Morphological and molecular identification of nike fish, Ophieleotris aporos in Tondano Lake, North Sulawesi, Indonesia

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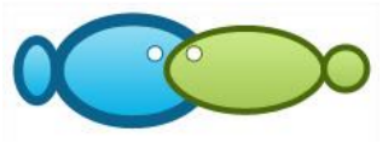

Morphological and molecular identification of nike fish, *Ophieleotris aporos* in Tondano Lake, North Sulawesi, Indonesia

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Abstract. This study aims to identify morphologically and molecularly the nike fish, *Ophieleotris aporos* in Tondano Lake. The small size of this fish in the larval, post-larva and juvenile stages causes difficulties in analyzing morphometric and meristic aspects. Therefore, the melanophore pattern approach and molecular analysis using the COI gene were used to identify and study the early life stages of the fish species. Sampling was carried out from January to February 2020, in Tondano Lake. The study was conducted based on activities of fishermen in Tondano Lake. Samples of nike fish were grouped based on differences in melanophore patterns on their bodies. It was found there were six different melanophore pattern groups coded A, B, C, D, E and F. The molecular analysis using the COI gene was done on 6 samples from the melanophore pattern groups. The results show that all the 6 analyzed samples belong to the same species, *Ophieleotris aporos*. Morphological changes have occurred due to an increase in the melanophore patterns of nike species when they become more mature.
Key Words: melanophore, migration, morphometric, Payangka fish, phylogenetic.

Introduction. "Nike" is a term for various minuscule fish species frequently found in Tondano Lake (Makmur et al 2015; Susanto et al 2017), and also in Gorontalo waters (Olii et al 2017, 2019; Sahami et al 2019a, 2019b). Nike fish are also found in Taal Lake, Luzon Island, in the Philippines (Masagca & Ordoñez 2007). At Tondano Lake, nike fish occupies a special ecological niche in the spawning ground, as planktonic larvae and young fish that dominates the fringing area, and also in the utilization of aquatic resources (Soeroto 1988). Nike fish is one of the important fishery resources in Tondano Lake, which has been known by the public for a long time. In general, people around Tondano Lake known this fish with two names, namely "Nike", and 'Wiiwir'. This fish is a treasured fish of the community around Tondano Lake, and even in Manado City, which makes this fish complementary to the traditional food menu of North Sulawesi and Manado.

Historically, the existence of nike fish in Tondano Lake was the result of an introduction from Limboto Lake, Gorontalo, that was carried out around 1912 (Soeroto 1988). One fish from the nike fish group is the Payangka fish (*Ophioleotris aporos*), when it is in the early life stages. The production of nike fish is estimated to reach around 35% of all existing fish production and dominates catches in Tondano Lake (Bataragoa & Tamanampo 2009). Payangka fish larvae have an average length between 12.61 to 22.4 mm (Susanto et al 2017). The number of captured nike fish needs to be supported by research on the existence of larval stages as part of the reproduction stages of Payangka fish in Tondano Lake. One of the reproductive factors that support the size of nike fish populations is fecundity. According to Bataragoa & Tamanampo (2009), the fecundity of Payangka fish in Tondano Lake with the size of 12.5 to 15.6 cm is between 30000 and

127000 eggs and the fecundity of Payangka fish with the size of 8.6 to 20.5 cm is between 30000 and 60000 eggs (Soeroto 1988).

To our knowledge, there has been no research regarding the identification of nike fish genetically in Tondano Lake. The present study was conducted to determine some characteristics of nike fish in Tondano Lake by molecular and morphological studies. Genetic characters can provide genetic information and support morphological characters data. This study aims to identify morphologically and molecularly nike fish in Tondano Lake.

Material and Method

Sample collection. The sampling of fish from fishermen was carried out randomly in 10 March 2020, from Tondano Lake. The sampling location is presented in Figure 1. The collected samples were placed in plastic sterile containers and into an icebox. The samples were sorted and grouped according to the melanophore pattern on the body of the fish at the Laboratory of the Faculty of Fisheries and Marine Sciences, Sam Ratulangi University, and Integrated Laboratory of the Faculty of Fisheries and Marine Sciences, Gorontalo State University. Samples of nike fish were sorted in 6 different groups, coded A, B, C, D, E and F. From each group of fish, 6 individuals were selected and preserved in sample containers with 70% alcohol. Furthermore, samples were analyzed for genetic identification in PT Genetika Science Indonesia, Jakarta. The small size of this fish in the larval, post-larva, juvenile stages causes difficulties in determining morphometric and meristic aspects. Therefore, the melanophore pattern approach and molecular analysis using the COI gene were used to identify the fish species.

Molecular analysis. DNA genome isolation of samples was conducted using an Isolation Kit by Geneaid - Genomic DNA Mini Kit (Tissue). The isolation method carried out refers to the product standard protocol. The PCR process was carried out using the primary pair (Baldwin et al 2009), namely (Forward) BCL Fish: 5'-TCAACYAATCAYAAAGATATYGGCAC3' and (Reverse) Fish BCH: 5'-ACTTCYGGGTGRCCRAARAATCA-3'. PCR products were then electrophoresed and photographed above an UV Transilluminator (Pacific image, Electronic). The nucleotide sequencing cycle is a method for determining the sequence of nucleotides contained in DNA. The DNA samples that had been amplified and electrophoresed were subsequently sequenced. The sequencing process was carried out at the First Base Laboratory in Malaysia by PT Genetics Science Indonesia. Samples consisting of 30 µL of PCR DNA products, 10 µL of forward primer and 10 µL of reverse primer were sent to the laboratory. Editing and proofreading sequences were performed using MEGA 6.0 software. The proofreading results from the forward and reverse sequences were combined into a sequence. Then the sequence results were analyzed to find genetic similarities. To find out the relationship level among samples, further analyses were carried out based on phylogenetic trees with the Maximum Likelihood Method with 1000 bootstraps using MEGA 6.0 software.

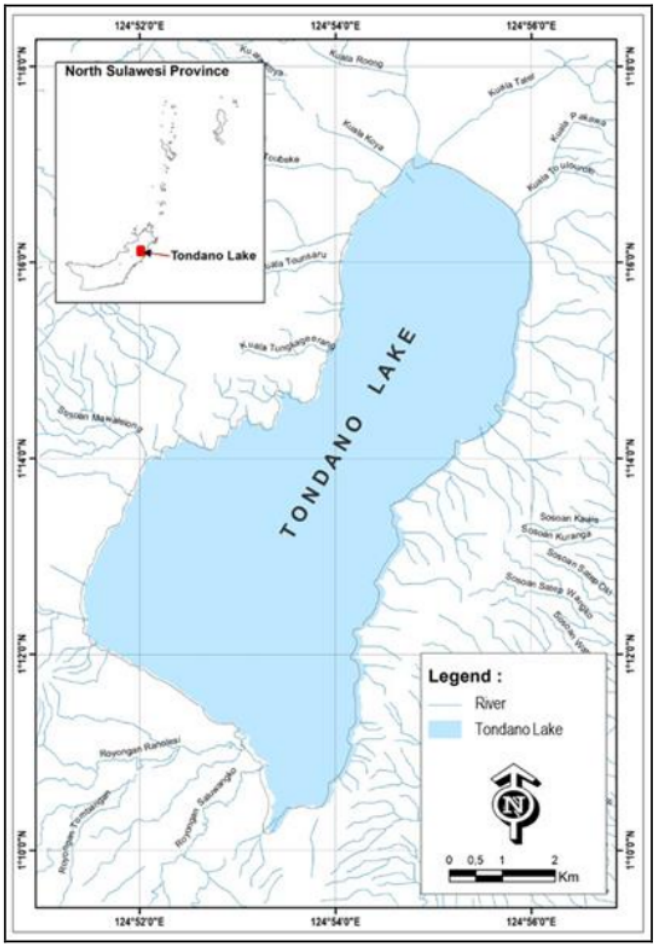

Figure 1. Map of sampling sites in Tondano Lake.

Results and Discussion. The results indicate that nike fish sampled consist of 2 morphologically dissimilar groups, with different melanophore patterns (Figure 2). Samples A, B, C, D, E and F have undergone alterations in the melanophore structure and body size. At first, the fish has no melanin. After the melanin is formed, it then spreads throughout the body. Furthermore, after melanophores spread proportionally throughout the body, there is a wider distance between melanophores because the fish body becomes larger. The research of Valade et al (2009) showed a change in the appearance of chromatophores in the body of *Sicyopterus langocephalus* larvae, starting from the head and spreading throughout the body during the larvae stage. The results of this study illustrate that there has been a variation in the melanophore pattern of *O. aporos* when becoming more mature. When still in larval stage (1.5 cm), the melanophore arrangement is not yet present, but when it enters the post-larval stage (1.7 to 2.5 cm) and juvenile stage (4.5 cm), the melanophore arrangement proportionally spreads along the body (Figure 2).

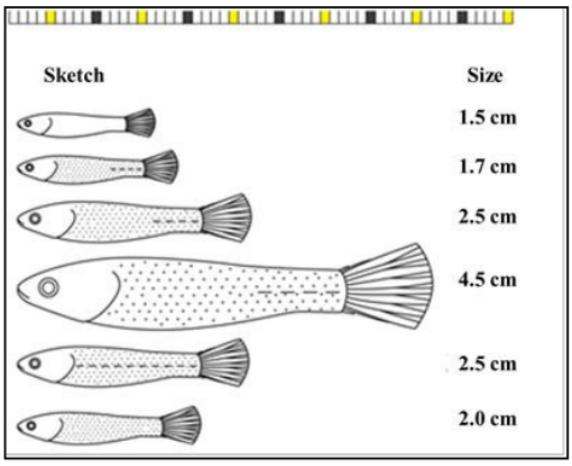

Figure 2. Schematic morphological alterations in nike fish samples, based on body size.

The differences in melanophore patterns from samples A, B, C, D, E and F and the differences in body size are schematically presented in Figure 3.

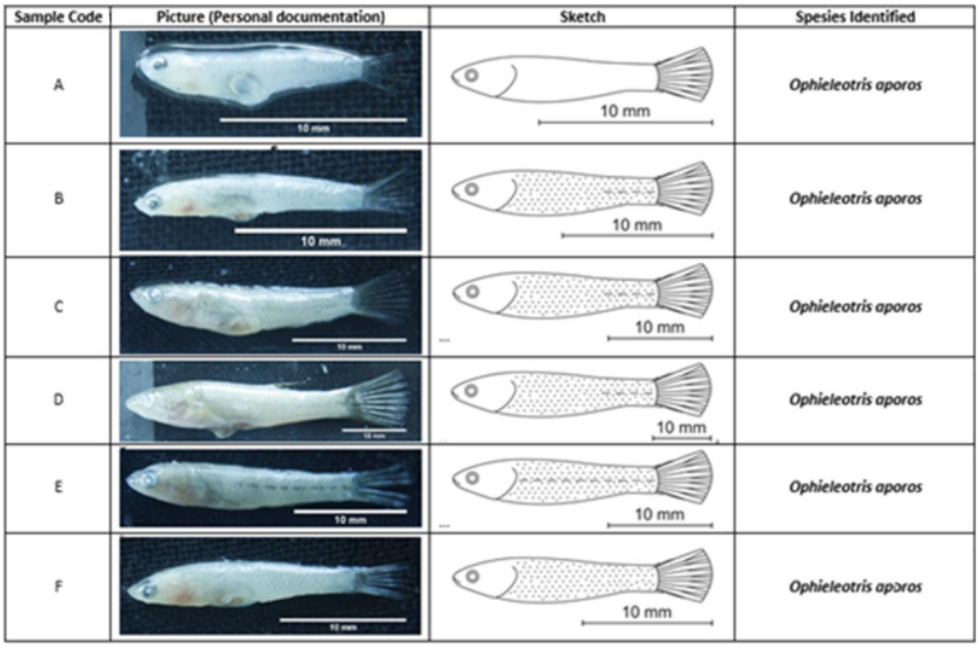

Figure 3. Schematic morphological alterations in samples, based on the melanophore pattern.

The proofreading results from the forward and reserve sequences combined with the sequence of the two samples (A, B, C, D, E and F) are presented in Table 1.

Table 1

Proofreading results of forward and reverse sequence of nike fish samples from A, B, C, D, E and F groups

Sample code	*Proof reading results*
A	CACCCTCTATCTTGTATTTGGTGCCTGAGCGGGAATAGTAGGCACGGCTTTAAGCCTGCTAATTCGAGCCGA ATTGAGTCAACCTGGCGCCCTGCTAGGAGATGACCAAATTTATAATGTCATCGTGACAGCTCATGCCTTCGT GATAATTTTCTTTATAGTAATACCAATTATGATTGGCGGCTTTGGAAATTGATTAGTGCCCCTAATGATTGGC GCCCCCGACATGGCCTTCCCCCGAATAAACAACATAAGCTTCTGACTCCTCCCCCCATCCTTCCTTCTTCTTC TAGCATCCTCTGGTGTAGAGGCGGGAGCTGGAACAGGGTGAACCGTCTACCCCCCCTCTAGCAGGAAACTTA GCCCACGCAGGGGCCTCCGTAGATCTGACCATTTTCTCTCTTCATCTTGCCGGTGTCTCTTCTATTTTAGGA GCTATCAACTTCATCACCACAATTATTAATATGAAACCTCCCGCCATTTCACAATACCAAACACCTCTGTTTG TATGGGCAGTCCTAATTACAGCTGTCCTTCTACTTCTATCCCTGCCAGTCCTAGCCGCTGGTATCACAATGCT TCTCACAGACCGAAACCTAAATACAACCTTCTTCGACCCTGCTGGAGGAGGTGACCCAATTCTATACCAACA TCTTTTCTGATTCTTC
B	GCACCCTCTATCTTGTATTTGGTGCCTGAGCGGGAATAGTAGGCACGGCTTTAAGCCTGCTAATTCGAGCC GAATTGAGTCAACCTGGCGCCCTGCTAGGAGATGACCAAATTTATAATGTCATCGTGACAGCTCATGCCTTC GTGATAATTTTCTTTATAGTAATACCAATTATGATTGGCGGCTTTGGAAATTGATTAGTGCCCCTAATGATTG GCGCCCCCGACATGGCCTTCCCCCGAATAAACAACATAAGCTTCTGACTCCTCCCCCCATCCTTCCTTCTTC TTCTAGCATCCTCTGGTGTAGAGGCGGGAGCTGGAACAGGGTGAACCGTCTACCCCCCCTCTAGCAGGAAAC TTAGCCCACGCAGGGGCCTCCGTAGATCTGACCATTTTCTCTCTTCATCTTGCCGGTGTCTCTTCTATTTTAG GAGCTATCAACTTCATCACCACAATTATTAATATGAAACCTCCCGCCATTTCACAATACCAAACACCTCTGTT TGTATGGGCAGTCCTAATTACAGCTGTCCTTCTACTTCTATCCCTGCCAGTCCTAGCCGCTGGTATCACAAT GCTTCTCACAGACCGAAACCTAAATACAACCTTCTTCGACCCTGCTGGAGGAGGTGACCCAATTCTATACCA ACATCTTTTCTGATTCTT
C	TCGGCACCCTCTATCTTGTATTTGGTGCCTGAGCGGGAATAGTAGGCACGGCTTTAAGCCTGCTAATTCGA GCCGAATTGAGTCAACCTGGCGCCCTGCTAGGAGATGACCAAATTTATAATGTCATCGTGACAGCTCATGC CTTCGTGATAATTTTCTTTATAGTAATACCAATTATGATTGGCGGCTTTGGAAATTGATTAGTGCCCCTAATG ATTGGCGCCCCCGACATGGCCTTCCCCCGAATAAACAACATAAGCTTCTGACTCCTCCCCCCATCCTTCCTT CTTCTTCTAGCATCCTCTGGTGTAGAGGCGGGAGCTGGAACAGGGTGAACCGTCTACCCCCCTCTAGCAGG AAACTTAGCCCACGCAGGGGCCTCCGTAGATCTGACCATTTTCTCTCTTCATCTTGCCGGTGTCTCTTCTATT TTAGGAGCTATCAACTTCATCACCACAATTATTAATATGAAACCTCCCGCCATTTCACAATACCAAACACCTC TGTTTGTATGGGCAGTCCTAATTACAGCTGTCCTTCTACTTCTATCCCTGCCAGTCCTAGCCGCTGGTATCAC AATGCTTCTCACAGACCGAAACCTAAATACAACCTTCTTCGACCCTGCTGGAGGAGGTGACCCAATTCTATA CCAACATCTTTTCTGATT
D	ATCGGCACCCTCTATCTTGTATTTGGTGCCTGAGCGGGAATAGTAGGCACGGCTTTAAGCCTGCTAATTCGA GCCGAATTGAGTCAACCTGGCGCCCTGCTAGGAGATGACCAAATTTATAATGTCATCGTGACAGCTCATGC CTTCGTGATAATTTTCTTTATAGTAATACCAATTATGATTGGCGGCTTTGGAAATTGATTAGTGCCCCTAATG ATTGGCGCCCCCGACATGGCCTTCCCCCGAATAAACAACATAAGCTTCTGACTCCTCCCCCCATCCTTCCTT CTTCTTCTAGCATCCTCTGGTGTAGAGGCGGGAGCTGGAACAGGGTGAACCGTCTACCCCCCTCTAGCAGG AAACTTAGCCCACGCAGGGGCCTCCGTAGATCTGACCATTTTCTCTCTTCATCTTGCCGGTGTCTCTTCTATT TTAGGAGCTATCAACTTCATCACCACAATTATTAATATGAAACCTCCCGCCATTTCACAATACCAAACACCTC TGTTTGTATGGGCAGTCCTAATTACAGCTGTCCTTCTACTTCTATCCCTGCCAGTCCTAGCCGCTGGTATCAC AATGCTTCTCACAGACCGAAACCTAAATACAACCTTCTTCGACCCTGCTGGAGGAGGTGACCCAATTCTATA CCAACATCTTTTCTGATTCTTCG
E	GCACCCTCTATCTTGTATTTGGTGCCTGAGCGGGAATAGTAGGCACGGCTTTAAGCCTGCTAATTCGAGCC GAATTGAGTCAACCTGGCGCCCTGCTAGGAGATGACCAAATTTATAATGTCATCGTGACAGCTCATGCCTTC GTGATAATTTTCTTTATAGTAATACCAATTATGATTGGCGGCTTTGGAAATTGATTAGTGCCCCTAATGATTG GCGCCCCCGACATGGCCTTCCCCCGAATAAACAACATAAGCTTCTGACTCCTCCCCCCATCCTTCCTTCTTC TTCTAGCATCCTCTGGTGTAGAGGCGGGAGCTGGAACAGGGTGAACCGTCTACCCCCCTCTAGCAGGAAAC TTAGCCCACGCAGGGGCCTCCGTAGATCTGACCATTTTCTCTCTTCATCTTGCCGGTGTCTCTTCTATTTTAG GAGCTATCAACTTCATCACCACAATTATTAATATGAAACCTCCCGCCATTTCACAATACCAAACACCTCTGTT TGTATGGGCAGTCCTAATTACAGCTGTCCTTCTACTTCTATCCCTGCCAGTCCTAGCCGCTGGTATCACAAT GCTTCTCACAGACCGAAACCTAAATACAACCTTCTTCGACCCTGCTGGAGGAGGTGACCCAATTCTATACCA ACATCTTTTCTGATTCTTCGGACACCCTGAAGTGTC
F	CCCTCTATCTTGTATTTGGTGCCTGAGCGGGAATAGTAGGCACGGCTTTAAGCCTGCTAATTCGAGCCGAAT TGAGTCAACCTGGCGCCCTGCTAGGAGATGACCAAATTTATAATGTCATCGTGACAGCTCATGCCTTCGTGA TAATTTTCTTTATAGTAATACCAATTATGATTGGCGGCTTTGGAAATTGATTAGTGCCCCTAATGATTGGCGC CCCCGACATGGCCTTCCCCCGAATAAACAACATAAGCTTCTGACTCCTCCCCCCATCCTTCCTTCTTCTTCTA GCATCCTCTGGTGTAGAGGCGGGAGCTGGAACAGGGTGAACCGTCTACCCCCCTCTAGCAGGAAACTTAG CCCACGCAGGGGCCTCCGTAGATCTGACCATTTTCTCTCTTCATCTTGCCGGTGTCTCTTCTATTTTAGGAG CTATCAACTTCATCACCACAATTATTAATATGAAACCTCCCGCCATTTCACAATACCAAACACCTCTGTTTGTA TGGGCAGTCCTAATTACAGCTGTCCTTCTACTTCTATCCCTGCCAGTCCTAGCCGCTGGTATCACAATGCTT CTCACAGACCGAAACCTAAATACAACCTTCTTCGACCCTGCTGGAGGAGGTGACCCAATTCTATACCAACAT CTTTTCTGATTCTTCGGACACCCTG

The sequences produced are then compared with sequences from the Gene Bank Deposits (NCBI nucleotide databases). The results are presented in Table 2.

Table 2

Comparison sequences of sample and NCBI nucleotide database

Species code	*Accession number*	*Species*	*Query cover*	*Identity*
A	AF391368.1	*Ophioleotris aporos*	100%	99.85%
B	AF391368.1	*Ophioleotris aporos*	100%	99.85%
C	AF391368.1	*Ophioleotris aporos*	100%	99.85%
D	AF391368.1	*Ophioleotris aporos*	100%	99.85%
E	AF391368.1	*Ophioleotris aporos*	99%	99.56%
F	AF391368.1	*Ophioleotris aporos*	99%	99.70%

The 6 samples are both *O. aporos* based on genetic testing with mitochondrial COI. This is a new discovery related to Nike fish data from Tondano Lake. The previous studies never reported about the larval, post-larva and juvenile stages of *O. aporos* from Tondano Lake. Further analysis was carried out with the phylogenetic tree to show the kindship relations between samples (A, B, C, D, E and F) and several species available in the NCBI database (Figure 4).

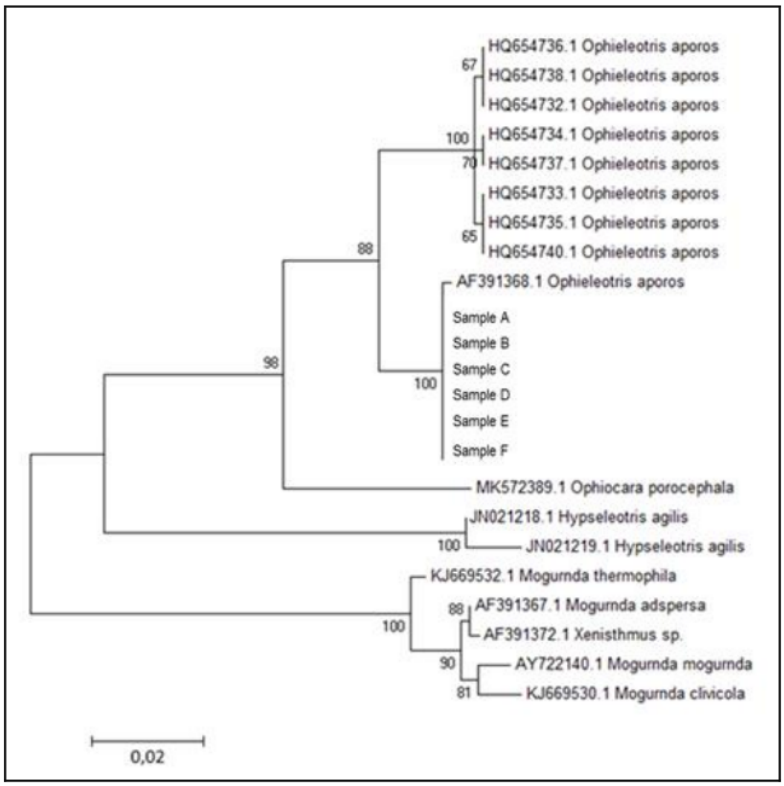

Figure 4. Phylogenetic tree of nike fish (samples A to F) compared with several species in the NCBI database.

The genetic analysis illustrates that the 6 samples are from the same species, even though they have different morphologies, particularly in the melanophore patterns. This modification appears in the early life stages of this species.

Sahami et al (2019a, 2019b) mentioned that the melanophore patterns of *Belobranchus segura* migrating from sea to rivers are characterized by a slight increase in coloration. This is visible when approaching the estuary, being either a form of adaptation in order to enter a new aquatic environment or a part of its development stages. Hobson et al (2007) informs that Hawaiian gobioid fish are amphidromous and have one life stage in the sea. They stay in the pelagic sea zone for several months before migrating in the river (Teichert et al 2016) and Olii et al (2017) showed that nike fish has a tendency to shift closer to the river mouth in the days after its appearance in the Gorontalo Bay, which indicates the migration of nike from sea water to fresh water. Apparently, the results of this research show that even though this nike fish is catadromous, where the spawning migration is from the sea to freshwater (Herre 1927), it has adapted well to the new environment, Tondano Lake, and it currently does not migrate for reproduction, because the lake does not allow it. It is due to the constraints of three water power plants (Tonsea Lama, Tanggari 1 and Tanggari 2) in Tondano River, which obstruct the migration pathways.

Various studies shows that the occurrence of migratory behavior has a genetic basis in freshwater fish (Lucas et al 2001). This migration could be related to the development stage (Fitzsimons et al 2007). Amphidromous fish spawn in freshwater and the larvae migrate to the sea, then back to freshwater (Milton 2009). The distribution of the species along the river is determined by post larva color aggregation (Nishimoto & Fitzsimons 1986). This study showed that the migration of Nike fish is only limited to the lake, either for the larvae, post larva or juvenile stages, especially on the fringing parts of Tondano Lake. This nike fish is active at night, which is why the fishing is carried out only at night or before dawn by using traditional fishing gear (*sero*) and LED light.

Conclusions. Samples of nike fish with different melanophore patterns are genetically the same species, *Ophioleotris aporos*. The morphological changes of this species from the nike fish group are indicated by the differences in melanophore patterns, with an increase in the number and spread of melanophores on the surface of the body when the species becomes more mature according to its early life stage.

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