STATUS OF INDIGENOUS OREOCHROMIS AND OTHER OREOCHROMINI SPECIES USING DNA BARCODING

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ABSTRACT

Nile tilapia (Oreochromis niloticus) and their hybrids has been used across the world for aquaculture. However, it can represent a threat to the genetic integrity of wild populations, as in the case of the Mozambique tilapia (Oreochromis mossambicus) in Southern Africa. Most of the available genetic data on Oreochromis comes from unknown wild source. DNA barcoding approach using mtDNA COI sequences was applied to improve the knowledge on biodiversity of the Oreochromis genus. The objectives include: assessing the genetic diversity of the tribe Oreochromini and test its monophyly; evaluate the genetic status of both O. mossambicus and O. niloticus focussing on the diversity of wild populations, and evaluating the genetic status of the genus Oreochromis within the tribe Oreochromini. The methodology involved retrieving mtDNA COI sequences from publicly available databases (BOLD and NCBI) and generating new sequencing on 81 specimens from 43 locations of the native ranges and 13 specimens from a hatchery. Maximum likelihood (ML) algorithm was used to analyse the combined sequences (532 bp fragment; N=560) in MEGA version 7.0.14. Results revealed clues of mtDNA introgression and polyphyly of species of the tribe Oreochromini. Cryptic diversity in wild Oreochromis populations, with multiple lineages of both O. mossambicus and O. niloticus. Oreochromis genus status need to be revisited based on the relationship between O. aureus and Sarotherodon with genetics distance ranging from 0.05 and 0.07. The overal average genetic distance in Oreochromis was 0.06. However, the genetic distances between O. mossambicus and O. niloticus was 0.06 while the distances between these species and O. aureus were 0.08 and 0.09 respectively. These results highlight the importance of revising the taxonomy of the tribe, assessing the phylogenetic relationships between taxa, and creating reference barcoding library that serves as an important molecular resource.

Keywords: Cryptic species, Misidentification, Mitochondria DNA, Phylogeography, Pseudocrenilabrinae,

INTRODUCTION

The tribe *Oreochromini* are mouth-brooding cichlids species divided into 10 genera (Dunz and Schliewen, 2013). Oreochromis is unique and especially diverse among these genera, with more than 43 extant species spread across rivers and lakes in Africa and the Middle East (Fricke *et al.,* 2020; Trewavas, 1983). *Oreochromis* is established as a valuable global aquaculture resource cantered around three species: *Oreochromis aureus* (Steindachner 1864), *Oreochromis mossambicus* (Peters 1852), and *Oreochromis niloticus* (Linnaeus 1758). The total amount of tilapia produced worldwide in 2018 was 5.5 million tonnes, valued at US $ 14.2 billion (FAO, 2020). Nile tilapia accounted for nearly 75% of global tilapia output (4525.4 thousand tons), or approximately 8.3% of total aquaculture production,while *O. mossambicus* and *O. aureus* contribution was 38,000 and 3,182 tonnes respectively (Samaddar *et al*., 2024). The domination of Nile tilapia has been attributed to its high growth rates and ability to withstand a variety of environmental factors, such as high temperatures and low dissolved oxygen (El-Sayed and Fitzsimmons 2023). Wild relatives of Nile tilapia, many of which support their own major fisheries, could be employed in selective breeding programs to improve productivity by introducing variations linked with critical aquaculture features (Ciezarek *et al.*, 2024). Due to the significant productivity of certain Oreochromis species in aquaculture and capture fisheries, they have been extensively introduced into tropical and subtropical freshwater ecosystems worldwide (Canonico *et al.,* 2005). Every year, fish consumption rises, placing strain on tilapia aquaculture in more than 140 countries (Deines *et al*., 2016). Despite their relevance for food security, many of the Oreochromis species are poorly understood (Ford *et al*., 2019). Furthermore, the identification of the origin of wild stocks used in aquaculture is limited by the possibility of hybridization in the wild and the lack of knowledge regarding the wild Indigenous populations of tilapia in aquaculture (Ciezarek *et al.*, 2024; Mojekwu *et al.*, 2020).

Genetic studies based on mtDNA have been carried out on *Oreochromis species* such as *O. aureus, O. mossambicus, O. niloticus and O. urolepies*. These research includes cross-reference check and validation of *Oreochromis* species (Muhala *et al*., 2024; Syaifudin *et al.,*2019), DNA barcodes for species diversity and identification in cichlids (Anbarasi *et al*., 2015; Bagley *et al*., 2017; Carvalho *et al*., 2018; Mashaphu, *et al*., 2024; Mojekwu *et al.*, 2020) and to detect mismatches between maternal origin species and its current offspring (June *et al*., 2016;

Muhala *et al*., 2024). Nevertheless, mtDNA can only reflect the evolution of the mitogenome, rather than that of the whole genome of the organisms. Hence, there is a need to combine it with reasonable numbers of nuclear markers to better understand the phylogeny of *Oreochromis* genus. The bulk of research done mostly utilized naturalized or farmed populations, with limited understanding of their population's origin (Mojekwu *et al.,* 2020).This is concerning, given a significant portion of the genetic data of Oreochromis originates from naturalized Asian and American populations (Maranan *et al*., 2015; Ordoñez *et al*., 2017; Pereira *et al*., 2011). This indicates that the specimens originate from an unidentified wild source, underscoring the necessity to acquire baseline reference data on the natural genetic diversity within the genus Oreochromis, particularly in Southern Africa (Mojekwu *et al.,* 2020). This is particularly significant for the indigenous populations of *O. mossambicus*, a species classified as endangered by the IUCN (Bills, 2019: Mashaphu, *et al*., 2024). Building a barcode reference library will contribute to a better understanding of the Oreochromis genus' biodiversity.

This study aims to improve the knowledge on biodiversity of Oreochromis genus using COI barcodes from wild populations of known origin.

MATERIALS AND METHODS

Taxonomic sampling and Mining COI sequences

The Cytochrome c Oxidase subunit I (COI) sequences, were generated from 94 specimens belonging to nine species of the genus *Oreochromis* from different river systems across Africa and hatchery stocks (Table 1.0).

In addition, all available COI sequences belonging to the tribe *Oreochromini* was mined from GenBank as of the 23rd March 2016. Oreochromini are mouth-brooders, comprising 10 genera of *Oreochromis, Alcolapia, Tristramella, Iranocichla, Sarotherodon, Pungu, Konia, Myaka, Stomatepia* and *Danakilia*. This tribe was included because previous work showed that this clade – formerly known as Tilapiine – involves several other genera, and that the species redbreast tilapia *Tilapia rendalli* (Boulenger 1897) and redbelly tilapia *Tilapia zillii* (Gervais 1848) belongs to the genus *Coptodon* (Dunz and Schliewen, 2013). The genus *Danakilia* was tentatively included in the analyses, as it is assumed to be closely related to *Iranocichla* (Schwarzer *et al*., 2017). *Coptodon rendalli*, mtDNA reference genomes of *Sarotherodon melanotheron, Coptodon zillii, .Heterotilapia buttikoferi, Neolamprologus*, *Pundamilia nyererei, Haplochromis burtoni* (Günther 1894), zebra mbuna *Maylandia zebra*, were added as outgroups. (Brawand *et al*., 2014). Details of the sequences and their haplotypes are shown in excel table 2.

DNA extraction and sequencing

Total genomic DNA was extracted from muscle tissues and fin clips preserved in 96% ethanol and stored at 4^0C using modified salting out method by Lopera-Barrero*et al*. (2008). The COI gene was amplified using the forward primer Oremos BCF (5'-CTT GAC GCT CAG CCA TCT TAC C-3') and reverse primer Oremos BCR (5'-GGG AGG ATA AGA ATG TAA ACT TCA GG-3') designed from the mitochondrial genome of *Oreochromis niloticus* (Brawand *et al*., 2014) using PRIMER3 version 0.4.0 website (http://bioinfo.ut.ee/primer3-0.4.0/). The PCR contained a final volume of 25 µl and sequencing was done on an ABI3500X1 automated sequencer (Applied Biosystems, Carlsbad, CA, USA).

Phylogenetic Analyses

These new sequences were combined with those retrieved from BOLD and NCBI databases and aligned all sequences using the online version of MAFFT v7.394 using the default settings (FFT-NS-2; Katoh *et al*., 2002: https://mafft.cbrc.jp/alignment/server/). The species *Tilapia rendalli* was changed to *Coptodon rendalli* following GenBank's latest taxonomic updates for the group. The sequences were ordered based on their similarity and brought into MEGA as an alignment for additional analyses. This includes mtDNA sequences associated with the reference genome of *O. niloticus*. MEGA was used to select the best model of evolution and then performed a phylogenetic tree using Maximum Likelihood algorithm (ML). 1000 bootstraps were selected with SPR5 swapping and moderate branch-swap filter.

RESULTS

Mining COI sequences

The combined dataset of 513 *Oreochromis* COI sequences were obtained from databases after filtering sequences that are duplicate, ambiguous or incomplete. The seven COI sequences from the sub-family Pseudocrenilabrinae served as an outgroup to the *Oreochromini* species. These datasets were combined with the 94 newly generated sequences to build a sequence dataset of 615 sequences (513 + 8 + 94) trimmed to a standard size of 532 bp, and that was further analysed using MEGA version 7.0.14. Removed 25 sequences with a size of 430bp and 28 duplicated sequences from *Danakilia* and two from *S. galilaeus*. This represents an *Oreochromini* dataset of 560 sequences (Table 1). The coding sequence (vertebrate mtDNA) were verified in MEGA using open reading frame, to check for the presence of stop codons or ambiguities before ML phylogenetic analyses.

Phylogenetic Analyses

The ML analyses that included 560 *Oreochromini* sequences dataset is characterized by 189 variable sites including 158 parsimony informative sites. The average GC content was 23.3% with an average transition: transversion ratio of 4.7. Topologies of the maximum likelihood tree showed evidence of cryptic diversity in *Oreochromis*, clues of mtDNA introgression in species of the Oreochromini tribe and that the tribe is not monophyletic (Figure 1). Details of the ML tree could be found in DOI: 10.13140/RG.2.2.26488.97282. Morphospecies of *O. aureus*×*O. niloticus, O. mossambicus, O. niloticus,* and some unidentified *Oreochromis* species were characterized by several lineages and were all represented among their lineages (Figure 1). Three lineages were observed in *Oreochromis niloticus* while the *O. mossambicus* comprises of the

Southern African and Eastern South African lineages. The Southern African lineage of *O. mossambicus* comprises of four other species *O. andersonii*, *O. macrochir, O. mortimeri* and *O. placidus* (Figure 1). However, the Eastern South African lineage of *O. mossambicus* and individuals sampled from the wild share the same haplotypes and are distributed along the coast. Hybrids of *O. aureus* maternal DNA (*O. aureus*×*O. niloticus*) were observed to be present in *O. mossambicus* and *O. niloticus* lineages 1. The result also indicated that unidentified *Oreochromis* species (*O. sp*) were found in *O. aureus, O. niloticus* and *O. urolepis* lineages. *Oreochromis aureus, O. mossambicus and O. niloticus* phenotypes were observed to occur in one another lineage. Morphological identified wild species of *O. aureus, O. niloticus* and *S. galilaeus* including a hatchery GIFT strain q603 (Hap 53) were found to be present in *O. aureus* lineage. The result also showed that Sarotherodon, excluding *S. caudomarginatus* clustered within *Oreochromis* near *O. aureus*.

DISCUSSION

The presence of three lineages of *Oreochromis niloticus* (Figure 1) can be attributed to cryptic diversity or large distribution range from different sources of introductions (Decru *et al*., 2016; Mojekwu *et al.*, 2020; Muhala *et al*., 2024; Syaifudin *et al*., 2019). The five species of the Southern African lineage have biogeographic affinity in Southern Africa as they co-occur in the wild across the, Limpopo system (South Africa), upper and middle Zambezi (Angola, Mozambique, Zambia and Zimbabwe), Okavango (Botswana) and Cunene river systems (Namibia) (Mojekwu and Hoareau, 2024; Nico and Neilson, 2017; Skelton, 2001; Zengeya *et al*., 2015, 2017). However, the sharing of same haplotypes and distribution along the coast in the Eastern South African lineage of *O. mossambicus,* were observed by previous reports which showed this species to cluster along the southern coast of South Africa, describing it as a recent southern radiation dating back to 17,800 yr before present (D'Amato *et al*., 2007). This shows likely migration from north to south, confirming that this lineage could be dispersing along the coast (Mojekwu and Hoareau, 2024; Skelton 2001; Zengeya *et al*., 2017). This geographical distribution could be driven by habitat and environmental conditions as this lineage thrives in water that is highly saline and at lower temperatures (James and Bruton, 1992; Mojekwu and Hoareau, 2024; Muhala *et al*., 2024). Hybrids of *O. aureus* maternal DNA (*O. aureus*×*O. niloticus*) present in *O. mossambicus* and *O. niloticus* lineages 1, were likely introduced for aquaculture from unkown wild sources. Similarly unidentified *Oreochromis* species (*O. sp*) found in *O. aureus, O. niloticus* and *O. urolepis* lineages, respectively, were either from farmed or feral populations in Australia, Canada. Phenotypic Identification of these species by phenotype might be difficult since several strains have been developed for aquaculture purposes (El-Zaeem *et al*., 2011; Frimpong, *et al*., 2016; Hamzah *et al*., 2014).

Several lineages contain more than one species associated to them by morphological assignment. *Oreochromis aureus, O. mossambicus and O. niloticus* phenotypes were observed to occur in one another lineage.This could be due to tilapiine species global translocation for stocking and aquaculture and having well established feral populations (Deines *et al*., 2016; Muhala *et al*., 2024) or adaptation to adverse environmental condition, which has taken place more than once within Oreochromis genus (Ford *et al*., 2019). Incomplete lineage sorting widely reported in cichlids could be another explanation but past hybridisation observed in Oreochromini tribe seems the most likely answer in some cases (Dunz and Schliewen, 2013). Morphospecies of wild *O. aureus, O. niloticus* and *S. galilaeus* including a hatchery GIFT strain q603 (Hap 53) were present in *O. aureus* lineage. This has been reported as differential introgression of mtDNA from *O. aureus to O. niloticus* in all West African area (Rognon and Guyomard, 2003). Misidentification could be another explanations to this discrepancy (Ordoñez *et al*., 2017) or genetic decline due to introgression (Gupta and Acosta, 2004; Mojekwu and Hoareau, 2024; Sukmanomon *et al*., 2012). It is worth noting that mtDNA alone cannot reliably detect hybrids but can give insight into mtDNA introgression if the number of sequences per species is sufficient.

Sarotherodon, excluding *S. caudomarginatus* are found clustering within *Oreochromis* near *O. aureus*, suggesting that the taxonomy needs revision. Several authors in the past have suggested the placement of Sarotherodon within *Oreochromis* (Klett and Meyer, 2002; Pouyaud and Agnèse, 1995). *Oreochromis* hybridizes with species that are congeneric, but no reported knowledge of any crosses between *Oreochromis* and *Sarotherodon* in the wild. Nevertheless, intergeneric hybridization with viable offsprings has been reported between the two species using in-vitro fertilisation (Bezault *et al*., 2012). Recent studies using nuclear DNA suggest likely mis-identification between *Sarotherodon galilaeus* and *O. aureus* (Ford *et al*., 2019; Syaifudin *et al.,*2019). However, when SNP-based data from greater than 1200 nuclear genomic markers were used there was clear separation of the two genera (Syaifudin *et al.,*2019). No hybrid of *Sarotherodon* and *Oreochromis* have been observed in the wild and based on the phylogenetic relationship between *S. boulengeri, S. galilaeus*, *S. lohbergeri, S. melanotheron* and *O. aureus*, there is the need for a review of *Sarotherodon* taxonomic status after a thorough, effective assessment using nuclear markers with robust sampling.

CONCLUSION

The genetic diversity of the tribe Oreochromini with overall average genetic distance of 0.06, showed clues of mtDNA introgression in species of the tribe and that it's not monophyletic. However, cryptic diversity in *Oreochromis* with more than one lineage were observed in both *O. mossambicus and O. niloticus though* no hybrid of it detected in Southern African rivers using mtDNA. This could be errors of identification, hybridization between feral populations, introgression \and selection within improved farm stocks (GIFT). Furthermore, the status of Oreochromis genus need to be revisited based on the relationship between *O. aureus and Sarotherodon* with

the suggestion for vast sampling of *O. aureus* to investigate its placement. Its worthy to note that a single gene approach alone is inadequate for detecting invading populations and we suggest the development of a reliable, replicable multilocus genomic approach in detection of hybrids within *O. mossambicus*. This study however gave us a background understanding of the phylogenetic structure in *O. mossambicus* from South Africa.

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AUTHORS CONTRIBUTION

MTO: literature search, experimental design, sample collection, laboratory work, data analysis and write-up. MJC:conceived the idea, experimental design, wrote the draft and supervised the work. TBH: Completed the work supervision, data analysis and write-up. All authors approve the manuscript submission.

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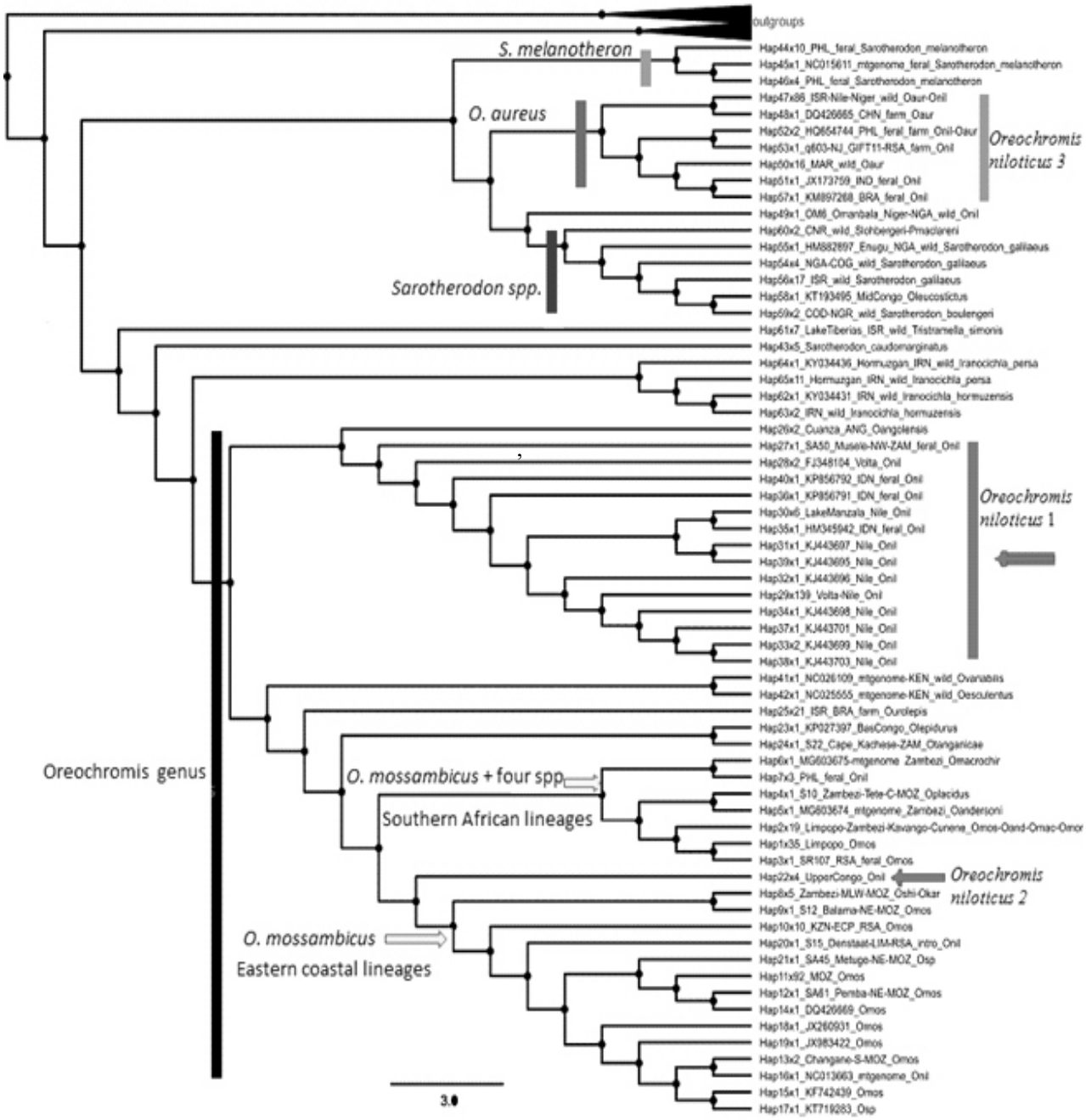

Fig 1. Cladogram of Oreochromini haplotypes (COI 532bp x 560 taxa). Dots on the branches represent values of posterior probability (>0.95). The thick black vertical bar indicates all the lineages of *Oreochromis* except *O. aureus* (purple), which were embedded in *Sarotherodon* (brown). Two distinct lineages of *O. mossambicus* are shown with yellow arrows to the right while the blue arrows to the left represent the different lineages of *O. niloticus*. Detail of the ML tree could be found in DOI: 10.13140/RG.2.2.26488.97282 https://www.researchgate.net/publication/386376758 as supplementary figure S1.

Table 1. New sequences of wild and hatchery samples

Sample ID	Species Phenotype	BIN	GenBank ID	Lat/ Long	Country/River
SA3	*O. mossambicus*	AD10792	MK497065	23,99 S 31,83 E	South Africa / Olifants
SA4	*O. mossambicus*	AD10792	MK497066	28,27 S 16,79 E	Namibia/ Orange
SA5	*O. mossambicus*	AD10792	MK497067	33,42 S 25,35 E	South Africa/ Sunday
SA6	*O. mossambicus*	AD10792	MK497068	22,36 S 29,28 E	SA/Denstaat farm (Limpopo)
SA7	*O. niloticus*	AD10792	MK497069	7,61 N 8,62 W	Liberia/ Dehn
Sa8	*O. andersonii*	AD10792	MK497070	17,26 S 11,76 E	Namibia/ Cunene Lagoon
SA9	*O. mossambicus*	AD10792	MK497071	27,04 S 17,86 E	Namibia / Lowen
SA10	*O. placidus*	AD10792	MK497072	16,31 S 33,73 E	Mozambique / Tete
SA11	*O. mossambicus*	AD10792	MK497073	25,52 S 28,23 E	South Africa/Apaies
SA12	*O. mossambicus*	ACE5030	MK497074	13,41 S 38,59 E	Mozambique/Namituko
SA13	*O. mossambicus*	AAA8511	MK497075	27,36 S 32,53 E	South Africa/ Sibaya
SA15	*O. niloticus*	AAA8511	MK497076	22,21 S 29,28 E	SA/Denstaat farm (Limpopo)
SA16	*O. mossambicus*	AD10792	MK497077	23,3 S 15,77 E	Namibia/ Kuiseb
SA17	*O. mossambicus*	AAA8511	MK497078	13,09 S 40,54 E	Mozambique/ Pemba
SA19	*O. macrochir*	AD10792	MK497079	17,05 S 19,53 E	Angola / Cuito
SA20	*Oreochromis*	ACE5030	MK497080	12,71 S 34,83 E	Mozambique/Ntumba kawile
SA22	*O. tanganicae*	ACZ6850	MK497081	8,49 S 30,47 E	Zambia / L. tanganyika
SA26	*O. mossambicus*	AD10792	MK497082	25,52 S 28,23 E	South Africa /Aapies
SA27	*O. mossambicus*	AD10792	MK497083	25,52 S 28,23 E	South Africa /Aapies
SA28	*O. andersonii*	AD10792	MK497084	18,43 S 21,9 E	Botswana / Okavango
SA33	*O. mossambicus*	AD10792	MK497085	23,26 S 30,77 E	South Africa/Nsama
SA35	*O. mossambicus*	AD10792	MK497086	24,49 S 17,85 E	Namibia / Hardap
SA36	*O. mortimeri*	AD10792	MK497087	18,06 S 26,63 E	Zimbabwe/ Zambezi
SA37	*O. mossambicus*	AAA8511	MK497088	22,94 S 33,67 E	Mozambique/ Changane
SA38	*O. andersonii*	AD10792	MK497089	15,17 S 19,2 E	Angola/ Cuito
SA39	*O. mossambicus*	AAA8511	MK497090	24,11 S 33,83 E	Mozambique/ Malawazi
SA42	*O. angolensis*	AAC4873	MK497091	9,69 S 14,42 E	Angola/ Cuanza
SA44	*O. niloticus*	AAA6537	MK497092	7,61 N 8,62 W	Liberia/ Dehn
SA45	*Oreochromis*	AAA8511	MK497093	13 S 40,39 E	Mozambique
SA46	*O. mossambicus*	AAA8511	MK497094	16,31 S 33,73 E	Mozambique/ Muarazi
SA47	*O.mossx O.nil*	AD10792	MK497095	23,11 S 30,12 E	South Africa/ Luvuvhu
SA49	*O. angolensis*	AAC4873	MK497096	9,17 S 13,41 E	Angola/ Kawa
SA50	*O. niloticus*	AAC9904	MK497097	12,15 S 25,39 E	Zambia/ Musangezle
SA51	*Oreochromis*	AAA8511	MK497098	13,1 N 40,45 E	Mozambique
SA52	*O. mossambicus*	AD10792	MK497099	25,52 S 28,23 E	South Africa/ Apies
SA53	*O. mossambicus*	AAA8511	MK497100	13,1 S 40,45 E	Mozambique
SA55	*O. mossambicus*	AD10792	MK497101	33,73 S 18,75 E	South Africa/Klapmuts
SA57	*O. andersonii*	AD10792	MK497102	17,51 S 20,06 E	Angola/ Okavango
SA60	*O. mossambicus*	AAA8511	MK497103	26,99 S 32,74 E	South Africa/Mahlampane
SA61	*O. mossambicus*	AAA8511	MK497104	13,09 S 40,54 E	Mozambique/Pemba
SA69	*O. shiranus*	ACE5030	MK497105	12,83 S 34,16 E	Malawi/ Bua
1154	*O. mossambicus*	AAA8511	MK497106	31,67 S 29,46 E	South Africa/Umngazi estuary
SR102	*O. mossambicus*	AD10792	MK497107	33,18 S 25,16 E	South Africa/ Sundays,ECP
SR103	*O. mossambicus*	AD10792	MK497108	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR104	*O. mossambicus*	AD10792	MK497109	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR107	*O. mossambicus*	AD10792	MK497110	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR108	*O. mossambicus*	AD10792	MK497111	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR111	*O. mossambicus*	AD10792	MK497112	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR112	*O. mossambicus*	AD10792	MK497113	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR113	*O. mossambicus*	AD10792	MK497114	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR119	*O. mossambicus*	AD10792	MK497115	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR120	*O. mossambicus*	AD10792	MK497116	33,18 S 25,16 E	South Africa/ Sundays ,ECP

SR121	*O. mossambicus*	AD10792	MK497117	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR122	*O. mossambicus*	AD10792	MK497118	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR123	*O. mossambicus*	AD10792	MK497119	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR125	*O. mossambicus*	AD10792	MK497120	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR127	*O. mossambicus*	AD10792	MK497121	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR130	*O. mossambicus*	AD10792	MK497122	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR137	*O. mossambicus*	AD10792	MK497123	33,18 S 25,16 E	South Africa/ Sundays ,ECP
SR139	*O. mossambicus*	AD10792	MK497124	33,18 S 25,16 E	South Africa/ Sundays ,ECP
TOM004	*O. mossambicus*	AD10792	MK497125	24,78 S 29,43 E	South Africa,Limpopo
TOM005	*O. mossambicus*	AD10792	MK497126	24,78 S 29,43 E	South Africa,Limpopo
TOM006	*O. mossambicus*	AD10792	MK497127	24,78 S 29,43 E	South Africa,Limpopo
TOM007	*O. mossambicus*	AD10792	MK497128	24,78 S 29,43 E	South Africa,Limpopo
TOM008	*O. mossambicus*	AD10792	MK497129	24,78 S 29,43 E	South Africa,Limpopo
TOM0109	*O. mossambicus*	AD10792	MK497130	22,63 S 30,4 E	South Africa,Limpopo
NASA8364	*O. mossambicus*	AD10792	MK497131	25,42 S 29,36 E	South Africa,Limpopo
NASA8537	*O. mossambicus*	AD10792	MK497132	23,92 S 29,45 E	South Africa/Phalaborwa Barrage
Q0574	*O. mossambicus "Red 5' strain*	AAA8511	MK497133	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0577	*O. mossambicus "Red 5' strain*	AAA8511	MK497134	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0578	*O. niloticus "Chitralada"*	AAC9904	MK497135	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0581	*O. niloticus "Chitralada"*	AAC9904	MK497136	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0582	*O. niloticus "Chitralada"*	AAC9904	MK497137	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0588	*O. niloticus "Chitralada"*	AAC9904	MK497138	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0589	*O. niloticus "Chitralada"*	AAC9904	MK497139	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0590	*Hybrid (O.moss x O.niloticus)*	AAA8511	MK497140	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0593	*Hybrid (O.moss x O.niloticus)*	AAA8511	MK497141	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0595	*Hybrid (O.moss x O.niloticus)*	AAA8511	MK497142	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0596	*Hybrid (O.moss x O.niloticus)*	AAA8511	MK497143	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0599	*Hybrid (O.moss x O.niloticus)*	AAA8511	MK497144	33,36 S 26,15 E	South Africa/ Hatchery,ECP
Q0603	*GIFT (O.niloticus*)	AAA6537	MK497145	33,36 S 26,15 E	South Africa/ Hatchery,ECP
OM3A	*O. niloticus*	AAA6537	MK497146	6,55 N 6,9 E	Nigeria,Anambra,Aguleri
OM4A	*O. niloticus*	AAA6537	MK497147	6,55 N 6,9 E	Nigeria,Anambra,Aguleri
OM5A	*O. niloticus*	AAA6537	MK497148	6,55 N 6,9 E	Nigeria,Anambra,Aguleri
OM6A	*O. niloticus*	AAA6537	MK497149	6,55 N 6,9 E	Nigeria,Anambra,Aguleri
OM9A	*O. niloticus*	AAA6537	MK497150	6,55 N 6,9 E	Nigeria,Anambra,Aguleri
ON10A	*O. niloticus*	AAA6537	MK497151	6,14 N 6,77 E	Nigeria, Anambra,Aguleri
ON12A	*O. niloticus*	AAA6537	MK497152	6,14 N 6,77 E	Nigeria, Anambra,Aguleri
ON13A	*O. niloticus*	AAA6537	MK497153	6,14 N 6,77 E	Nigeria, Anambra,Aguleri
SG3	*S. galilaeus*	AAA6537	MK497154	32,79 N 35,54 E	Israel, Galilee
SG4	*S. galilaeus*	AAA6537	MK497155	32,79 N 35,54 E	Israel, Galilee
BI	*O. mossambicus*	AD10792	MK497156	19,52 S 29,25 E	Zimbabwe,Shangani
B2	*O. mossambicus*	AAC9904	MK497157	19,52 S 29,25 E	Zimbabwe,Shangani
B3	*O. mossambicus*	AD10792	MK497158	19,52 S 29,25 E	Zimbabwe,Shangani