The DNA Barcoding of Tilapia Species from Wasile, East Halmahera, North Maluku, Indonesia

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Abstract

Tilapia (Oreochromis niloticus) is one of the most in-demand freshwater commodities for consumption purposes because of its delicious taste and relatively affordable price. This commodity is often cultivated because it is easy to maintain, grows fast, and is resistant to changes in air quality. To scrutinize the difference between the domestic and foreign Tilapia, the DNA barcoding was done by identifying species using gene sequences from the genome to identify morphologically similar species. This study aims to determine the genetic variation of red and black tilapia from East Halmahera, Indonesia. Samples of red and black tilapia were analyzed at the Molecular Biology Laboratory of the Bogor Freshwater Aquaculture Research Institute (BRPBAT). Employing the experimental method, samples of tilapia collected in bio floc ponds were taken. The extraction was done by taking DNA through fish organs from the fins using the Phenol-Chloroform method. The samples were vortexed for 1 minute and incubated at 37ºC for 24 hours. The samples were then centrifuged at 10,000 rpm for 10 minutes and vortexed for 1 minute until the white lumps were seen. The precipitated DNA was centrifuged at 10,000 rpm for 10 minutes; the samples were then dried at room temperature. The DNA pellet was dissolved with 100 l Tris-EDTA (TE) buffer and stored at 4ºC. The results of the study showed that when the Wasile fish blast samples were compared to samples of tilapia from Myanmar, Merauke, and Malang, the four samples were tilapia with the type of Oreochromis niloticus.

Keywords: DNA barcoding, tilapia, east halmahera wasile

1. INTRODUCTION

East Halmahera Regency is located in the eastern part of North Maluku Province, Indonesia, between latitudes 1° 4' and 0° 40' South and Longitudes 126° 45' and 130° 30' East. The regency is dominated by coastal and mountainous or hilly terrain. In addition, the majority of the villages are directly facing the bay or open sea where 75% of them have a coastline and the remaining 25% are mountainous areas. Administratively, East Halmahera has an area of 14,202.01 km^2 consisting of about 6,506.19 km^2 (650,619 hectares) of land and about 7,695.82 km^2 of the sea.

Tilapia is one of the most extensively cultivated fish in Indonesia, particularly the Wasile transmigration area, where the majority of the residents are expert cultivators from East Java, West Java, and Central Java. Carp, tilapia, catfish, and milkfish are among the variety of fish cultivated (BKPM North Maluku, 2007). Most of the red and black tilapia were imported from the Philippines and Thailand in 1981 and 1989, respectively. In addition, the comparative advantage of this species is

that it is easy to breed, has a more rapid growth rate, very strong, can adapt to the environment, as well as resistant to pests and diseases. Tilapia is also resistant to climate change and adverse weather conditions. Its cultivation is highly profitable due to its quick development and the simple technology used. Several regions in Indonesia have significantly increased the cultivation of this species enabling it to penetrate the export market to several Asian countries, including Singapore and Malaysia, as well as Japan, the United States, and Europe. It is exported whole, filleted, and in some cases alive (Iskandariah et al., 2011).

Tilapia, also known as freshwater chicken, has essential world economic value. Commercially, this fish can be cultivated in ponds or floating net cages, in brackish, freshwater, and even in coastal waters (Gustiano et al., 2008). It is an African fish that has been imported into many countries due to its disease resistance, ease of breeding, and tolerance of low water quality, including low dissolved oxygen levels.

Red tilapia (Oreochromis niloticus) is a strain of tilapia that is tolerant of brackish waters. It has high resistance to various diseases, tolerance to low and high temperatures, feed efficiency, and fast growth. In addition, red tilapia is preferred by many people due to its delicious taste. It was introduced from the Philippines (1981) and Thailand (1989). This species has several advantages including fast breeding and fast growth. Tilapia is also resistant to pests and diseases and quite tolerant of environmental changes (Iskandariah et al., 2011). This is demonstrated in tilapia cultivation as an indicator of rapid growth. In Indonesia, cultivation has expanded for export to several countries such as Europe, Singapore, Japan, and the United States.

Tilapia exports are sent alive and in the form of fresh fillets. This fish can be cultivated in ponds, cages, floating nets, and in rice fields (with the Mina Padi system) in fresh, brackish, as well as saltwater. Tilapia is a cultivated commodity in most areas in Indonesia. However, poor quality control can significantly reduce fish quality (Arifin et al., 2007). The decline in the genetic quality of this species is generally characterized by traits such as slow growth, high mortality rate, gonad maturity at an early age, and small individual size.

Low genetic diversity reduces growth, size diversity, stability of organ development, survival rate, and adaptation to environmental changes (Leary et al., 1985). Tilapia has many advantages, one of which is its ease of breeding. Furthermore, the fish farming system has developed quite rapidly in several areas in Indonesia due to its relatively quick growth, resistance to pests and diseases, and fairly broad tolerance to environmental changes.

The high public interest in this fish affects changes in genetic quality. Good parent quality affects the quality of the resulting offspring. Physiologically, the decline in the genetic quality of fish can be characterized by slower growth, high mortality rates, gonad maturity at a very early age, and stunted size.

In Indonesia, studies on genetic improvement for black tilapia have been conducted extensively, however, few have

been conducted on red tilapia. Nugroho and Maskur (2002) conducted a study on tilapia using the mtDNA method and discovered that the parent of red tilapia was derived from black tilapia and that the red tilapia is a hybrid of black and white tilapia. However, these findings have not been supported by other studies. This gives the red tilapia a well-deserved place in the hearts of Indonesian consumers.

2. METOHODOLOGY

Test Fish

The samples used for the test were 2 strains of red and black tilapia taken from the biofloc nutrition system pond in Ternate City and other fish samples from Bumi Restu Village, Wasile district, East Halmahera with a size of 18-20 cm (8 samples). The tilapia sample used for DNA testing was part of the fins which were cut and stored in 70% alcohol solution for use in the analysis process.

DNA extraction

Extraction begins with taking fish DNA by taking fish organs from fins using the Phenol-Chloroform method (Nugroho, 2001). The sample used in this study was 5-10 mg which was then put into a microtube with a size of 1.5 ml and filled with 70% alcohol, then 10 µl of protein kinase was added. The next stage was vortexing for 1 minute, then the samples were incubated at 37°C for 24 hours. Then add 1000 µl of Phenol Chloroform solution and vortex for 1 minute and the sample is incubated at 37 ºC for 24 hours. The next step is centrifuging the sample at 10,000 rpm for 10 minutes. Then the supernatant was taken and put into a new microtube, then added 1000 µl 90% ethanol and 10 µl CH3COONa. Then the sample was vortexed for 1 minute until white lumps were seen. Precipitated DNA was centrifuged at 10,000 rpm for 10 minutes, then the sample was dried at room temperature. The DNA pellet was dissolved with 100 µl Tris-EDTA (TE) buffer and stored at 4°C before being used in the next step.

Random Amplified Polymorphism DNA (RAPD)

The amplification process was carried out using the Polymerize Chain Reaction (PCR) method with the reaction composition: 1µl DNA, 1.5µl primer, 12.5µl 2X PCR Master Mix and 10µl H2O; with a total volume of 25 µl. The primer used was OPA-03 with the base sequence AGT CAG CCA C. The next step is to insert a thermocycler with 1 denaturation cycle at 94 ºC for 2 minutes, 35 doubling cycles consisting of denaturation at 94 ºC for 1 minute, annealing at 36 ºC for 1 minute and elongation at 72 ºC for 2.5 minute; and final elongation at 72 ºC for 7 minutes. The PCR results were then electrophoresed using 1% agarose gel in 1% buffered Tris-Boric-EDTA (TBE). The results were then observed with a UV illuminator and the images printed with Polaroid. The next stage is data analysis using the Tools For Population Genetic Analysis (TFPGA) software. Genetic variation between populations used Molecular Analysis of Variance (AMOVA) and Fst, while kinship between populations used genetic distance (D) which was analyzed based on Wright (1978) modified by Rogers (1972).

3. RESULTS AND DISCUSSION

The sampling location was Wasilei District, East Halmahera Regency, North Sulawesi Province, Indonesia. The tilapia seeds were developed in Bumi Restu Village, Wasilie Sub-district. Furthermore, the parent seeds were all imported from East Java and grew rapidly up to this point. The map of the sampling locations in the Wasile Sub-district is shown in Figure 1.

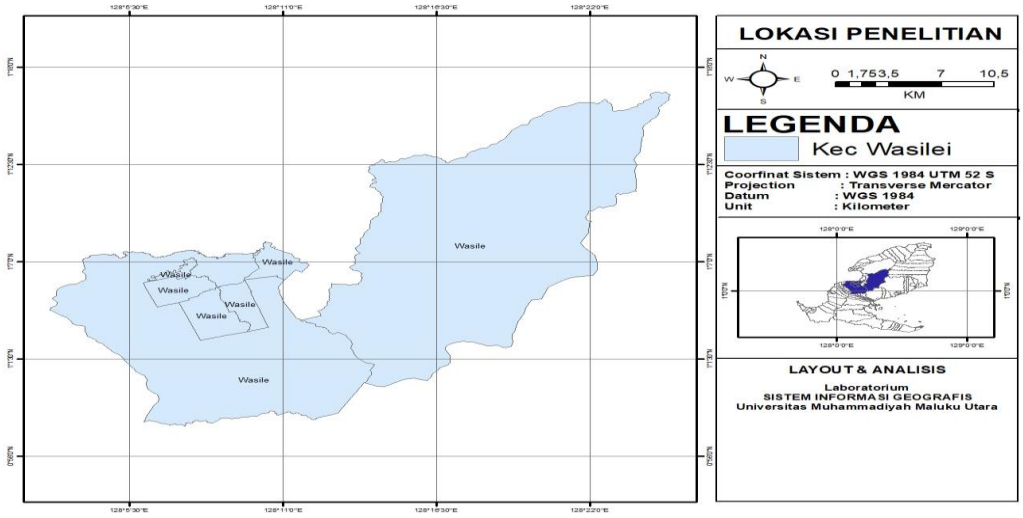

Figure 1. Map of tilapia sampling locations

The DNA barcoding of Wasile tilapia showed a relationship between Wasile tilapia and tilapia originating from Myanmar resulting from the sequencing of red and black tilapia. DNA barcoding is a system designed to quickly and accurately identify the nucleotide base sequence of a standardized short marker gene, namely the Cytochrome Oxidase Subunit I (COI) gene. In addition, the COI gene is one of the coding genes in the mtDNA genome known to have many advantages, one of which is that the COI gene undergoes very few deletions and insertions in its sequence and many parts are conserved. Therefore, it can be used as a DNA barcode, particularly for the peculiarities of each species (Hebert et al. 2003). This was demonstrated by the DNA Barcoding between tilapia originating from Wasile and Myanmar which is shown in Figure 2.

DNA barcoding of red and black tilapia from Wasile compared with black tilapia from Merauke Papau and Malang, East Java, revealed a kinship between tilapia Wasile and Myanmar. The results of DNA Barcoding between tilapia originating from Merauke Papau and Malang East Java are shown in Figure 3. This result is consistent with Yang and Rannala (2012) study on molecular phylogenetics, a method used in practically all branches of biology for genome comparisons and determining the relationship between species based on the tree of life using statistical calculations. Base sequence DNA barcoding and Molecular phylogenetics have been a part of various studies in molecular biology, population genetics, developmental biology, and evolutionary biology (Kumar and Filipski 2001).

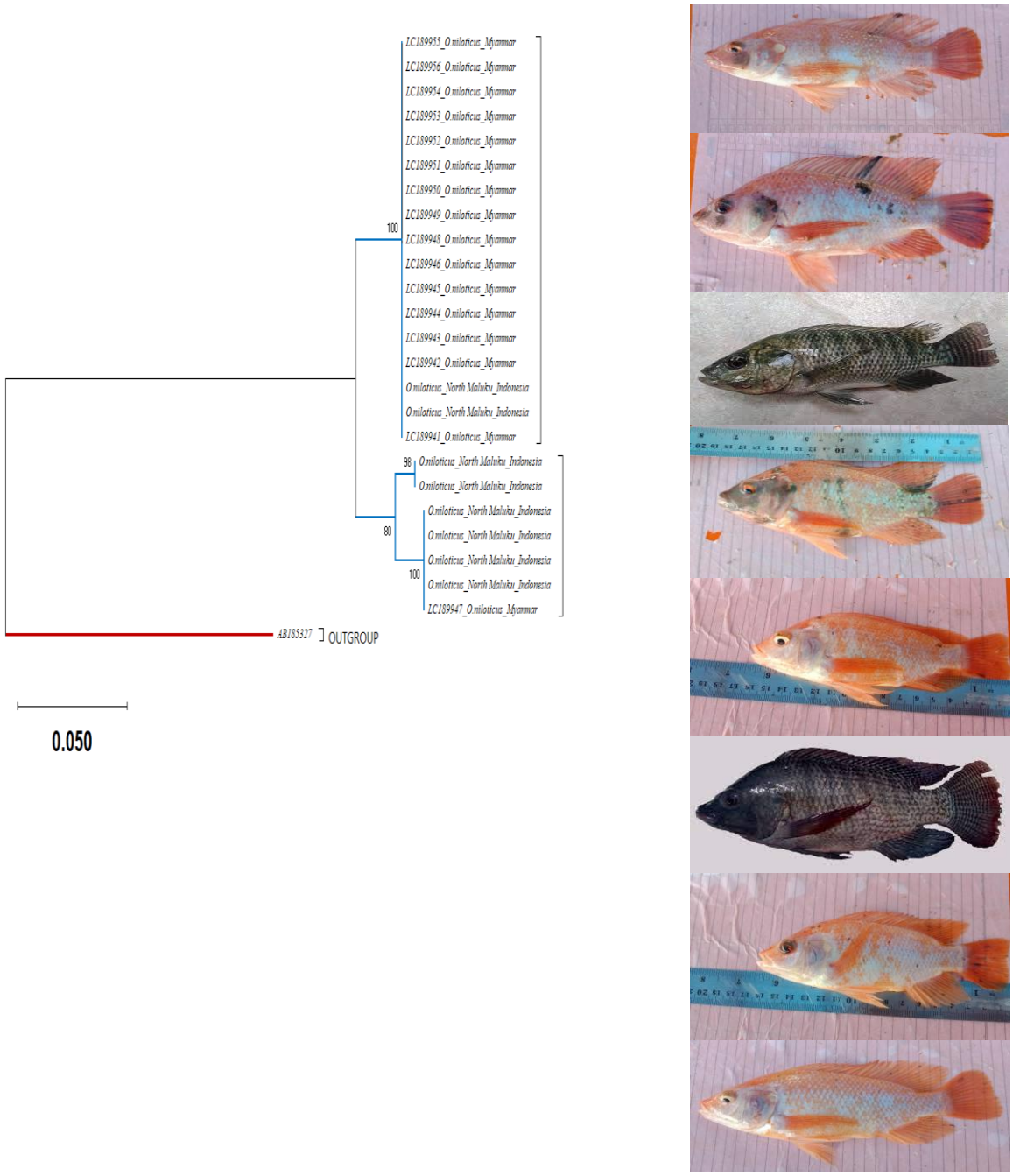

Figure 2. Phylogenetics of Wasile Tilapia, East Halmahera, North Maluku Province, Indonesia

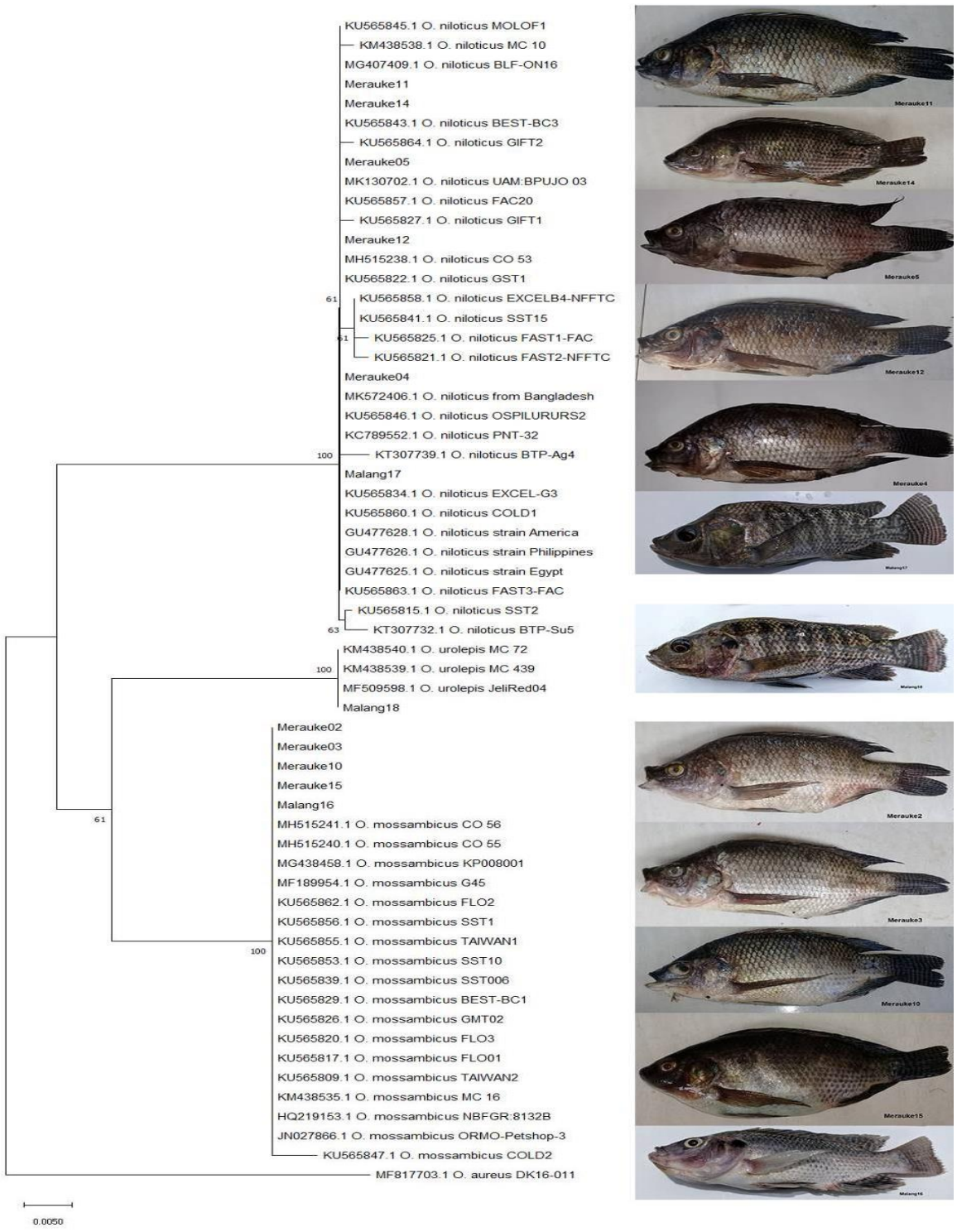

Figure 3. The phylogenetic results of tilapia from Merauke and Malang

Tilapia (Niloticus mossambicus) originating from Merauke and Malang show many similarities because they have a similar phylogeny. A phylogenetic tree was

created by incorporating sequence data from GenBank. Furthermore, the result of using blast on the eight samples from North Maluku revealed that the samples were tilapia species Oreochromis niloticus. Based on the phylogenetic tree or kinship, Indonesian tilapia originating from North Maluku is related to those from Inle Lake in Myanmar.

Similarly, black tilapia samples from Merauke and Malang shared the same phylogenetic tree or kinship with tilapia samples from Wasile and Myanmar. The diversity of each species is indicated by their morphological differences. This morphology is the outcome of the phenotype, a product of the interaction between genetic factors and their environment (Prehadi et al., 2015). According to Rafsanjani (2011), kinship in a population or species is typically studied using a morphological approach. However, a genetic approach using the DNA Barcode technique must also be used.

The segment near the 5' end of the COI along about 650 bases is widely used as a DNA barcode in a variety of species, including the catfish (Wong et al., 2011, Pratama, 2017), tilapia (Syaifudin et al., 2015), sharks (Peloa et al., 2015), baung fish (Syaifudin et al., 2017), and snakehead fish (Chen et al., 2003, Tan et al., 2010). The application of DNA barcodes is vital for obtaining basic information on genes that have great diversity, making them useful for the selection process in fish breeding (Arifin and Kurniasih, 2007).

This is supported by Dunham (1995), which stated that the success of the selection program in fish breeding is influenced by the level of genetic diversity and the potential for genetic diversity. In addition, DNA barcoding can function as a taxonomic tool to reveal genetically diverse snakehead species precisely and accurately, the nucleotide sequence of a species and its comparison with species, and determine the phylogenetic structure, particularly snakeheads from the Keleka River.

4. CONCLUSION

The results of research using DNA barcoding techniques on red and black tilapia from samples of biofloc nutrient system ponds in Ternate City and samples of value fish from Bumi Restu village, Wasile District, East Halmahera and two samples of black tilapia from Merauke and Malang and show similarities because they have one similar phylogeny. Furthermore, the results of squeezing eight samples from North Maluku indicated that these samples were Oreochromis niloticus tilapia. Then, when viewed from the phylogeny or kinship tree, it can be seen that the Indonesian tilapia samples from North Maluku, Merauke and Malang have one kinship with the tilapia samples from Inle lake from Myanmar.

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