INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
ISSN Print: 1560–8530; ISSN Online: 1814–9596
23–0377/2024/31–4–243–249
DOI: 10.17957/IJAB/15.2138
http://www.fspublishers.org

Full Length Article

Identification, Genetic Diversity and Phylogenetics of Germplasm using COX1 Marker: Preliminary Study for New Tilapia Breeding Scheme

Irmawati Irmawati[1,2*], Rosmiati Rosmiati[3], Darmawan Setia Budi[4], Kamaruddin Kamaruddin[3], Ince Ayu Khairana Kadriah[3], Mohammad Faizal Ulkhaq[4], Siti Aslamyah[2], Siti Halimah Larekeng[5] and Iswanto Iswanto[5]

1*Research and Development Centre for Biotechnology, Institute of Research and Community Service, Universitas Hasanuddin, Makassar, Indonesia*
2*Faculty of Marine Science and Fisheries, Hasanuddin University, Makassar, Indonesia*
3*Fisheries Research Center, National Research and Innovation Agency, Cibinong, Indonesia*
4*Study Program of Aquaculture, School of Health and Life Sciences, Universitas Airlangga, Banyuwangi, Indonesia*
5*Natural Heritage and Biodiversity Research Group, Institute of Research and Community Service, Universitas Hasanuddin, Makassar, Indonesia*
[*]For correspondence: trif.ahwa@gmail.com
Received 19 September 2023; Accepted 22 January 2024; Published 06 March 2024

Abstract

This study aims to analyze the genetic biodiversity of domesticated tilapia (kekar, sultana, DSLU) and wild tilapia (WPLU) in the context of the tilapia breeding program in South Sulawesi, Indonesia. Kekar and sultana tilapia strains were tilapia broodstock from Java Island introduced to South Sulawesi in 2022, while DSLU and WPLU tilapia strains are tilapia from Salulemo hatchery and wild tilapia caught in Poreang Creek in North Luwu, South Sulawesi, respectively. The other four Kekar are the first generation of Kekar from the broodstock introduced from Java to South Sulawesi (22PFG1). The alignment of 656 bp mt-DNA COX1 sequences showed that specimens originally thought to be *Oreochromis niloticus* were identified as *O. niloticus* and *O. mossambicus*, while the WPLU strain was identified as *O. aureus*. The 64 single nucleotide polymorphisms (SNPs) identified resulted in seven haplotypes that formed four clades. Tilapia strains were spread across four clades, indicating that tilapia has high genetic diversity. Salulemo tilapia had a genealogical relationship with sultana and kekar *O. niloticus* strains. These results suggest that tilapia introductions have included *O. mossambicus* and *O. niloticus*. The wild *O. aureus* in Poreang Creek, North Luwu, is a first record for *O. aureus* in Indonesia. The discovery of *O. aureus* is useful for designing tilapia breeding programs to produce all-male seeds. © 2024 Friends Science Publishers

Keywords: mt-COX1; Haplotype; *Oreochromis*; Biodiversity; Tilapia

Introduction

Nile tilapia, [*Oreochromis niloticus* (Linnaeus 1758)], is the second most widely cultivated species globally after cyprinids (FAO 2020; Makwinja and Geremew 2020), being farmed now in over 120 countries (Charo-Karisa 2022). Female tilapia have high fecundity, and their gonads mature at a relatively small size, while male tilapia generally have a faster growth rate, causing a demand for monosex (all male) seed for cultivation (El-Greisy and El-Gamal 2012). Various efforts have been made to improve the efficiency of tilapia production, including through the design of good breeding strategies and hybridization, as well as through male monosex cultivation systems (Ghosal and Chakraborty 2020). *O. niloticus* is an important global aquaculture commodity that has the potential to form a basis for the freshwater aquaculture industry in Indonesia.

In South Sulawesi, Indonesia, tilapia farming has developed rapidly in the last three years. However, the problems encountered include the inconsistent supply of quality and good seeds by the broodstock centers, which are still centralized in Java, and the poor management of genetic germplasm, with uncontrolled hybridization, introgression, and lack of understanding of breeding strategies among cultivators. Uncontrolled crossing of strains by cultivators occurs because controlled and universal management practices have not yet been implemented in Indonesia. Sustainable aquaculture is one key to achieving the Zero Hunger Sustainable Development Goal (SDG) and food safety in the future. However, it will not be easy to achieve without the support of proper and good-quality seed production management (Naylor *et al.* 2021) and the adequate and sustainable production of superior seeds (Mala *et al.* 2023).

To cite this paper: Irmawati I, DS Budi, SH Larekeng, R Rosmiati, K Kamaruddin, IAK Kadriah, MF Ulkhaq, S Aslamyah, I Iswanto (2024). Identification, genetic diversity and phylogenetics of germplasm using COX1 marker: preliminary study for new tilapia breeding scheme. *Intl J Agric Biol* 31:243–249

Genetic diversity information is crucial in selective breeding programs because it is the basic data for obtaining pedigree information and controlling inbreeding. In hatchery management, integrated pedigree information to avoid inbreeding is vital for genetic improvement strategies because poor pedigree management will impact productivity, growth, and sustainability (Hollenbeck and Johnston 2018; Liu *et al.* 2022). The Wallacea region is famous for its native genetic diversity (Yanuarita *et al.* 2020). However, the genetic diversity of introduced species, such as tilapia hat, has not yet been identified and described. Furthermore, no studies or reports show that genetic diversity is a consideration in the tilapia breeding conducted in South Sulawesi. The analysis of genetic diversity can be performed using mitochondrial DNA markers, particularly cytochrome c oxidase 1 (mt-COX1) (Lee *et al.* 2012). Prior to our study, the mt-COX1 had been used as a first step to identify and analyze the genotypic diversity of the *Channa striata* as a source of genotypes for use in breeding programs (Irmawati *et al.* 2018; Mala *et al.* 2023). The mt-COX1 marker has also been used to analyze the genetic diversity of the genus *Nodularia* (Choi *et al.* 2020) and *Cyprinus carpio* L. (Torgunakova *et al.* 2012). Genetic diversity studies using microsatellite DNA markers have been used in breeding programs for tilapia (Montoya-López *et al.* 2019) and barramundi (Loughnan *et al.* 2016), including to plan the conservation of germplasm and management of tilapia fisheries (Soliman *et al.* 2017).

This study identified and analyzed the genetic diversity and genealogical evolution of introduced tilapia germplasm and wild-type tilapia in South Sulawesi for sustainable seed production and conservation management. This research is important for improving production performance and developing the new MaJaCea tilapia strain. Improving production performance in aquaculture is crucial for reducing production costs and achieving competitive products. Although the data presented in the current study came from a limited number of samples, scientific genetic data related to identifying genetic diversity, the pedigree of tilapia in South Sulawesi, and the evolution of tilapia in Indonesia are reported for the first time. This research is a preliminary study to assemble basic genetic information on tilapia populations in the Wallacea Region of South Sulawesi as a first step to producing superior tilapia fry named the MaJaCea strain.

Materials and Methods

Fish samples

The research design used descriptive-analytical methods. Data were obtained by analyzing the genotypes of 13 tilapia specimens: 12 domesticated and one wild tilapia. The domesticated tilapia comprised a sample of male broodstock (015F4) from the fourth generation of kekar produced in 2015 at the Hatchery of Nila Kekar (HNK) Pasuruan; a female broodstock (22SPB) of sultana strain; prospective broodstock specimens 021F1_K1, 021F1_K2 and 021F1_K3 from the first generation of kekar tilapia produced in 2021 by the HNK Pasuruan; first generation (22PFG1) produced in 2022 by Polobete Fishfarm; tilapia seeds 2522DSLU, 2622DSLU and 2722DSLU produced by Salulemo hatchery, Sukamaju District, North Luwu Regency; and one wild tilapia (0222WPLU) specimen obtained from brackish waters in Poreang Village, North Luwu Regency. Each muscle organ and fin specimen were taken and fixed with a 96% ethanol solution for DNA analysis.

DNA extraction

Genomic DNA was isolated from muscle and fin samples from the 13 fish specimens using the CTAB-DTAB method (GeneReach Biotechnology Corp., Taiwan) following the manufacturer's instructions. Genomic DNA was measured using a Nanodrop 8000 spectrophotometer (ND Technologies, Wilmington, DE) at 260/280 nm wavelengths. The genomic DNA subjected to the subsequent analysis exhibits a purity range of 1.82–1.96 and a concentration within the range of 78.00–82.50 ng/mL.

Amplification and visualization of DNA bands

The tilapia COX1 gene was amplified using the universal primary pair FishF2 and FishR2. DNA amplification used a polymerase chain reaction (PCR) method. The PCR reaction volume of 50 μL contained 25 μL of MyTaq HS red-mix enzyme (Bioline, UK), 20 μL of nuclease-free water, two μL of each primer and one μL of sample DNA template. The PCR reaction cycle comprised denaturation at a temperature of 95°C for 3 min, followed by 40 cycles with denaturation at 95°C for 30 s, annealing at 55°C for 30 s and elongation at 72°C for 30 s. Once the 40 cycles were completed, the final stage was elongated at 72°C for one min, after which the amplification product was kept at 4°C for ~ (infinite) time until the operator stopped the cycle. The amplification product was then verified through electrophoresis on 1% agarose gel to view the results (Fig. 1).

Sequencing

The DNA amplification products obtained were sent to the 1st Base laboratory (Malaysia) for sequencing on an ABI3500 Genetic Analyzer machine (Applied Biosystems, USA) according to the company's protocol. DNA strands were sequenced in both directions using the FishF2 and FishR2 primers for each specimen to ensure data accuracy.

Data analysis

The partial mitochondrial COX1 gene sequences were edited using Gene Studio TM Professional software to ensure no ambiguous bases. Tilapia identification was

conducted by aligning the nucleotide sequences obtained from the tilapia samples with tilapia COX1 gene nucleotide sequences deposited in the National Center for Biotechnology Information (NCBI) GenBank (www.ncbi.nlm.nih.gov) nucleotide repository using the Basic Local Alignment Search Tool BLASTn program. The alignment results were tabulated as % query cover, % identity, and e-value significance values. Alignment and phylogenetic reconstruction of sample sequences and out-groups were performed using ClustalW (Thompson *et al.* 1997) in MEGA (Molecular Evolutionary Genetics Analysis) software v. 11 (Kumar *et al.* 2018). Phylogeny reconstruction used the iTOL v. 6.7 (interactive Tree of Life) (Letunic and Bork 2007). Genetic distances between specimens were analyzed using the pairwise distance function in MEGA v 11. Genetic variation was analyzed using the DnaSP v. 6.12.03 program (Librado and Rozas 2009), while haplotype distribution and connectivity were analyzed using Popart (Leigh and Bryant 2015).

Results

Molecular identification

This study isolated 13 partial sequences of the tilapia COX1 gene from three locations, namely North Luwu in South Sulawesi, Pasuruan in East Java and Sukabumi in West Java. The wild genotype from North Luwu (0222WPLU) was identified as *O. aureus* (ID: KU565831.1). The domesticated genotype from North Luwu (2722DSLU) was identified as *O. mossambicus* (ID: KM438534.1). In contrast, the seven remaining genotypes were all identified as *O. niloticus*. The query cover and identity of the 13 tilapia genotypes in this study ranged from 99–100% and 99.07–100%, respectively (Table 1).

Genetic variation, haplotype and FST

This study successfully isolated 656 bp of a nucleotide of the COX1 tilapia gene that encodes 218 amino acids. The analysis revealed seven haplotypes among the 13 specimens with 64 polymorphic sites, a nucleotide diversity (π) of 0.0185, 33 parsimony sites, 29 singleton sites and two indel sites. Genetic differentiation among the tilapia specimens in HNK Pasuruan, Polobete Fishfarm Pinrang and North Luwu varied from 0.0000 to 0.1869. The lowest FST values were between tilapia in HNK Pasuruan and Polobete Fishfarm, meanwhile the higher FST value were between tilapia from North Luwu and Pinrang (Table 2).

In North Luwu, South Sulawesi, three haplotypes (Hap_1, Hap_2 and Hap_3) were found, while in HNK Pasuruan, four haplotypes (Hap_4, Hap_5, Hap_6 and Hap_7) were identified. Hap_3 was detected in Sukabumi-West Java and the Salulemo hatchery in North Luwu. Hap_1 and Hap_2 differed by 45 mutations, differing from Hap_3 by 47 mutations, Hap_4 by 50, Hap_5 by 48, Hap_6 by 46, and Hap_7 by 49 mutations. Hap_2 and Hap_3 differ by 22 mutations and differ from Hap_4 by 25 mutations, while Hap_2 and Hap_6 differed by only two mutations and Hap_3 and Hap_4 differed by just three mutations (Fig. 2 and Table 3). The AT content of the tilapia COX1 gene is greater (51.91–53.06%) than the GC content (46.94–48.09%), as is the largest GC base content in the third base, which ranges from 57.14–57.80% (Table 3).

Phylogenetic tree and genetic distance

The phylogenetic tree of the 13 tilapia genotypes in this study and tilapia genotypes from geographical areas outside Indonesia using Neighbor-Joining are presented in Fig. 3. The phylogenetic tree resulting from the partial sequence of mt-COX1 distinguishes *O. aureus* from Poreang, *O. aureus* from the Philippines (KU565831.1), *O. niloticus* from Guangzhou, China (MW829393.1). Four of the 13 study samples, namely the sultana genotype, two hatchery genotypes of North Salulemo Luwu (2522DSLU &; 2622DSLU), and one stocky genotype (021F1 K1), each formed a different clade, and the others formed a clade with tilapia from geographical areas outside Indonesia.

In general, the genetic distances between the tilapia genotypes in this study were relatively small (< 0.003) except for the *O. aureus* genotype (0222WPLU), the Salulemo genotype (2722DSLU), and the kekar genotype (021F1-K2) whose genetic distance with other genotypes was > 0.050. The genetic distance of the *O. aureus* sample (0222WPLU) was very close to *O. aureus* from the Philippines KU565831.1 (0.002) and *O. niloticus* MW829393.1 (0.003) from Guangzhou, China. Meanwhile, the genetic distance of the kekar genotype (021F1-K2) and the Salulemo genotype (2722DSLU) with *O. mossambicus* from Zimbabwe (ID: KM438534.1) was 0.000, indicating that 021F1-K2 and 2722DSLU were *O. mossambicus*. Similarly, the genetic distance between kekar (021F1-K2) and *O. mossambicus* from Thailand was 0.003, indicating that the kekar genotype (021F1-K2) may belong to the Mozambique tilapia rather than the Nile tilapia.

Discussion

Understanding genetic diversity and genealogy patterns is critical to efficient germplasm management and improved aquaculture production performance. Diverse germplasm is essential for fish genetic improvement. Fish transfers have not been regulated well in Indonesia, posing a threat to the sustainability of tilapia production due to the loss of pure strains and inbreeding. This study used 64 SNPs markers derived from partial sequences of the mt-COX1 gene to assess the genetic diversity of domesticated tilapia and a wild type from a naturalized introduced population in South Sulawesi, Indonesia. No previous studies have reported tilapia germplasm in South Sulawesi, even in Indonesia. This study is the first to identify and partially characterize the mt-

Table 1: Percentage similarity, query cover, and e-value of closest GenBank accession matched the nucleotide sequences of the 13 tilapia specimens in this study

Specimen code	Query cover (%)	E-value	Identity (%)	Accession number and country
015F4	100	0.00	100	MF509597.1 – *O. niloticus*, Kelantan, Malaysia
22SPB	100	0.00	99.85	MK130702.1 – *O. niloticus*, Nigeria
021F1_K1	100	0.00	99.70	MK130702.1- *O. niloticus*, Nigeria
021F1_K2	100	0.00	99.56	MF509597.1- *O. niloticus*, Kelantan, Malaysia
021F1_K3	99	0.00	100	KU565826.1 – *O. niloticus*, BFAR-National Freshwater Fisheries Technology Center, Philippine
22PFG1-1	99	0,00	100	MK130702.1- *O. niloticus*, Nigeria
22PFG1-2	99	0,00	100	MK130702.1- *O. niloticus*, Nigeria
22PFG1-3	99	0,00	100	MK130702.1- *O. niloticus*, Nigeria
22PFG1-4	99	0,00	100	MK130702.1- *O. niloticus*, Nigeria
2522DSLU	100	0.00	99.85	MK130702.1- *O. niloticus*, Nigeria
2622DSLU	100	0.00	99.85	MF509597.1 – *O. niloticus*, Kelantan, Malaysia
2722DSLU	100	0.00	99.85	KM438534.1 – *O. mossambicus*, Zimbabwe
0222WPLU	100	0.00	99.70	KU565831.1 – *O. aureus*, Philippines

Note: 015F4, 021F1, 22PFG1, 22SPB, and DSLU = domesticated tilapia, WPLU = wild tilapia

Table 2: Genetic variation within the tilapia specimens in this study

Variation	n	Base pairs	Haplotype	Conserved sites	Variable sites	Parsimony sites	Singleton sites	InDel sites	Diversity (π)
Nucleotides	9	656	7	593	62	33	29	2	0.0185
Amino acids	9	218	7	147	53	28	25		
F_{ST}	0.0000 – 0.1869								

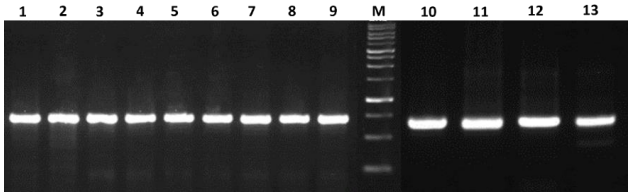

Fig. 1: The PCR product amplification of mtDNA COX1 gene region of 13 muscle samples of tilapia. 1: 015F4; 2: 22SPB; 3: 021F1_K1; 4: 021F1_K2; 5: 021F1_K3; 6: 2522DSLU; 7: 2622DSLU; 8: 2722DSLU; 9: 0222WPLU; 10-13: 22PFG1; M: 1kb DNA ladder

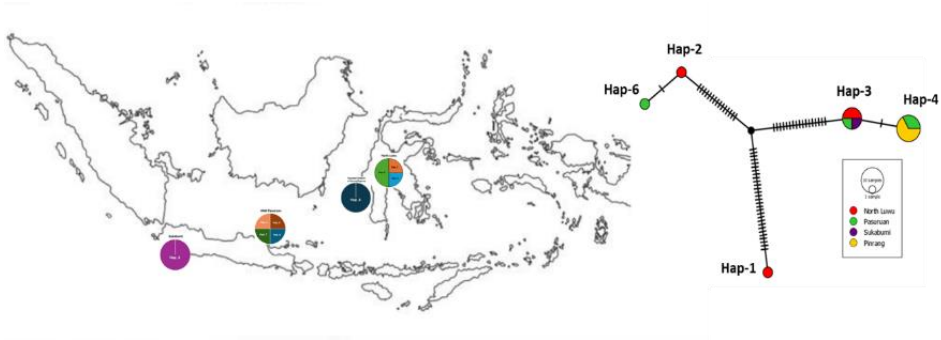

Fig. 2: Haplotype median-network joining described distribution (left) and haplotype evolution (right) of introduced and wild tilapia stock for hatchery broodstock in South Sulawesi

COX1 tilapia gene sequence and its evolution in this country.

From an aquaculture perspective, ensuring that the germplasm of the individuals selected as broodstock for seed production has a high genetic diversity is very important. Genetic diversity data is also essential in managing wild tilapia germplasm. The results showed low to moderate genetic differentiation between tilapia in HNK Pasuruan, Polobete Fishfarm Pinrang, and wild tilapia from North Luwu (FST = 0.0000 – 0.1869). Although there could be geographical isolation, gene flow between populations is thought to have occurred long before the introduction activities mentioned in this study were carried out. In addition to being strengthened by the FST value, common ancestry is also reflected in the genetic distance and the phylogenetic tree where sultana and kekar tilapia strains (from broodstock centers in Java Island) are in one clade with several tilapia from the Salulemo hatchery in North Luwu, South Sulawesi, with genetic distances between individuals from different populations ranging from 0.000 to 0.053.

Table 3: Haplotype nucleotide base variations, GC and AT content composition (%), and GC composition (%) for each codon position in the COX1 gene

Haplo-type	Variation nucleotide base sites	Quantity (%)		G+C% and codon position		
		G+C	A+T	1st	2nd	3rd
	1111111222222222222223333333333344444444444445555555566666 2337992456889002334456667889011224556691123344568899012236922222 2923725598758139709284369247951439406258168170987251406143691235 7					
Hap_1	GCGCCGTTGGCAACTTGCTCCTCCCCTATCGACGTTGTCGCCCTACGCTTAAGTTTTACTATACT	48.09	51.91	44.75	42.20	57.34
Hap_2	.TAT...C.ATG.AACAT.T.CT.T.CGGTAGTAC..CT.TTT.CTATCCC.A.CCA.AC-AT..	47.09	52.91	44.75	39.45	57.14
Hap_3	.TA.TACCA...C.ACATCTTCTTTT....AGT..CA.TATTTCCT.TCC.GACCC.GAC-AT.A	47.09	52.91	44.75	38.99	57.60
Hap_4	TTA.TACCA...C.ACATCTTCTTTT....AGT..CA.TATTTCCT.TCC.GACCC.GAC..C-A	46.94	53.06	44.50	38.99	57.34
Hap_5	.TA.TACCA...C.ACATCTTCTTTT....AGT..CA.TATTTCCT.TCC.GACCC.GACCAT.A	47.18	52.82	44.75	38.99	57.80
Hap_6	TTAT...C.ATG.AACAT.T.CT.T.CGGTAGTAC..CT.TTT.CTATCCC.A.CCA.AC-AT..	46.94	53.06	44.75	38.99	57.14
Hap_7	TTA.TACCA...C.ACATCTTCTTTT....AGT..CA.TATTTCCT.TCC.GACCC.GAC..C.A	47.02	52.98	44.75	38.99	57.34

Note: Hap_1: *O. aureus* (0222WPLU); Hap_2: *O. mossambicus* (2722DSLU); Hap_3: *O. niloticus* (2522DSLU and 2622DSLU), and *O. niloticus* (22SPB); Hap_4: *O. niloticus* (015F4 and 22PFG1-4); Hap_5: *O. niloticus* (021F1_K1); Hap_6: *O. niloticus* (021F1_K2); Hap_7: *O. mossambicus* (021F1_K3)

Table 4: Pairwise genetic distance (below) and P-value (above) between tilapia specimens from different populations based on COX1 genetic marker sequences using the Kimura 2-parameter distance model with 1000 bootstrap replicates

		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1	O. *aureus* Poreang (0222WMLU)		0.012	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.013	0.001	0.013	0.002
2	O. *mossambicus* Salulemo (2722DSLU)	0.076		0.010	0.010	0.010	0.010	0.010	0.001	0.010	0.010	0.010	0.010	0.010	0.010	0.001	0.003	0.010	0.012	0.000	0.013
3	O. *niloticus* Salulemo (2522DSLU)	0.080	0.053		0.000	0.000	0.001	0.000	0.010	0.001	0.001	0.001	0.001	0.001	0.001	0.010	0.011	0.001	0.013	0.010	0.013
4	O. *niloticus* Salulemo (2622DSLU)	0.080	0.053	0.000		0.000	0.001	0.000	0.010	0.001	0.001	0.001	0.001	0.001	0.001	0.010	0.011	0.001	0.013	0.010	0.013
5	O. *niloticus* Sultana (22SPB)	0.080	0.053	0.000	0.000		0.001	0.000	0.010	0.001	0.001	0.001	0.001	0.001	0.001	0.010	0.011	0.001	0.013	0.010	0.013
6	O. *niloticus* Kekar (015F4)	0.082	0.055	0.002	0.002	0.002		0.001	0.010	0.000	0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.013	0.010	0.013
7	O. *niloticus* Kekar (021F1_K1)	0.080	0.053	0.000	0.000	0.000	0.002		0.010	0.001	0.001	0.001	0.001	0.001	0.001	0.010	0.011	0.001	0.013	0.010	0.013
8	O. *niloticus* Kekar (021F1_K2)	0.078	0.002	0.055	0.055	0.055	0.053	0.055		0.010	0.010	0.010	0.010	0.010	0.010	0.000	0.002	0.010	0.012	0.000	0.013
9	O. *niloticus* Kekar (021F1_K3)	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053		0.000	0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.013	0.010	0.013
10	O. *niloticus* Kekar (22PFG1-1)	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053	0.000		0.000	0.000	0.000	0.000	0.010	0.010	0.000	0.013	0.010	0.013
11	O. *niloticus* Kekar (22PFG1-2)	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053	0.000	0.000		0.000	0.000	0.000	0.010	0.010	0.000	0.013	0.010	0.013
12	O. *niloticus* Kekar (22PFG1-3)	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053	0.000	0.000	0.000		0.000	0.000	0.010	0.010	0.000	0.013	0.010	0.013
13	O. *niloticus* Kekar(22PFG1-4)	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053	0.000	0.000	0.000	0.000		0.000	0.010	0.010	0.000	0.013	0.010	0.013
14	MK130702.1_*O. niloticus* _Nigeria	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053	0.000	0.000	0.000	0.000	0.000		0.010	0.010	0.000	0.013	0.010	0.013
15	KU565865.1_*O. niloticus* _Philippine	0.078	0.002	0.055	0.055	0.055	0.053	0.055	0.000	0.053	0.053	0.053	0.053	0.053	0.053		0.002	0.010	0.012	0.000	0.013
16	MG438458.1_*O. mossambicus* _Thailand	0.082	0.005	0.059	0.059	0.059	0.057	0.059	0.003	0.057	0.057	0.057	0.057	0.057	0.057	0.003		0.010	0.013	0.002	0.013
17	KU565863.1_*O. niloticus* _Philippine	0.082	0.055	0.002	0.002	0.002	0.000	0.002	0.053	0.000	0.000	0.000	0.000	0.000	0.000	0.053	0.057		0.013	0.010	0.013
18	KU565831.1_*O. aureus* _Philippine	0.002	0.076	0.081	0.081	0.081	0.079	0.081	0.074	0.079	0.079	0.079	0.079	0.079	0.079	0.074	0.078	0.079		0.012	0.001
19	KM438534.1_*O. mossambicus* _Zimbabwe	0.076	0.000	0.054	0.054	0.054	0.054	0.054	0.000	0.054	0.054	0.054	0.054	0.054	0.054	0.000	0.003	0.054	0.075		0.013
20	MW829393.1_*O. niloticus* _Guangzhou_China	0.003	0.078	0.083	0.083	0.083	0.081	0.083	0.076	0.081	0.081	0.081	0.081	0.081	0.081	0.076	0.076	0.081	0.002	0.077	

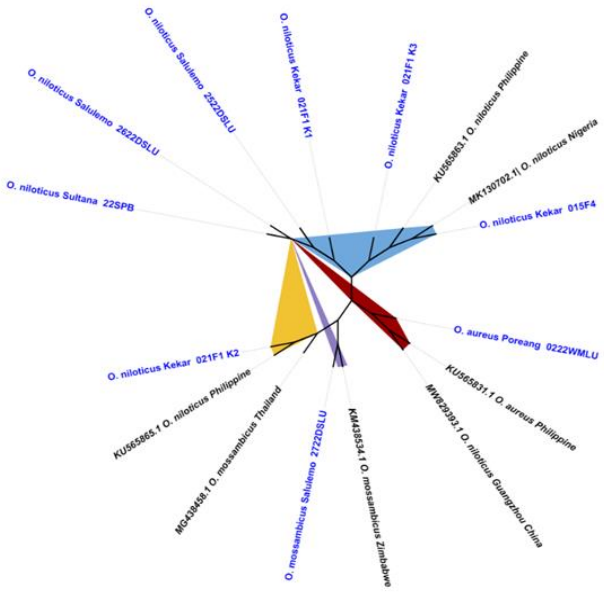

Fig. 3: Phylogenetic tree constructed with Indonesian tilapia mt-COX1 gene sequences and GenBank tilapia accessions from other countries using the Kimura 2-parameter distance Maximum Likelihood model with 1000 bootstrap replicates.

Despite this close ancestry, the mutation rate is also relatively high (Fig. 2), indicating that tilapia tend to form new populations, indicated by moderate FST values and phylogenetics that separate distributed the 13 specimens from four populations into different clades. The FST value in this study was much lower than the FST value in six Tanzanian farmed tilapia stocks (Kajungiro *et al.* 2019), Nile tilapia in East Africa natural and stocked population with FST = 2.1 (Tibihika *et al.* 2020). Furthermore, Kajungiro *et al.* (2019) state that data on the distribution of genetic variation among tilapia stocks or populations is essential to maximize genetic diversity in designing breeding strategies for forming basic populations. The Polobete is a hatchery that introduced kekar and sultana tilapia in 2022 to be used as broodstock for producing seeds. Therefore, the data in this study can be used as a guide to assemble the MaJaCea tilapia strain by the Polobete hatchery. Other broodstock centers can use the data for genetic improvement and breeding management.

Although the samples analyzed in this study were limited, this study revealed three types of tilapias germplasm in South Sulawesi: *Oreochromis niloticus*, *O. aureus*, and *O. mossambicus.* The results obtained in this study are relatively good. A study to identify ichthyofauna germplasm using eDNA metabarcoding conducted in ten rivers and lakes across Indonesia only succeeded in identifying *Oreochromis niloticus* and *Oreochromis* sp. (ongoing, unpublished). Data from genotype 0222WPLU captured in the Poreang Creek identified this specimen as either *O. aureus* or *O. niloticus* with genetic distances (GD) of 0.002 and 0.003, respectively. Similarly, genotype 021F1-K2 was identified as *O. mossambicus* (GD: 0.000) or *O. niloticus* (GD: 0.003). These cases indicate that introgression may have occurred. The accidental introgression of *O. mossambicus* into farmed *O. niloticus* stock in Asia has been reported; meanwhile, the introgression of *O. niloticus* into farmed *O. aureus* stock has been done intentionally (Syaifudin *et al.* 2019). Introgression by *O. mossambicus* into other tilapia species, including the Nile tilapia *O. niloticus*, has also been reported (Gupta and Acosta 2004).

This study's genetic diversity of tilapia germplasm was relatively high, with seven haplotypes among the 13 specimens of tilapia analyzed (Table 2, 3) and 64 polymorphic sites (Table 2). Research on three aquaculture farms in Ghana using microsatellite markers detected just five polymorphic microsatellite loci with four alleles (Diyie *et al.* 2021). The kekar strain tilapia in this study had high genetic variation, evidenced by the fact that the four individuals observed all had different haplotypes and were spread across three distinct clades (Fig. 2). Interspecific introgression of *O. mossambicus* was detected in populations of kekar strain tilapia (Table 1 and 4), which is thought to cause increased genetic variation relative to the original kekar strain. HNK Pasuruan maintains high genetic diversity in the seeds produced using broodstock from various genetic sources (haplotypes). High genetic diversity is the basis for expanding the selection spectrum for genetic improvement to improve the adaptability of tilapia to the closed population and new cultivation systems and environments.

In contrast to the kekar tilapia strain, Sukmanomon *et al.* (2012) showed that three out of four generally analyzed GIFT-derived populations managed to maintain pure strains of GIFT tilapia in Thailand, despite slight changes resulting in genetic variation. Changes in genetic variation are frequent in hatcheries and are usually accompanied by loss of alleles due to small effective population size (Ne) during spawning (Aho *et al.* 2006; McKinna *et al.* 2010). Small effective population sizes in seed production systems cause the inbreeding effect to accumulate (Falconer and Mackay 1996; Romana-Eguia *et al*. 2005).

Conclusion

Distinguishing tilapia species, hybrids, and introgressions is critical in aquaculture and wild populations. Identification, genetic diversity, and phylogenetics of germplasm are the first steps toward producing superior seeds. The DNA-based identification and analysis of genetic diversity and tilapia lineage in this study indicate that the germplasm of farmed tilapia and wild tilapia in South Sulawesi consists of *Oreochromis niloticus*, *O. mossambicus,* and possibly *O. aureus*. These tilapia species formed seven COX1 haplotypes, showing that the genetic diversity of tilapia germplasm in South Sulawesi is quite high. The results of this study can be used as basic data for designing tilapia breeding programs to overcome the scarcity of quality seeds and to produce quality seeds independently from South Sulawesi.

Acknowledgments

The authors are grateful to the Ministry of Research, Technology, and Higher Education of Indonesia [Grant No. 686/E1.1/KS.06.02/2022] and Public Service Agency of Universitas Hasanuddin in accordance with the Rector's Decree Number: 1474/UN4.22/PT.01.03/2022] for the financial assistance provided. The authors are also grateful to Dr. Abigail Mary Moore from England for her careful and thorough language editing of this manuscript.

Author Contributions

II: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Visualization, Writing-original draft, and Writing-review and editing. DSB: Formal analysis, Validation, Project administration, Supervision, Writing – original draft, and Writing-review and editing. SHL: Formal analysis, Supervision, Validation, Visualization, and Writing –original draft. RR: Investigation, Formal analysis, Resources, Visualization, Software, Writing –original draft, Writing-review and editing. KK: Investigation, Project administration, and

Methodology. IAKK: Conceptualization, Investigation, and Resources. MFU: Investigation and Formal analysis. SA: Project administration and Investigation. IsI: Resources and Investigation. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

There were no reported conflicts of interest related to this article.

Data Availability

The datasets for this study can be made available by the corresponding author upon a reasonable request.

Ethics Approval

This study conformed to the guidance of animal ethical treatment for the care and use of experimental animals. This research has followed the standard operating procedure of the tilapia experimental of Universitas Hasanuddin.

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