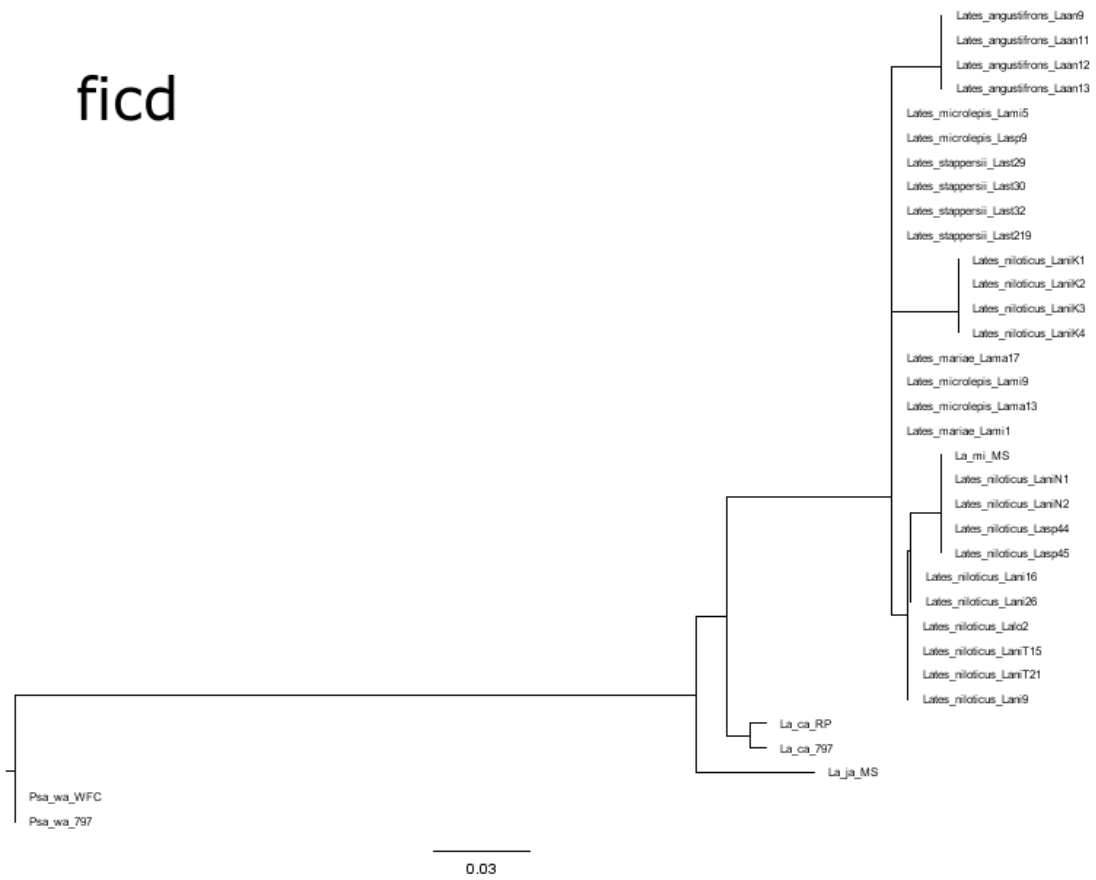
SLC	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,063										
Lates_calcarifer	0,067	0,025									
Lates_niloticus_West_Africa	0,064	0,019	0,022								
Lates_mariae	0,064	0,019	0,022	0,003							
Lates_microlepis	0,064	0,019	0,022	0,003	0,000						
Lates_stappersii	0,064	0,019	0,022	0,003	0,000	0,000					
Lates_angustifrons	0,064	0,019	0,022	0,003	0,000	0,000	0,000				
Lates_niloticus_Congo	0,064	0,019	0,021	0,002	0,000	0,000	0,000	0,000			
Lates_niloticus_Nile	0,062	0,017	0,020	0,002	0,002	0,002	0,002	0,002	0,002		
Lates_niloticus_Turkana/East_Africa	0,063	0,017	0,019	0,000	0,002	0,002	0,002	0,002	0,002	0,000	

sreb2	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,041										
Lates_calcarifer	0,039	0,012									
Lates_niloticus_West_Africa	0,040	0,013	0,010								
Lates_mariae	0,039	0,012	0,009	0,003							
Lates_microlepis	0,039	0,012	0,009	0,003	0,000						
Lates_stappersii	0,039	0,011	0,009	0,003	0,000	0,000					
Lates_angustifrons	0,039	0,011	0,009	0,003	0,000	0,000	0,000				
Lates_niloticus_Congo	0,041	0,011	0,012	0,006	0,002	0,002	0,002	0,002			
Lates_niloticus_Nile	0,041	0,014	0,011	0,005	0,004	0,004	0,004	0,004	0,006		
Lates_niloticus_Turkana/East_Africa	0,041	0,013	0,011	0,005	0,003	0,003	0,003	0,003	0,005	0,000	

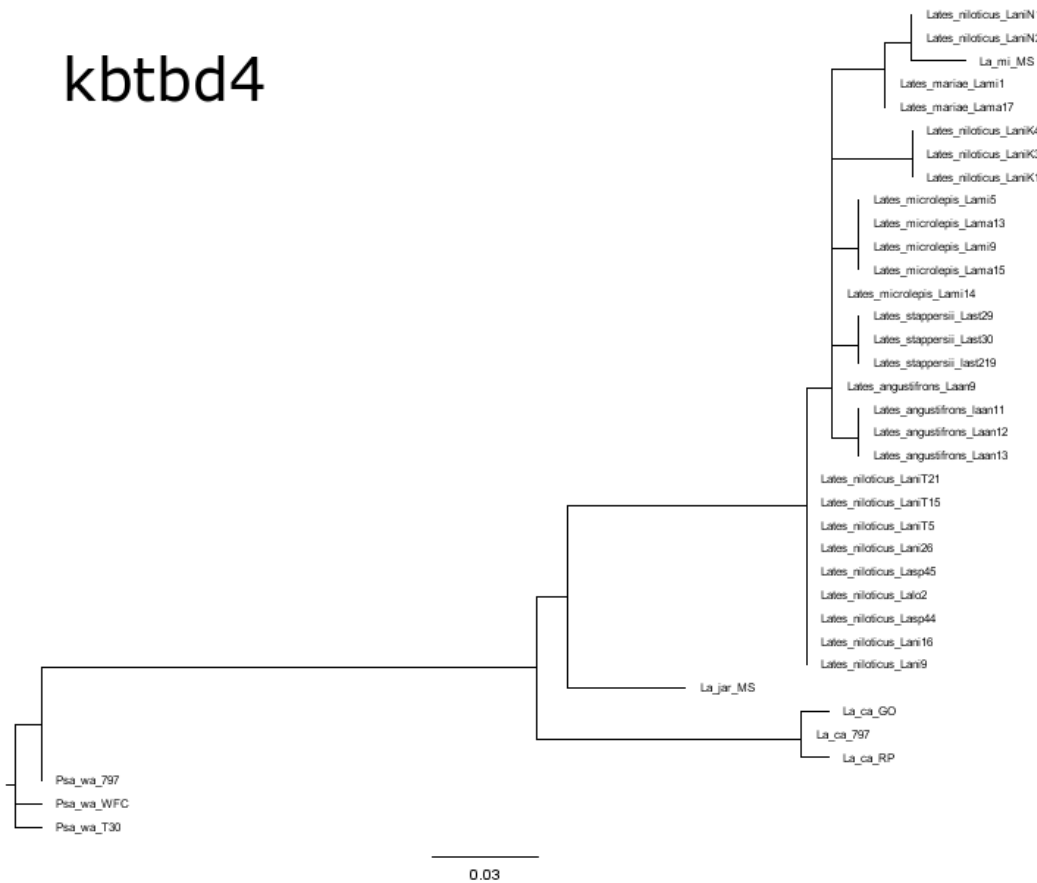
zic1	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,014										
Lates_calcarifer	0,024	0,008									
Lates_niloticus_West_Africa	0,013	0,000	0,009								
Lates_mariae	0,014	0,000	0,008	0,000							
Lates_microlepis	0,014	0,000	0,008	0,000	0,000						
Lates_stappersii	0,014	0,000	0,008	0,000	0,000	0,000					
Lates_angustifrons	0,014	0,000	0,008	0,000	0,000	0,000	0,000				
Lates_niloticus_Congo	0,015	0,002	0,010	0,002	0,002	0,002	0,002	0,002			
Lates_niloticus_Nile	0,014	0,000	0,008	0,000	0,000	0,000	0,000	0,000	0,002		
Lates_niloticus_turkana/East_Africa	0,014	0,000	0,008	0,000	0,000	0,000	0,000	0,000	0,002	0,000	

ZNF	Psa_wa	La_ja	La_ca	La_ni_W	La_ma	La_mi	La_st	La_an	La_ni_C	La_ni_N	La_ni_T
Psammoperca_waigiensis											
Lates_japonicus	0,043										
Lates_calcarifer	0,045	0,010									
Lates_niloticus_West_Africa	0,044	0,007	0,011								
Lates_mariae	0,043	0,006	0,010	0,003							
Lates_microlepis	0,044	0,006	0,010	0,003	0,002						
Lates_stappersii	0,044	0,007	0,011	0,004	0,001	0,003					
Lates_angustifrons	0,044	0,007	0,011	0,004	0,001	0,003	0,002				
Lates_niloticus_Congo	0,042	0,005	0,009	0,002	0,001	0,001	0,002	0,002			
Lates_niloticus_Nile	0,042	0,007	0,013	0,000	0,003	0,002	0,004	0,003	0,002		
Lates_niloticus_Turkana/East_Africa	0,044	0,007	0,011	0,002	0,003	0,001	0,004	0,004	0,002	0,002	

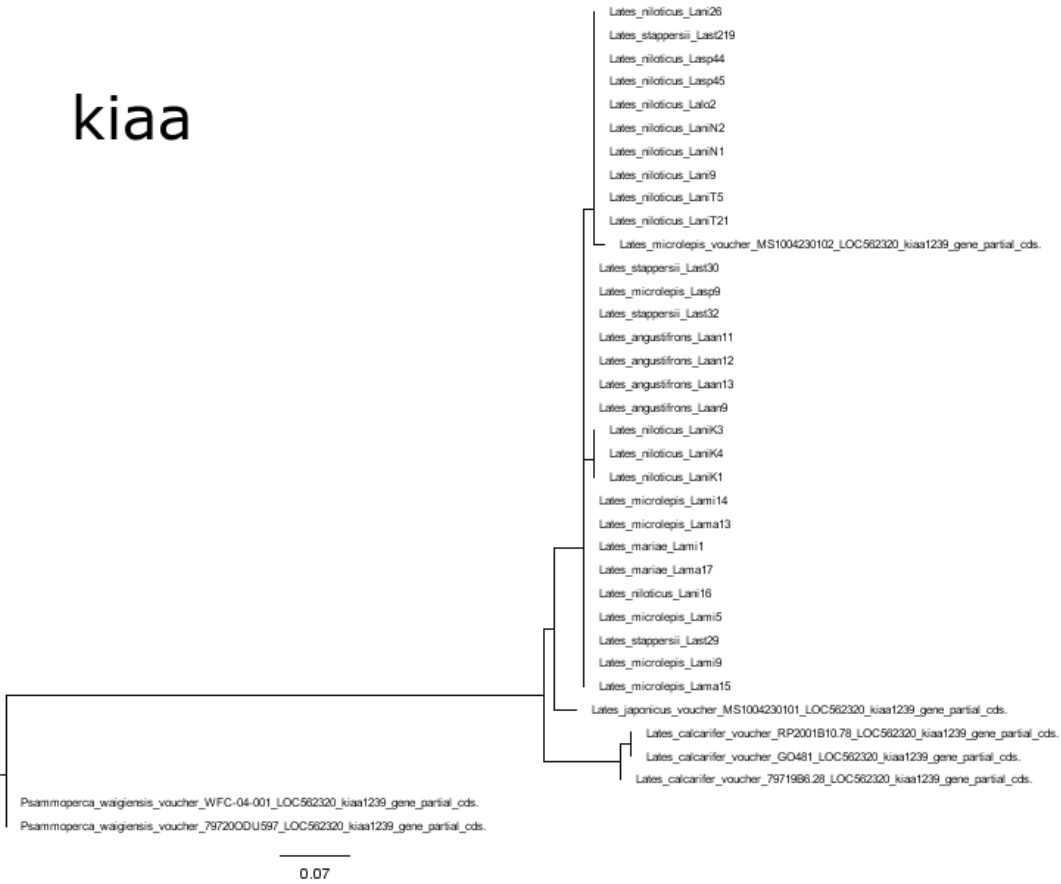
ficd



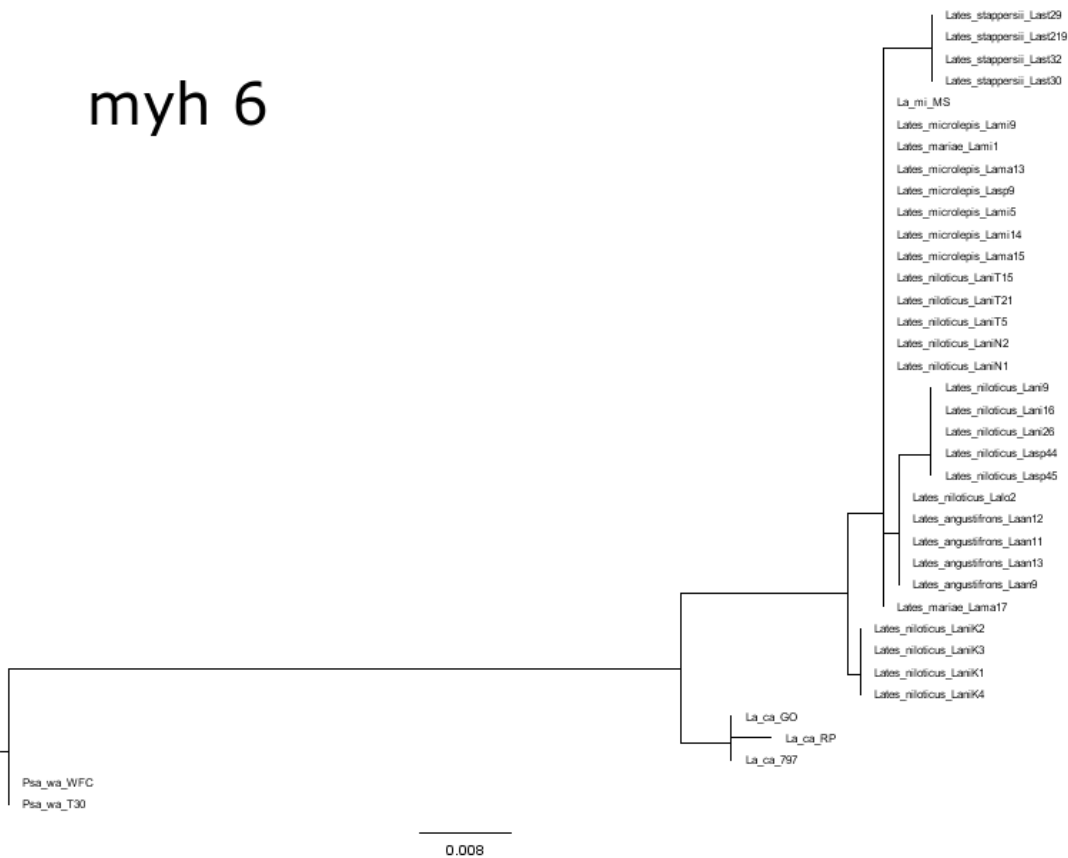
kbtbd4



kiaa



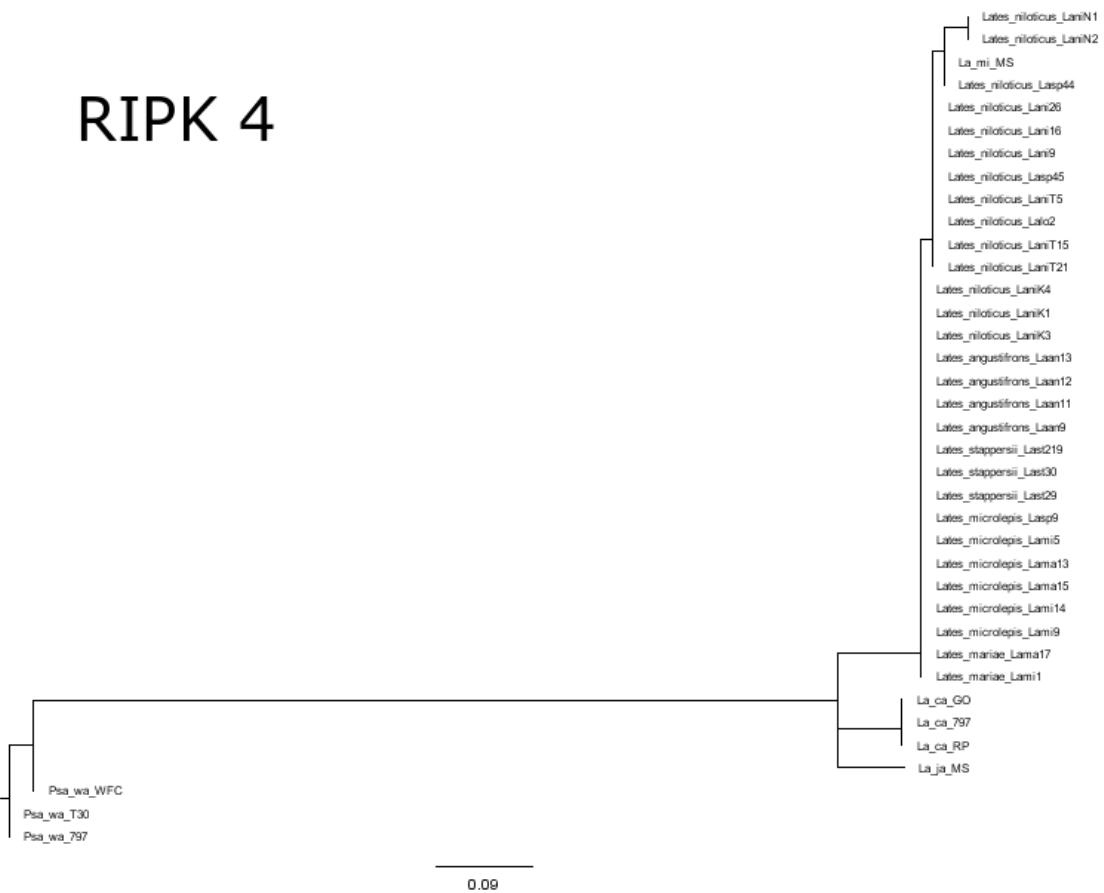
myh 6



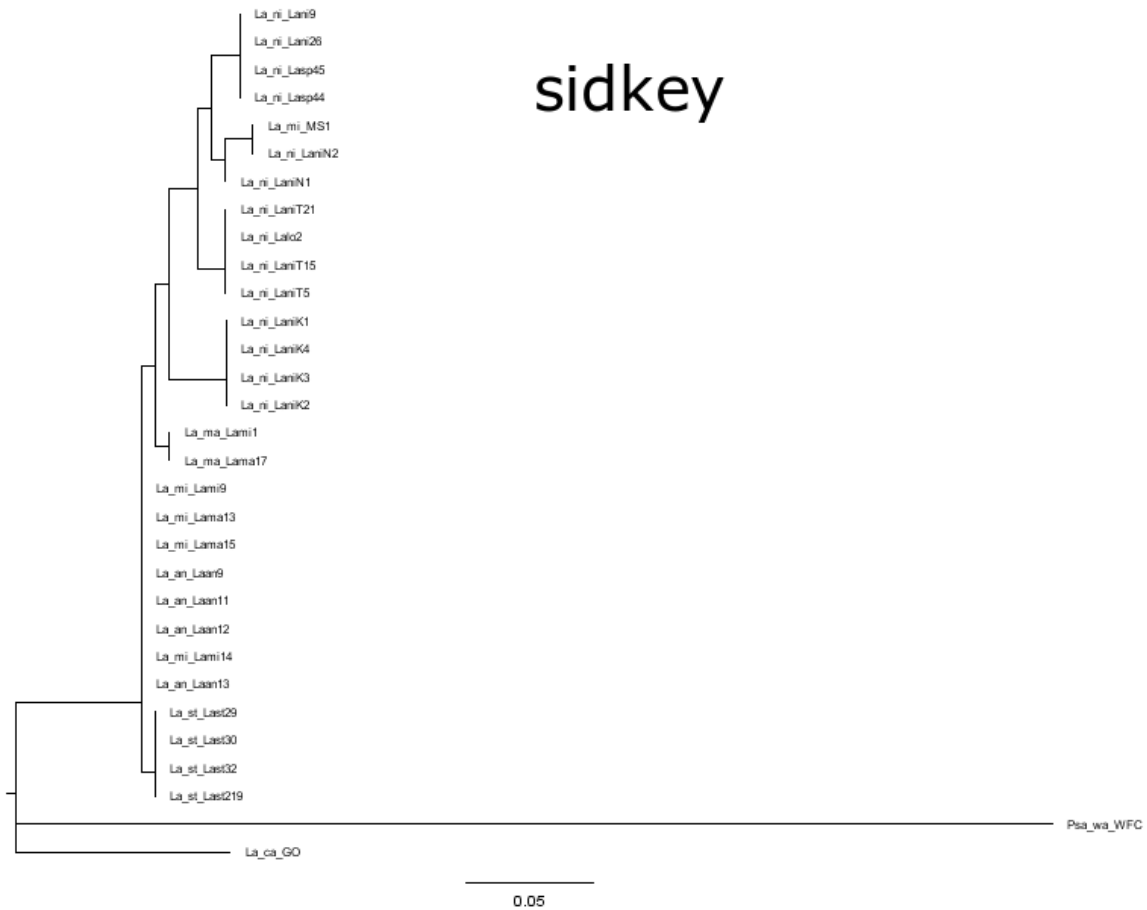
plagl 2



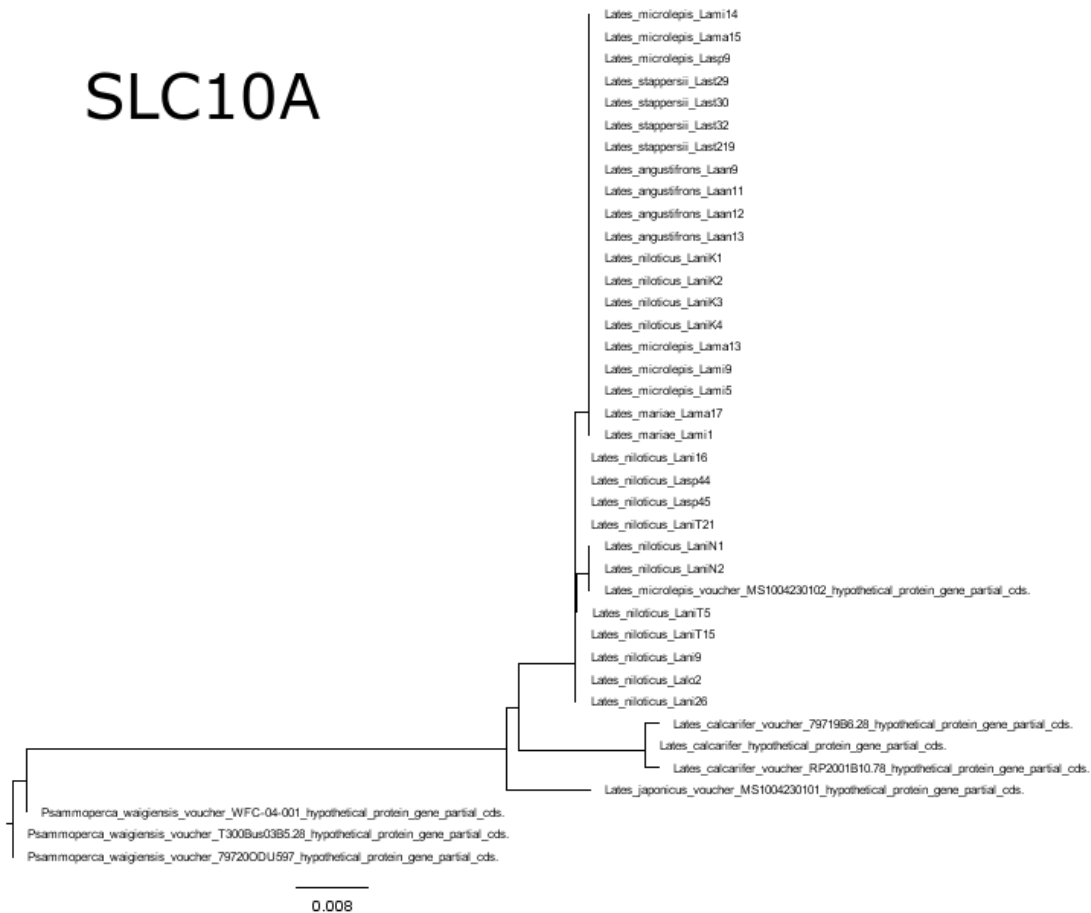
RIPK 4



sidkey



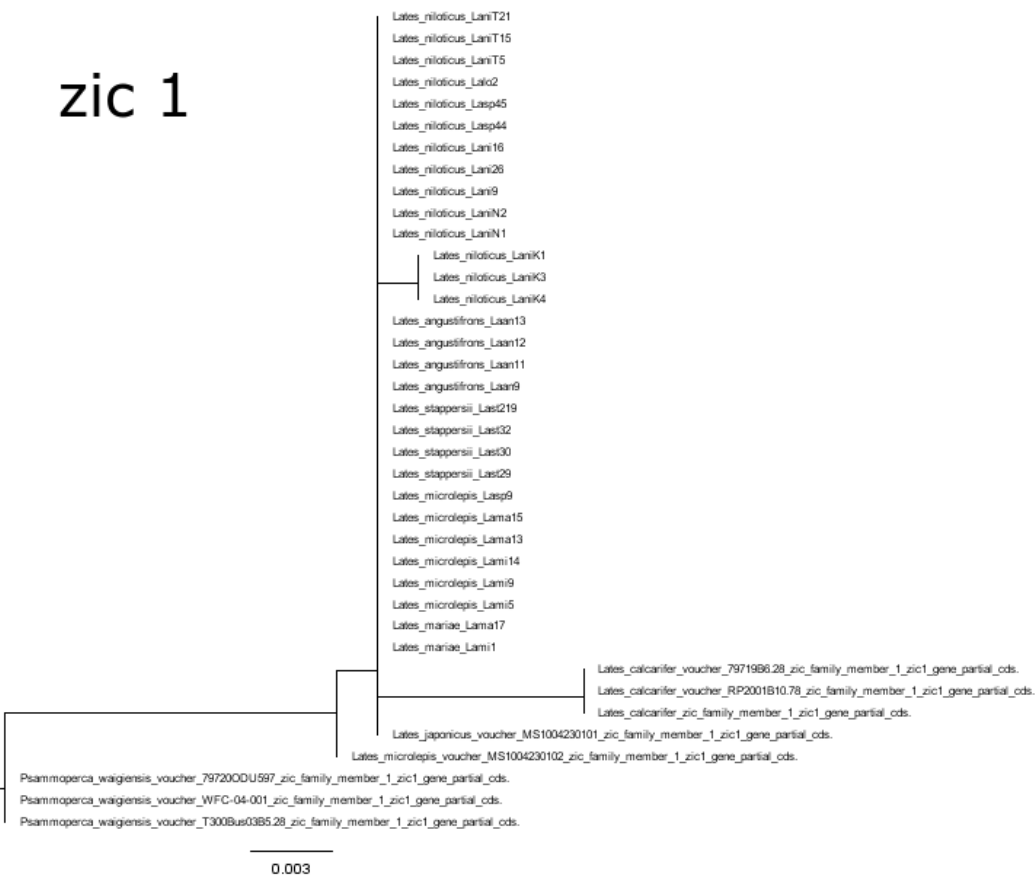
SLC10A



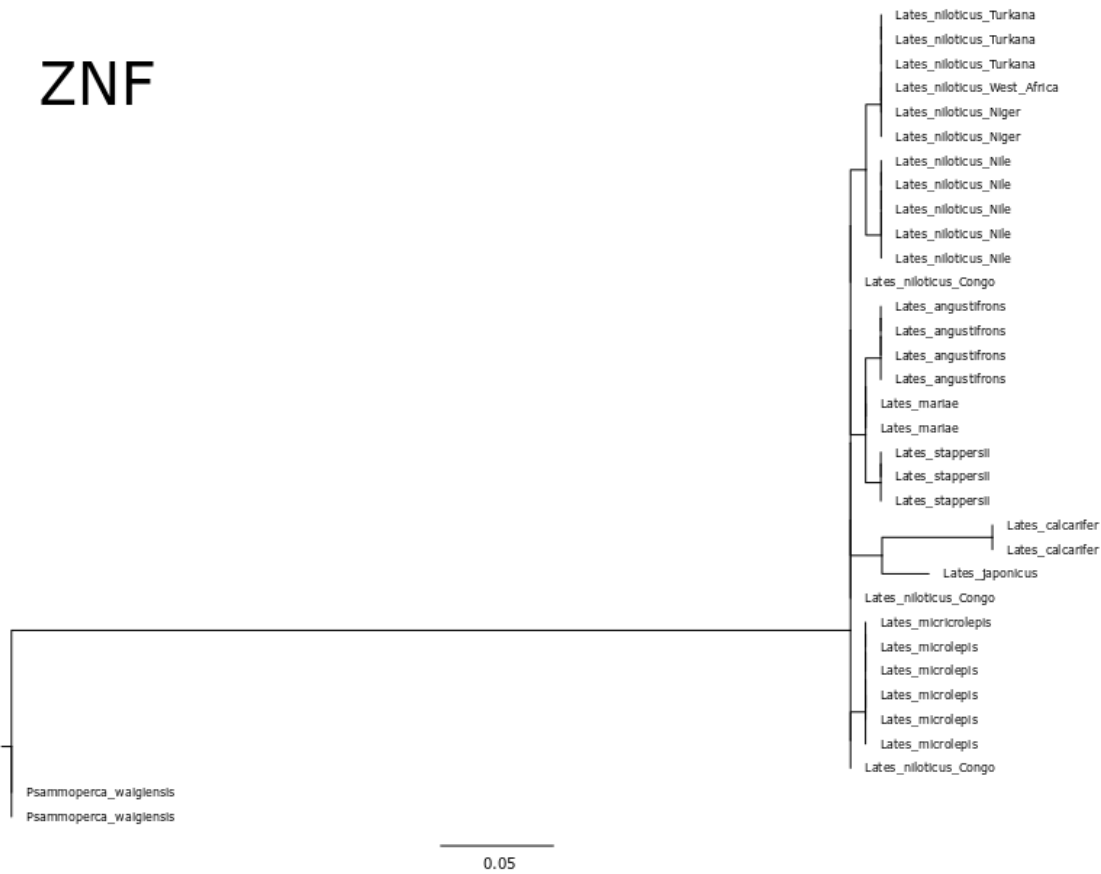
sreb2



zic 1



ZNF



Legend:

Psa_wa = *Psammoperca waigiensis*

La_ja = *Lates japonicus*

La_ca = *Lates calcarifer*

La_ma = *Lates mariae*

La_mi = *Lates microlepis*

La_st = *Lates stappersii*

La_an = *Lates angustifrons*

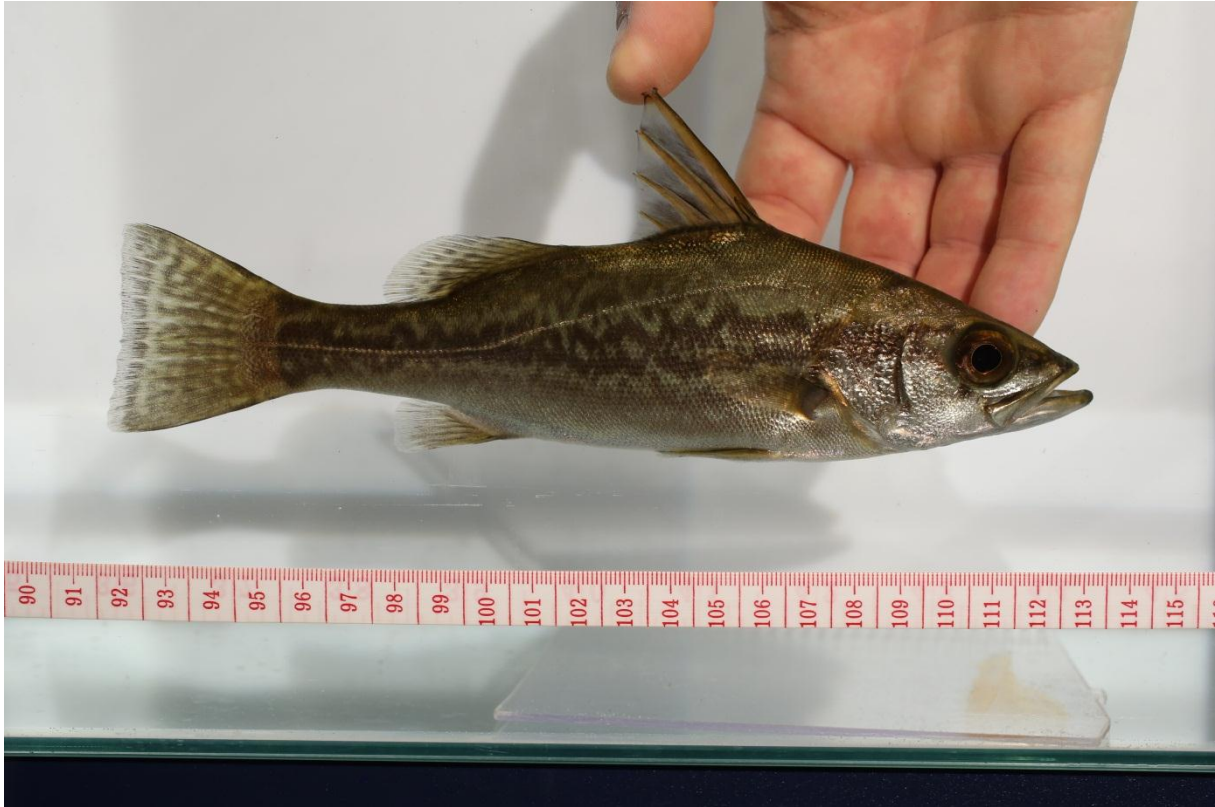
La_ni = *Lates niloticus* (Nile lineage)

La_lo = *Lates longispinis* (mistake, *Lates niloticus* for real)

LaniT = *Lates niloticus* - Turkana/Omo lineage

LaniN = *Lates niloticus* - West African lineage

LaniK = *Lates niloticus* - Congo lineage



Supplementary figure 2: Lateral view of a juvenile *Lates microlepis*; Photo by: Wolfgang Gessl



Supplementary figure 3: Lateral view of a juvenile *Lates niloticus*; Photo by: Wolfgang Gessl



Supplementary figure 4: Mouth of a lates perch (Lates stappersii) - Lates perches are predators which can also swallow large prey



Supplementary figure 5: Lates perches are an important food source for the local population of large lakes and rivers in Africa.

Photo: Lates angustifrons at Lake Tanganyika / Zambia.

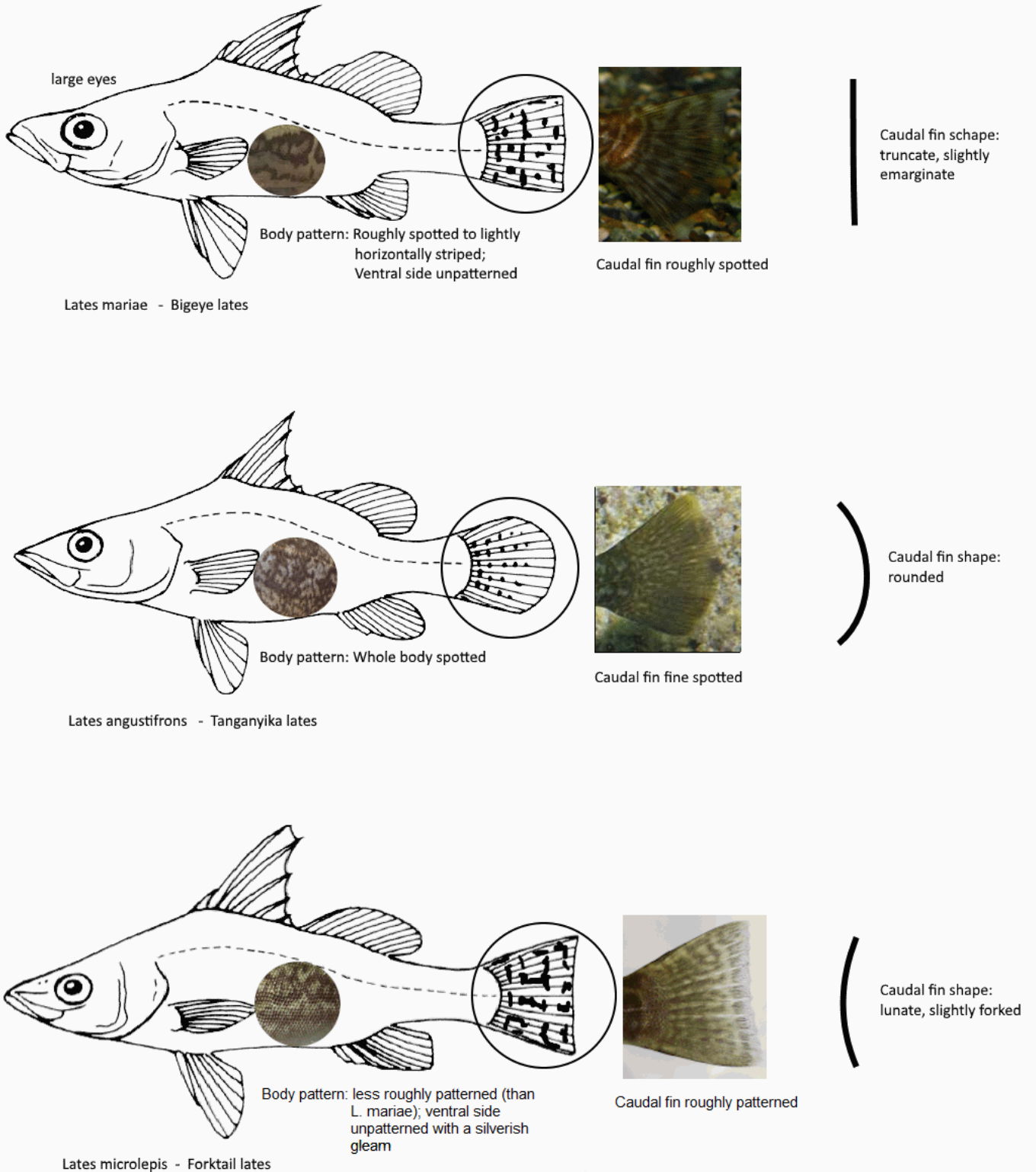


Supplementary figure 6: Seven *Lates perches* on a fish market on Lake Tanganyika; Six *Lates angustifrons* and one *Lates mariae* (3rd from the right)



Supplementary figure 7: Sampling on lates perches on Lake Tanganyika

Distinction of juvenile Lates perches



Supplementary Figure 8: A Distinction Aid for juvenile Lates of Lake Tanganyika

Searching for *Lates longispinis* and *Lates macrophthalmus*

A sampling report from
Clemens J. Lorber

Searching for the two putative Species of *L. longispinis* in Lake Turkana and *L. macrophthalmus* in Lake Albert was futile in terms of not finding a single fish, morphologically and/or genetically distinct to *L. niloticus*. While I didn't manage to come along a specimen that seemed to be outside the intra-species variation, I still collected some information from talking to local fishmongers, fishermen and biologists that might prove valuable for anybody planning to take up the quest again, for genetically or morphologically resolving the dispute over the phylogenetic place of the putative species respectively subspecies, varieties, ecotypes or races.

As the taxonomic Information I had from the literature was not exactly helpful in the communication with the locals, I used pictures (see Figures 1 and 2) showing two specimens, found to be of the respective species as prototypes for recognition.



Figure 1: The picture of *Lates longispinis* as found on the website fishbase.se.

Lake Turkana, Kenya

The variation of the genus *Lates* that inhabits Lake Turkana was summed up in case of color as follows by two Officers of the Department of fisheries, Mr. Timothy Lokuruka Ekal and Mr. Barre Saidi Alexander: There are yellow specimens from the northern region where the river Omo flows into the lake; black ones from the Island where the rock is black and white-silverish ones from everywhere else. The white-silverish *L. niloticus* was abundant in the fish markets on the Western Shore, I visited.

The picture of the *L. longispinis* (Figure 1) was recognized by the Officers and I was told that only sometimes, very rarely, a specimen of *Lates longispinis* goes astray to end up in the net of a fisherman. They are hardly ever seen, but still seen in the market; with somewhat higher frequency in the first/last part of the year, when the water level of the lake is lower.

Mr. James Last A. Keyombe, PhD-student at the Kenya Marine and Fisheries Research Center and also an Officer of the Department of Fisheries gave some more detailed information about *L. longispinis*. He said that it is sometimes referred to as the *yellow lates* (contrary to the aforementioned black color) and lives mostly in the region of the rocky shores around the Island. This region is an environmentally protected area (South Island National Park) and Fishermen are not allowed to fish there, which is the reason that it can't be found at the fish markets.

Some fishermen supported this information and one immediately recognized the specimen on the picture as belonging to the kind of fish that he, growing up on the Island, used to catch in his youth. He noted contrary to the literature that the fish does get very big, even bigger than the *L. niloticus*. Though this impression might only be due the overfishing in the unprotected areas that leads to smaller and smaller average specimens of *L. niloticus* but can also be an incitation for reevaluating whether the literatures information about the relatively smaller maximum size of *L. longispinis* is correct.

If one was trying to solve the riddle of the genetic status of *L. longispinis* he should at first make contact with the Kenyan Marine and Fisheries Research Center in Kalokol/Lodwar to suggest a research partnership and must at least calculate one week to organize the trip to the Island as there are permissions to be applied for at the administration of the national park, in order to enter the protected area and to fish there.

Lake Albert, Uganda

Again, working with the prototypic picture (Figure 2), the only characteristic I could find, is the mouth, that is less terminally oriented than that of a normal *Lates niloticus*, but is pointing somewhat more upwards.



Figure 1: The picture of *Lates macrophthalmus* as found on the website *fishbase.se*.

I showed the picture to people, emphasizing this characteristic and was told that this kind of fish exists in the lake and is called *gem utshok* [ge:m u:ʃok]. While a *gem* is any young *Lates*, *utshok* refers to a mouth that is pointing upwards. They are seldomly found at the central eastern coast of Lake Albert where I searched for it (i. e. the border region of Buliisa and Hoima Districts), but fisherman told me, they are far more common in the northern region, close to the City Bulisa. Also, a Congolese Fisherman, selling his catch in Uganda, told me that this kind of fish is far more frequently seen along the western coast.

If somebody was planning to undertake a genetic analysis of the *L. macrophthalmus*, the northern and western coast might be worthwhile starting the search.