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Research Article

DNA barcoding of wild and culture tilapia based on cytochrome c oxidase subunit I (*COI*) gene

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Abstract

Tilapia is an introduced fish for aquaculture that spreads across Indonesia's water resources. The study aimed to determine the mitochondrial DNA *COI* sequence, the genetic distance and phylogenetic of tilapia from Lake Toba, Lake Ranau, and the *Balai Riset Pemuliaan Ikan* (BRPI) Sukamandi, West Java. Five individuals were collected from each site, either wild and culture tilapia; strains of blue tilapia and red tilapia resulting from genetic development at BRPI. Basic Local Alignment Search Tool nucleotide (BLASTN) indicated that *Oreochromis mossambicus* and *O. niloticus* exist in natural water resources and culture in Sumatra and Java; *O. aureus* exists in natural water resources of Lake Toba and Lake Ranau, however, *O. Urolepsis* is only present at the research center of BRPI. The phylogenetic tree indicated four different subclusters of *O. niloticus, O. mossambicus, O. aureus,* and *O. urolepsis;* however, all are still in the same cluster with a bootstrap value of 88%.

Introduction

Tilapia is a general name for cichlid fish of which there are hundreds of varieties. The types of tilapia found in Indonesia include Nile tilapia (*Oreochromis niloticus*), blue tilapia (*Oreochromis aureus*), mujair fish (*Oreochromis mossambicus*), and tilapia fish strains resulting from genetic development such as red tilapia, GIFT and BEST tilapia (Priambodo et al., 2024). According to Trewavas (1983), tilapia in Indonesia was first introduced to the islands of Sumatra, Kalimantan, Java, and Sulawesi. Tilapia are widely distributed in Indonesia, one of which is on the island of Sumatra, for instance in Toba Lake and Ranau Lake. Toba Lake in the North Sumatra is the largest lake in Southeast Asia, meanwhile, Ranau Lake is the second largest lake on the island of Sumatra, located in the region of Lampung and South Sumatra Province. Nile tilapia have been introduced to at least 100 countries for aquaculture. At the same time, it is currently recognized as one of the most dangerous invasive species globally due to their invasion having reduced the trophic status, shortened the food chain, and affected the isotopic diversity of native fish species (Shuai and Li, 2022). However, it continues to experience development through hybridization by genetic breeding research institutes in Indonesia. The development of aquaculture is directed at increasing the production of fishery products to overcome high fishing activities (Arifin and Kurniasih, 2007). With the development of fishery activities, more and more fishery products are marketed, so that product mislabeling is very likely to occur (Wong et al., 2011).

Nile tilapia and mujair are invasive fish, and genetic mixing of the two often occurs resulting in a decrease in genetic diversity due to hybridization (Firmat et al., 2013). The basis for genetic conservation efforts for wild and cultivated tilapia can be done by knowing the genetic characteristics and analyzing mitochondrial DNA. A commonly used means of identification of species is through morphological approaches and species characteristics. However, this technique is subjective, resulting in overlapping information on the characteristics of adjacent taxa (Rasmussen and Kellis, 2007). Therefore, it is necessary to identify at the molecular level using DNA barcoding techniques based on the *COI* (Cytochrome C Oxidase Subunit I) gene. All the nucleotide sequences are also pivotal to be submitted in the international database such as BOLDSystems and GenBank. The Barcode of Life Data System (BOLDSystems) is a freely available web platform used specifically for DNA barcoding, which aids in the publication of records that meet the quality of the international nucleotide sequence databases (Ratnasingham and Hebert, 2013).

Identification and phylogenetic analysis of tilapia has been carried out, among others, from groups of tilapia taken from several waters in Africa and the Middle East (Syaifudin et al., 2019a), tilapia from Northeastern Nigeria (Sogbesan et al., 2017), native Oreochromis species (Mojekwu et al., 2021), and tilapia from the Brazilian market (Nascimento et al., 2023). It was reported that only one species of *O. niloticus* inhabits natural rivers in Korea; however, based on a study using *COI* gene,

the *O. aureus* natural population was identified (Wang et al., 2023). Therefore, it is necessary to determine the percentage of species similarity, genetic distance and phylogenetic between wild and culture tilapia in Sumatra and Java water resources based on the *COI* gene.

Materials and methods

Sample collection

Wild and culture tilapia samples were collected from three locations. Four individuals of wild tilapia were collected from Ranau Lake, Warkuk, South Ranau District, South Sumatra (2°52'38,31"S; 104°0'34,77"T) (sample code TRA) and five individuals from Toba Lake, Ajibata District, North Sumatra (2°39'18,31"U; 98°56'0,39"T) (sample code TTA) (Fig. 1). Ten specimens of culture tilapia were collected from Ranau Lake (4°52'36,25"S; 104°0'32,60"T) (sample code TRB) and Toba Lake (2°39'16,48"U; 98°56'0,13"T) which is coded TTB. Another nine samples of tilapia culture were collected from the Fish Breeding Research Institute (BRPI) Sukamandi, West Java (6°22'6,17"S; 107°37'24, 60"T), which represents the red tilapia strain (TRR) and blue tilapia species (TBR). Samples were collected from September 2022-January 2023. The samples were observed morphologically, morphometrically, and meristically, then the pectoral fins were taken and put into a tube containing 96% ethanol until DNA extraction.

DNA barcoding

A total of 30 representative samples were extracted using the genome DNA extraction kit (GeneAid) by following the method in the extraction manual. The *COI* gene fragment was amplified using Primers FishF2 (5' TCGACTAATCATAAAGATATCGGCAC 3') and FishR2 (5' ACTTCAGGGTGACCGAAGAATCAGAA 3') according to Ward et al. (2005). The PCR test volume was 50 µL, which contained a mixture of 22 µL ddH2O, 20 µL go tag green master mix 2X, 1 µL FishF2 primer, 1 µL FishR2 primer, and 6 µL DNA template. The amplification stages include the initiation cycle at 94℃ for 1 min, denaturation at 94 ℃ for 30 seconds, annealing at 52℃ for 45 s, extension at 72℃ for 15 s and post extension at 72℃ for 3 min. PCR products were visualized using 1% agarose gel through electrophoresis for 35 minutes at 75 volts. DNA was visualized using a UV transilluminator on the documentation gel (Geldoc Go Gel Imaging System from Bio-Rad); the size of the DNA target was measured using a 100 bp marker. PCR products of known sizes were then sequenced with Sanger DNA Sequencing method at Apical Scientific Sdn. Bhd in Malaysia using the services of Genetica Sains in Jakarta.

Data analysis

The *COI* sequences were saved in fasta format and then manually aligned, edited and assembled using version XI of MEGA. All the sequences have been deposited in the BOLDSYSTEMS (BOLD:ACR7163 for *O. urolepsis*; BOLD:AAC9904 for *O. niloticus*; BOLD:AAA8511 for *O. mossambicus*; and BOLD:AAA6537 for *O. aureus*). Based on the *COI* gene sequences, the identity percentage from the GenBank (NCBI) database was retrieved using the

BLAST (Basic Local Alignment Search Tool). The genetic distance was examined using the pairwise distance technique p-distance model and the neighbor joining (NJ) method of the maximum composite likelihood model to create the phylogenetic tree between tilapia (Stecher et al., 2020; Tamura et al., 2021) with 1000 replications.

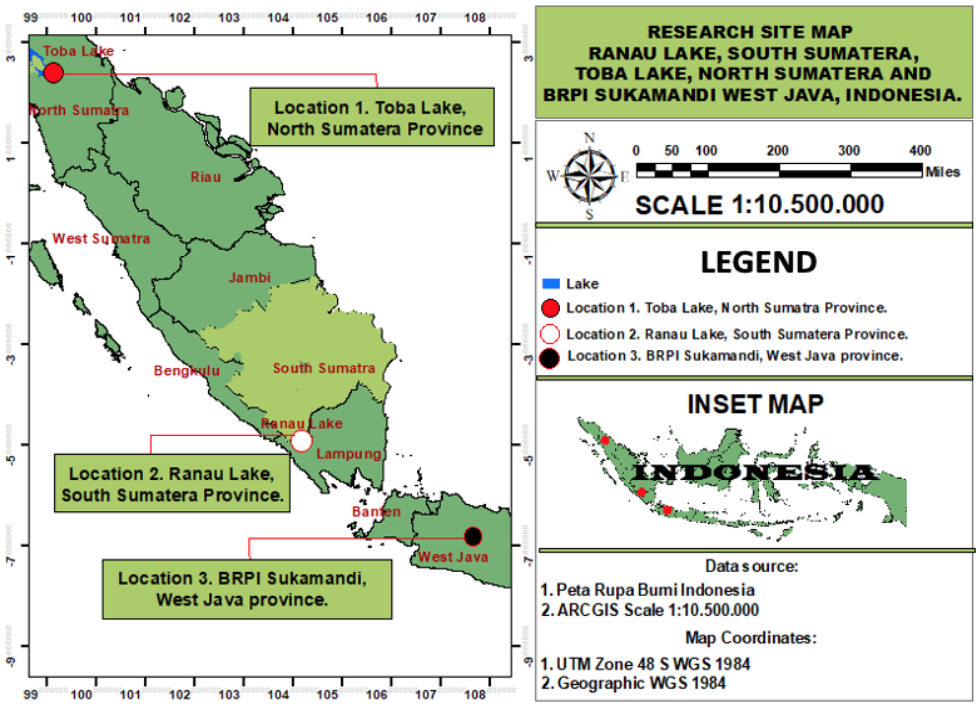


Figure 1: Map of research locations of tilapia.

Results

Nucleotide similarity

A total of 30 nucleotide sequences were successfully amplified, but only 28 samples were subjected to further analysis because of the low read of TRA2 and TRR2. The nucleotide length of the *COI* gene in tilapia samples was 690 base pairs (bp) after trimming and aligning the sequences. The percentage of nucleotide identity was recorded between 99.42 to 100% where the Nile tilapia (*O. niloticus*) had a high similarity of 99.42-100% to the same species from Nigeria (Table 1).

The accuracy value of tilapia sequences showed a significant similarity with the data in the GenBank. The Mujair (*O. mossambicus*) fish with a high resemblance of 99.42% to species from the Philippines, which was discovered in wild samples from Toba Lake (TTA1, TTA3, and TTA4), Ranau (TRA5), and culture specimen (TRB3). However, the wild samples from Toba Lake (TTA5) and Ranau Lake (TRA1) were identified as blue tilapia (*O. aureus*) having a high similarity of 99.70-100% to species from the Philippines and Nigeria. Ranau Lake culture samples (TRB1, TRB2 and TRB5) and red tilapia strain samples from BRPI (TRR4) were identified as *O. urolepis* having a high similarity of 99.42-100% to species from Malaysia.

Table 1: The highest percentage of nucleotide identity in tilapia samples.

No.	Sample code	Description	Identity (%)	Accession Code	Sample Origin
1.	TTA2, TTB1, TTB2, TTB3, TTB4, TTB5, TRA3, TRA4, TRB4, TRR1, TRR2, TRR3, TRR5, TBR1, TBR2, TBR3, TBR4 and TBR5.	*O. niloticus*	99.42-100	MK130702.1	Nigeria
2.	TTA1, TTA3, TTA4, TRA5 and TRB3.	*O. mossambicus*	99.42	KU565826.1	Philippines
3.	TTA5 and TRA1.	*O. aureus*	99.70-100	KU565831.1	Philippines Nigeria
4.	TRB1, TRB2, TRB5 and TRR4.	*O. urolepsis*	99.42-100	MF509598.1	Malaysia

Genetic distances and phylogenetic

The genetic distance of tilapia samples and GenBank databases were constructed using the MEGA 11 with the maximum composite likelihood model at bootstrap 1000 replications. The genetic distance between *O. niloticus* and *O. mossambicus* was 0.03 (3%), while between *O. aureus* and *O. urolepsis* it was 0.04 (4%). The genetic distance between *O. mossambicus* and *O. aureus* was 0.04 (4%), and with *O. urolepsis* it was 0.03 (3%). The *O. aureus* had a genetic distance of 0.04 (4%) with *O. urolepsis*. The *Sarotherodon galilaeus* had a very close genetic distance 0.00-0.01 (0-1%) to the blue tilapia (*O. aureus*) from Toba Lake Wild (TTA5), Ranau Lake Wild (TRA1) and with species in the GenBank. The genetic distance within the population of tilapia (Table 2.) showed that Toba Lake culture (0.000) and Ranau Lake culture (0.0006) indicated lower genetic distance in comparison to the wild population from Toba (0.0392) and Ranau Lake (0.0471). Blue tilapia and red tilapia indicated higher genetic distance (0.0312 and 0.0262) in comparison to *O. niloticus* (0.0013), *O. mossambicus* (0.0012), *O. aureus* (0.0013), and *O. urolepsis*, *S. galilaeus*, *C. zillii* (0.000) from the GenBank database.

Table 2: Genetic distance within population of tilapia.

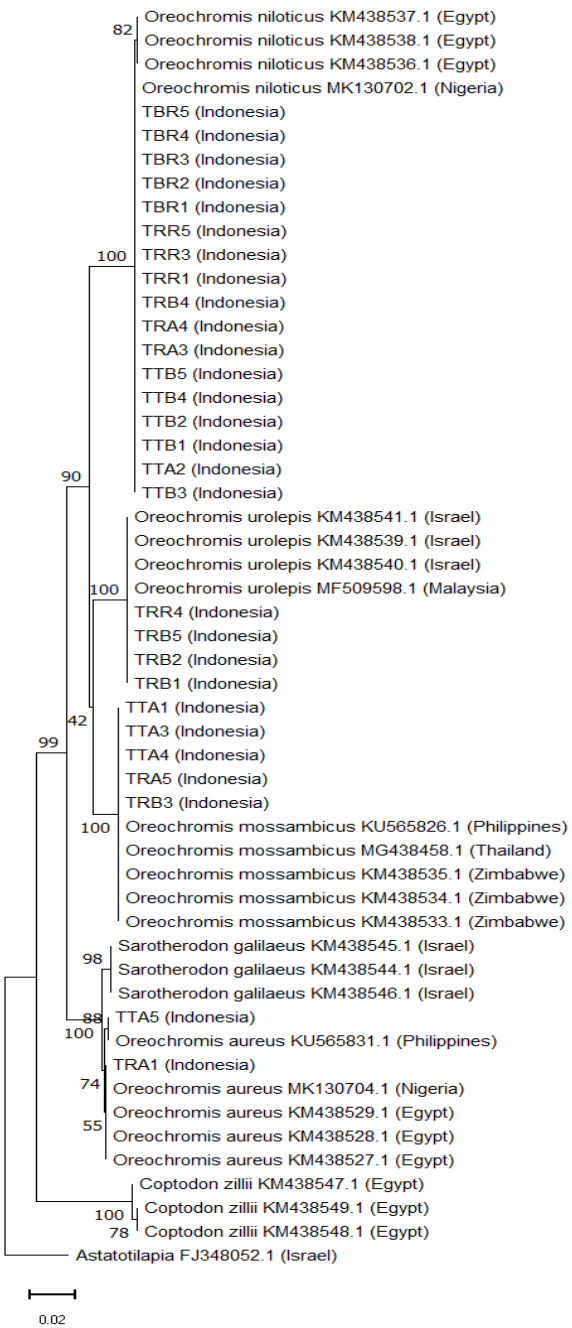
No	Population	P-Distance	SE
1	Toba Lake Wild	0.0392	0.0049
2	Toba Lake Culture	0.0000	0.0000
3	Ranau Lake Wild	0.0471	0.0059
4	Blue Tilapia Culture	0.0312	0.0046
5	Red Tilapia Culture	0.0262	0.0043
6	Ranau Lake Culture	0.0006	0.0006
7	*O. niloticus*	0.0013	0.0009
8	*O. mossambicus*	0.0012	0.0007
9	*O. aureus*	0.0013	0.0008
10	*O. urolepsis*	0.0000	0.0000
11	*S. galilaeus*	0.0000	0.0000
12	*C. zillii*	0.0000	0.0000
13	Astatotilapia	n/c	n/c

The genetic distance between populations (Table 3) indicated that the wild population of Toba Lake had the closest distance with *O. mossambicus* (0.023); Toba Lake culture and Ranau Lake culture denoted the adjacent distance with *O. niloticus* (0.002); blue tilapia culture had the nearest distance to *O. urolepsis* (0.018); red tilapia culture and Ranau Lake culture indicated the closest distance to *O. niloticus* (0.013 and 0.002).

Table 3: Genetic distance between populations of tilapia.

No	Population	1	2	3	4	5	6	7	8	9	10	11	12	13
1	Toba_Lake_Wild													
2	Toba_Lake_Culture	0.041												
3	Ranau_Lake_Wild	0.039	0.028											
4	Blue_Tilapia_Culture	0.039	0.041	0.044										
5	Red_Tilapia_Culture	0.042	0.013	0.033	0.035									
6	Ranau_Lake_Culture	0.041	0.000	0.028	0.041	0.013								
7	*O._niloticus*	0.044	0.002	0.031	0.044	0.015	0.002							
8	*O._mossambicus*	0.023	0.048	0.040	0.032	0.046	0.049	0.051						
9	*O._aureus*	0.054	0.069	0.051	0.066	0.068	0.069	0.071	0.067					
10	*O._urolepsis*	0.046	0.054	0.052	0.018	0.040	0.054	0.057	0.038	0.067				
11	*S._galilaeus*	0.057	0.068	0.054	0.068	0.069	0.070	0.071	0.070	0.009	0.069			
12	*C._zillii*	0.121	0.127	0.121	0.120	0.124	0.127	0.128	0.122	0.111	0.117	0.114		
13	Astatotilapia	0.117	0.131	0.122	0.114	0.125	0.131	0.130	0.115	0.109	0.108	0.115	0.126	

All populations denoted the great distance to *C. zillii* and Astatotilapia. Phylogenetic tree construction of tilapia showed three main clusters. The first cluster consisted of five subclusters with a bootstrap value of 88%. The first subcluster was classified to *O. niloticus* from thTTA2, TTB1, TTB2, TTB3, TTB4, TTB5, TRA3, TRA4, TRB4, TRR1, TRR2, TRR3, TRR5, TBR1, TBR2, TBR3, TBR4, TBR5), Nigeria and Egypt. The second subcluster belonged to *O. urolepsis* from this study (TRB1, TRB2, TRB5, TRR4), Malaysia and Israel. The third subcluster was *O. mossambicus* from Indonesia (TTA1, TTA3, TTA4, TRA5, and TRB3), tilapia from the Philippines, Thailand, and Egypt. The fourth subcluster was *Sarotherodon galilaeus*, while the fifth subcluster belonged to *O. aureus* from the current study (TRA1, TTA5), Nigeria, the Philippines and Egypt, respectively. The second cluster, *Coptodon zillii*, had a bootstrap value of 96%. The third cluster was the Astatotilapia species and was selected as an outgroup species, so this genus is separate from other genera. The construction of the phylogenetic tree of the tilapia and the existing samples in the GenBank database are presented in Figure 2.



Information:

1. TTA1 Toba Lake Wild (Indonesia)*
2. TTA2 Toba Lake Wild (Indonesia)*
3. TTA3 Toba Lake Wild (Indonesia)*
4. TTA4 Toba Lake Wild (Indonesia)*
5. TTA5 Toba Lake Wild (Indonesia)*
6. TTB1 Toba Lake Culture (Indonesia)*
7. TTB2 Toba Lake Culture (Indonesia)*
8. TTB3 Toba Lake Culture (Indonesia)*
9. TTB4 Toba Lake Culture (Indonesia)*
10. TTB5 Toba Lake Culture (Indonesia)*
11. TRA1 Ranau Lake Wild (Indonesia)*
12. TRA3 Ranau Lake Wild (Indonesia)*
13. TRA4 Ranau Lake Wild (Indonesia)*
14. TRA5 Ranau Lake Wild (Indonesia)*
15. TRB1 Ranau Lake Culture (Indonesia)*
16. TRB2 Ranau Lake Culture (Indonesia)*
17. TRB3 Ranau Lake Culture (Indonesia)*
18. TRB4 Ranau Lake Culture (Indonesia)*
19. TRB5 Ranau Lake Culture (Indonesia)*
20. TRR1 BRPI Sukamandi (Indonesia)*
21. TRR3 BRPI Sukamandi (Indonesia)*
22. TRR4 BRPI Sukamandi (Indonesia)*
23. TRR5 BRPI Sukamandi (Indonesia)*
24. TBR1 BRPI Sukamandi (Indonesia)*
25. TBR2 BRPI Sukamandi (Indonesia)*
26. TBR3 BRPI Sukamandi (Indonesia)*
27. TBR4 BRPI Sukamandi (Indonesia)*
28. TBR5 BRPI Sukamandi (Indonesia)*
29. *Oreochromis niloticus* MK130702.1 (Nigeria)
30. *Oreochromis niloticus* KM438536.1 (Egypt)
31. *Oreochromis niloticus* KM438537.1 (Egypt)
32. *Oreochromis niloticus* KM438538.1 (Egypt)
33. *Oreochromis mossambicus* KU565826.1 (Philippines)
34. *Oreochromis mossambicus* MG438458.1 (Thailand)
35. *Oreochromis mossambicus* KM438535.1 (Zimbabwe)
36. *Oreochromis mossambicus* KM438534.1 (Zimbabwe)
37. *Oreochromis mossambicus* KM438533.1 (Zimbabwe)
38. *Oreochromis aureus* MK130704.1 (Nigeria)
39. *Oreochromis aureus* KU565831.1 (Philippines)
40. *Oreochromis aureus* KM438529.1 (Egypt)
41. *Oreochromis aureus* KM438528.1 (Egypt)
42. *Oreochromis aureus* KM438527.1 (Egypt)
43. *Oreochromis urolepsis* MF509598.1 (Malaysia)
44. *Oreochromis urolepsis* KM438540.1 (Israel)
45. *Oreochromis urolepsis* KM438541.1 (Israel)
46. *Oreochromis urolepsis* KM438539.1 (Israel)
47. *Sarotherodon galilaeus* KM438546.1 (Israel)
48. *Sarotherodon galilaeus* KM438545.1 (Israel)
49. *Sarotherodon galilaeus* KM438544.1 (Israel)
50. *Coptodon zillii* KM438549.1 (Egypt)
51. *Coptodon zillii* KM438548.1 (Egypt)
52. *Coptodon zillii* KM438547.1 (Egypt)
53. *Astatotitilapia* FJ348052.1 (Israel)

* specimen of this study

Figure 2: Phylogenetic tree of tilapia.

Discussion

Based on the sequence of the COI gene, *Oreochromis mossambicus* and *O. niloticus* exist in natural water resources (Toba and Ranau Lake) and culture in both lakes at Sumatra and Java islands; *O. aureus* exists in natural water resources of two lakes; however, *O. Urolepsis* is only present at the research center of BRPI. The high similarities between *O. mossambicus* of this study to specimen from Philippines (KU565826.1) was supported by Puliin (1988) who stated that all Asian *O. mossambicus* populations could be derived from Java, the origin of all of the feral populations of this species established throughout the world (Pullin, 1988). The Nile tilapia could be spread across the Indonesian archipelago after being introduced from Taiwan in 1969, followed by black tilapia, Chitralada, from Thailand in 1989, GIFT (Genetic Improvement of Farmed Tilapia) from the Philippines in 1994, and the Thai red tilapia strain (NIFI) from Thailand in 1989 (Naim, 2010). In most Asian countries, tilapia farmers have changed from using *O. mossambicus* or *O. mossambicus*/*O.hornorum* hybrids to *O. niloticus* or *O. niloticus*/*O. aureus*. Red hybrid tilapia, which are the Taiwanese, Florida, and Israel strains, are produced from selected tilapia species of the genus *Oreochromis* which have an attractive red coloration as a result of continuous selective breeding (Mohamad et al., 2021). Mutant reddish-orange female *Oreochromis mossambicus* (Peters, 1852) and a normal-colored *Oreochromis urolepis* (Norman, 1922) female were propagated with a red-gold male *O. mossambicus* to produce a Florida strain (Behrends et al., 1982). Furthermore, red Nile tilapia originating from Egypt were crossed with wild-type blue tilapia, *Oreochromis aureus* (Steindachner, 1864) to yield an Israeli strain (Hulata et al., 1995).

This study is in accordance with that of Fiteha et al. (2020), which stated the usefulness of the mitochondrial *COI* gene for fish species identification and how to estimate genetic relationships of the common Egyptian Tilapiine, especially when the morphological characteristics are unreliable or inaccurate. The high similarities of the sequences indicated a closer relationship. However, the morphological and genetic identification might have different results due to the morphological similarities of the species observed (Jefri et al., 2015). Currently, the base population for the GIFT tilapia strain has been widely cultivated in many countries and is thought to have experienced introgression with wild *O. mossambicus* (Acosta and Gupta, 2010; McKinna et al., 2010). Morphological identification tends to be subjective, giving rise to overlapping information on the characteristics of adjacent taxa (Rasmussen et al., 2009), besides that in tilapia, genetic mixing often occurs, resulting in a decrease in genetic diversity due to hybridization (Firmat *et al.*, 2013).

Genetic distance is used to investigate the genetic relationship between one species and another. The value describes the numerical quantity used to measure the difference in the level of gene differences between species and populations to determine the level of kinship (Liu et al., 2015). The genetic distance between

populations of tilapia (Table 3) showed that the wild population of Toba Lake had the closest distance with *O. mossambicus* (0.023), followed by Ranau Lake wild and blue tilapia culture (0.039), Ranau and Toba Lake culture (0.041), *O. niloticus* (0.044), *O. urolepsis* (0.046), and *O. aureus* (0.054). Meanwhile, the furthest distance was *C. zillii* (0.121), followed by Astatotilapia, an outgroup species (0.117), and *S. galilaeus* (0.057). It is confirmed that most individuals of wild tilapia in Toba Lake are *O. mossambicus*, in contrast to Ranau Lake, where *O. niloticus* is dominant. Kornfield et al. (1979) compared *T. zilli*, *S. galilaeus* and *O. aureus* and found no significant morphological differences. The genetic material indicated that the chromosomes of *O. aureus* and *S. galilaeus* contained the same centromere heterochromatin but were not found in *T. zillii* species with an interspecific similarity value of 0.25. However, the number of samples in each population was still quite low to represent the genetic variation of the population. Furthermore, the average decreases in levels of genetic diversity are proportional to the decline in population (Petit-Marty et al., 2022). The highest percentage was detected in Egyptian tilapiine between populations of *T. zillii* and *S. galilaeus*, *O. niloticus*, and *T. zillii*, and finally the variation between the population of *O. niloticus* and *S. galilaeus* (Fiteha et al., 2020).

Genetic variation within the same species is generally less than 2% or even in many cases less than 1% (Shen et al., 2013). The low genetic distance values indicated that these species have close kinship. According to (Hebert et al. 2003) a genetic distance value above 3% indicates that the species is different. However, in some cases, a value below 3% resulted in species differences even with a low genetic distance. Genetic distance shows the possible influence of geographic isolation on a population. The greater the value of the genetic distance (p-distance) between an individual or population, the more isolated they will be from one another (Laltanpuii et al., 2014). The smaller the genetic distance value, the smaller the diversity between species or populations. Genetic diversity refers to the interpretation of isolation results ecologically, behaviorally, and physically, which includes the limited number of individuals and the selection of certain traits (Mignon-Grasteau et al., 2005).

Phylogenetic construction is used to determine lineage, migration, evolution, and kinship which is aimed at maintaining the identity of a population from genetic mixing (Torres and Artoni 2019). In the wild population (Toba and Ranau Lake), there is a high chance of a hybrid between *O. mossambicus*, *O. niloticus*, and *O. aureus*. In the culture, blue tilapia was in the same subcluster with *O. niloticus*, while red tilapia is composed of *O. niloticus* and *O. urolepsis*. All *Oreochromis* were in the same cluster but separated by different subclusters. Phylogenetic construction had a scale of 0.02, indicating a nucleotide change twice per 100 bp of genetic distance. The bootstrap value in the main branch ranged from 88-99% indicating the higher the level of confidence in phylogenetic tree construction. The phylogenetics of all

tilapia form four separate subclusters. The first subcluster is *O. niloticus*, the second is *O. urolepsis*, the third is *O. mossambicus*, and the fourth is *O. aureus*. However, *S. galilaeus* and *C. zillii*, which were retrieved from the GenBank database were in a separate cluster from *Oreochromis*. It indicated a similar result to the study by Fiteha et al. (2020), which successfully determined the genetic relationship among *O. niloticus*, *T. zillii*, and *S. galilaeus*. Wu and Yang (2012) succeeded in identifying cultivated tilapia and wild using the *COI* gene and found a hybrid species identified as a cross of *O. niloticus* X *O. mossambicus*. DNA barcoding has denoted that African freshwater cichlid fishes (*O. niloticus*, *Neolamprologus brichardi/pulcher*, *Metriaclima zebra*, *Pundamilia nyererei*, and *Astatotilapia burtoni*) have rapid species divergence and adaptive radiation, through wild hybridization and natural selection (Brawand et al., 2015). Furthermore, it was also successfully used in African fisheries to solve the problems of fish species authentications, evolutions, population divergence, and biogeographic distributions (Elsaied et al., 2021). Despite the wide use of the COI gene, either in the wild or culture of tilapia, there is a limitation in use for studies of hybridization and introgression as mtDNA is maternally inheritance (D'Amato et al., 2007; Wu and Yang, 2012). Therefore, species authentication and phylogenetic study will be pivotal to combine with nuclear DNA markers, for instance to estimate species composition in the commercially important tilapia species in the Molobicus breeding program (Bartie et al., 2020) using a set of 10 species-specific diagnostic SNP markers (Syaifudin et al., 2019b), which is developed based on a double digest variant of RADseq (Peterson et al., 2012). DNA barcoding has been accurately applied for the rapid identification of various taxa using *COI* gene between wild and culture tilapia from two lakes (Toba and Ranau Lake), and the research center facility at BRPI Sukamandi.

Conclusions

The wild population of tilapia in Toba Lake had the closest distance with *O. mossambicus* (0.023), meanwhile, Toba Lake culture and Ranau Lake culture denoted the adjacent distance with *O. niloticus* (0.002). The blue and red tilapia culture had the nearest distance to *O. urolepsis* (0.018) and *O. niloticus* (0.013) respectively. The phylogenetics of tilapia from Lake Toba, Lake Ranau, and BRPI Sukamandi formed four separate subclusters, namely the subclusters *O. niloticus*, *O. urolepis*, *O. mossambicus*, and *O. aureus*, but all four species are in the same cluster.

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Conflicts of interest

The authors declare that there are no competing interests that could have appeared to influence the article.

References

Acosta, B.O. and Gupta, M.V., 2010. The genetic improvement of farmed tilapias project: Impact and lessons learned. In: Silva, S.S. and Davy, F.B. (Eds.) Success Stories in Asian Aquaculture. Dordrecht: Springer, Netherlands. pp. 149–171. DOI:10.1007/978-90-481-3087-0_8

Arifin, Z.A. and Kurniasih, T., 2007. Genetic variation of three populations of tilapia (*Oreochromis niloticus*) based on mt-DNA polymorphism. *Jurnal Riset Akuakultur*, 2(**1**), 67–75.

Bartie, K.L., Taslima, K., Bekaert, M., Wehner, S., Syaifudin, M., Taggart, J.B., de Verdal, H., Rosario, W., Muyalde, N., Benzie, J.A.H., McAndrew, B.J. and Penman, D.J., 2020. Species composition in the Molobicus hybrid tilapia strain. *Aquaculture*, 526, 735433. DOI:10.1016/j.aquaculture.2020.735433

Behrends, L.L., Nelson, R.G., Smitherman, R.O. and Stone, N.M., 1982. Breeding and culture of red-gold color phase of tilapia. *Journal of the World Aquaculture Society*, 13, 210–220.

Brawand, D., Wagner, C.E., Li, Y.I., Malinsky, M., Keller, I., Fan, S., Simakov, O., Ng, A.Y., Lim, Z.W., Bezault, E., Turner-Maier, J., Johnson, J., Alcazar, R., Noh, H.J., Russell, P., Aken, B., Alföldi, J., Amemiya, C., Azzouzi, N., Baroiller, J.F., Barloy-Hubler, F., Berlin, A., Bloomquist, R., Carleton, K.L., Conte, M.A., D'Cotta, H., Eshel, O., Gaffney, L., Galibert, F., Gante, H.F., Gnerre, S., Greuter, L., Guyon, R., Haddad, N.S., Haerty, W., Harris, R.M., Hofmann, H.A., Hourlier, T., Hulata, G., Jaffe, D.B., Lara, M., Lee, A.P., MacCallum, I., Mwaiko, S., Nikaido, M., Nishihara, H., Ozouf-Costaz, C., Penman, D.J., Przybylski, D., Rakotomanga, M., Renn, S.C.P., Ribeiro, F.J., Ron, M., Salzburger, W., Sanchez-Pulido, L., Santos, M.E., Searle, S., Sharpe, T., Swofford, R., Tan, F.J., Williams, L., Young, S., Yin, S., Okada, N., Kocher, T.D., Miska, E.A., Lander, E.S., Venkatesh, B., Fernald, R.D., Meyer, A., Ponting, C.P., Streelman, J.T., Lindblad-Toh, K., Seehausen, O. and Di Palma, F., 2015. The genomic substrate for adaptive radiation in African cichlid fish. *Nature*, 513(**7518**), 375–381. DOI:10.1038/nature13726

D'Amato, M.E., Esterhuyse, M.M., Van Der Waal, B.C.W., Brink, D. and Volckaert, F.A.M., 2007. Hybridization and phylogeography of the Mozambique tilapia *Oreochromis mossambicus* in southern Africa evidenced by mitochondrial and microsatellite DNA genotyping. *Conservation Genetics*, 8(**2**), 475–488. DOI: 10.1007/s10592-006-9186-x

Elsaied, H., Soliman, T., Abdelmageed, A.A. and Abu-Taleb, H.T., 2021.

Applications and challenges of DNA barcoding and metabarcoding in African fisheries. *Egyptian Journal of Aquatic Research,* 47(**1**), 1–12. DOI:10.1016/j.ejar.2021.02.003

Firmat, C., Alibert, P., Losseau, M., Baroiller, J.F. and Schliewen, U.K., 2013. Successive invasion-mediated interspecific hybridizations and population structure in the endangered cichlid *Oreochromis mossambicus*. *PLoS One,* 8(**5**). DOI:10.1371/journal.pone.0063880

Fiteha, Y.G., Magdy, M., Elhifnawy, H.T., El-Keredy, A., Ali, R.A.M. and Rashed, M.A., 2020. Cytochrome oxidase subunit I gene-based identification of the common Egyptian tilapiine species. *Egyptian Journal of Genetics and Cytology,* 49, 1–12.

Hebert, P.D.N., Ratnasingham, S., & de Waard, J. R. (2003). Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1), S96-S99. https:// doi.org/10.1098/rsbl.2003.0025.

Hulata, G., Karplus, I. and Harpaz, S., 1995. Evaluation of some red tilapia strains for aquaculture: growth and colour segregation in hybrid progeny. *Aquaculture Research,* 26(**10**), 765–771. DOI:10.1111/j.1365-2109.1995.tb00869.x

Jefri, E., Zamani, N.P., Subhan, B. and Madduppa, H.H., 2015. Molecular phylogeny inferred from mitochondrial DNA of the grouper Epinephelus spp. In Indonesia collected from local fish market. *Biodiversitas,* 16(**2**), 254–263. DOI:10.13057/biodiv/d160221

Kornfield, I.L., Ritte, U., Richler, C. and Wahrman, J., 1979. *Biochemical and Cytological Differentiation Among Cichlid Fishes of the Sea of Galilee*. Available at: http://www.jstor.orgURL:http://www.jstor.org/stable/2407360http://www.jstor.org/stable/2407360?seq=1&cid=pdf-reference#references_tab_contents (Accessed on 20 November 2024)

Laltanpuii, Kumar, N.S. and Mathai, M.T., 2014. Molecular and phylogenetic analysis of the genus *Orthetrum* (odonata: anisopera: libellulidae) using mitochondrial COI gene. *Science Vision,* 14(**3**), 152–157.

Liu, L., Xi, Z., Wu, S., Davis, C.C. and Edwards, S.V., 2015. Estimating phylogenetic trees from genome-scale data. *Annals of the New York Academy of Sciences,* 1360(**1**), 36–53. DOI:10.1111/nyas.12747

McKinna, E.M., Nandlal, S., Mather, P.B. and Hurwood, D.A., 2010. An investigation of the possible causes for the loss of productivity in genetically improved farmed tilapia strain in Fiji: Inbreeding versus wild stock introgression. *Aquaculture Research,* 41(**11**), e730–e742. DOI:10.1111/j.1365-2109.2010.02539.x

Mohamad, S.N., Noordin, W.N.M., Ismail, N.F. and Hamzah, A., 2021. Red hybrid tilapia (*Oreochromis* spp.) broodstock development programme in malaysia: Status, challenges and prospects for future development. *Asian Fisheries Science,* 34(**1**), 73–81. DOI:10.33997/j.afs.2021.34.1.008

Mojekwu, T.O., Cunningham, M.J., Bills, R.I., Pretorius, P.C. and Hoareau, T.B., 2021. Utility of DNA barcoding in native Oreochromis species. *Journal of Fish Biology*. 98, 498–506. DOI: 10.1111/jfb.14594

Naim, S., 2010. *74 Years of Tilapia Culture and Development in Indonesia (1936-2010)*. Arizona: University of Arizona.

Nascimento, B.M., Silva de Paula, T. and Brito, P.M.M., 2023. DNA barcode of tilapia fish fillet from the Brazilian market and a standardized COI haplotyping for molecular identification of *Oreochromis* spp. (Actinopterygii, Cichlidae). *Forensic Science International: Animals and Environments*, 3(**100059**). https://doi.org/10.1016/j.fsiae.2022.100059

Peterson, B.K., Weber, J.N., Kay, E.H., Fisher, H.S. and Hoekstra, H.E., 2012. Double digest RADseq: An inexpensive method for de novo SNP discovery and genotyping in model and non-model species. *PLoS ONE*, 7(**5**). DOI:10.1371/journal.pone.0037135

Petit-Marty, N., Liu, M., Tan, I.Z., Chung, A., Bàrbara Terrasa, B., Guijarro, B., Francesc Ordines, F., Ram´ırez-Amaro, S., Massut, I.E. and Schunter, C., 2022. Declining Population Sizes and Loss of Genetic Diversity in Commercial Fishes: A Simple Method for a First Diagnostic. *Frontiers in Marine Science*, 9. DOI:10.3389/fmars.2022.872537

Priambodo, R., Faizal, I., Abinawanto, A. and Bowolaksono, A., 2024. Transferrin gene profiling from various species and strains of tilapia in Indonesia. In: *AIP Conference Proceeding*, 2710(**1**). AIP Publishing, p. 040021. DOI:10.1063/5.0144071

Pullin, R.S.V., 1988. Tilapia genetic resources for aquaculture. The Second International Symposium on Tilapia in Aquaculture. Bangkok, Thailand. *ICLARM Conference Proceeding*, **15**, xii, 623 P.

Rasmussen, M.D. and Kellis, M., 2007. Accurate gene-tree reconstruction by learning gene- and species-specific substitution rates across multiple complete genomes. *Genome Research*, 17(**12**), 1932–1942. DOI:10.1101/gr.7105007

Rasmussen, R.S., Morrissey, M.T. and Hebert, P.D.N., 2009. DNA barcoding of commercially important salmon and trout species (*Oncorhynchus* and *Salmo*) from North America. *Journal of Agricultural and Food Chemistry*, 57(**18**), 8379–8385. DOI:10.1021/jf901618z

Ratnasingham, S. and Hebert, P.D.N., 2013. A DNA-Based Registry for All Animal Species: The Barcode Index Number (BIN) System. *PLoS One*, 8(**7**). DOI:10.1371/journal.pone.0066213

Shen, Y.Y., Chen, X. and Murphy, R.W., 2013. Assessing DNA Barcoding as a Tool for Species Identification and Data Quality Control. *PLoS One*, 8(**2**). DOI:10.1371/journal.pone.0057125

Shuai, F. and Li, J., 2022. Nile Tilapia (*Oreochromis niloticus* Linnaeus, 1758) Invasion Caused Trophic Structure Disruptions of Fish Communities in the South China River—Pearl River. *Biology*, 11(**11**). DOI:10.3390/biology11111665

Sogbesan, O.A., Sanda, M.K., Jaafar, N.J. and Adebowale, H.A., 2017. DNA Barcoding of Tilapia Species (Pisces: Cichlidae) from North-Eastern Nigeria. *Journal of Biotechnology & Biomaterials*, 7(**4**), 1-4, 1000277. https://doi. org/10.4172/2155-952x.1000277.

Stecher, G., Tamura, K. and Kumar, S., 2020. Molecular evolutionary genetics analysis (MEGA) for macOS. *Molecular Biology and Evolution*, 37(**4**), 1237–1239. DOI:10.1093/molbev/msz312

Syaifudin, M., Bekaert, M., Taggart, J. B., Bartie, K. L., Wehner, S., Palaiokostas, C., Khan, M. G. Q., Selly, S. L. C., Hulata, G., D'Cotta, H., Baroiller, J. F., McAndrew, B. J. and Penman, D. J., 2019a. Species-specific marker discovery in tilapia. *Scientific Reports*, 9(**1**). DOI:10.1038/s41598-019-48339-2

Syaifudin, M., Bekaert, M., Taggart, J. B., Bartie, K. L., Wehner, S., Palaiokostas, C., Khan, M. G. Q., Selly, S. L. C., Hulata, G., D'Cotta, H., Baroiller, J. F., McAndrew, B. J. and Penman, D. J., 2019b. Species-specific marker discovery in tilapia. *Scientific Reports*, 9(**1**), 1–11. DOI:10.1038/s41598-019-48339-2

Tamura, K., Stecher, G. and Kumar, S., 2021. MEGA11: Molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution*, 38(**7**), 3022–3027. DOI:10.1093/molbev/msab120

Torres, R.A. and Artoni, R.F., 2019. Editorial: Genetics, Evolution, and Conservation of Neotropical Fishes. *Frontiers in Genetics*, 10. DOI:10.3389/fgene.2019.01124

Trewavas, E., 1983. Tilapiine fishes of the genera *Sarotherodon, Oreochromis, and Danakilia*. London British Museum (Natural History), 583 P.

Wang, J.H., Choi, H.K., Lee, H.J. and Lee, H.G., 2023. On the Species Identification of Two Non-Native Tilapia Species, Including the First Record of a Feral Population of *Oreochromis aureus* (Steindachner, 1864) in South Korea. *Animals*, 13(**8**). DOI:10.3390/ani13081351

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R. and Hebert, P.D.N., 2005. DNA barcoding Australia's fish species. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 360(**1462**), 1847–1857. DOI: 10.1098/rstb.2005.1716

Wong, L. L., Peatman, E., Lu, J., Kucuktas, H., He, S., Zhou, C., Na-nakorn, U. and Liu, Z., 2011. DNA barcoding of catfish: Species authentication and phylogenetic assessment. *PLoS One*, 6(**3**), 1–7. DOI:10.1371/journal.pone.0017812

Wu, L. and Yang, J., 2012. Identifications of captive and wild tilapia species existing in Hawaii by mitochondrial DNA control region sequence. *PloS One*, 7(**12**), e51731. DOI:10.1371/journal.pone.0051731.