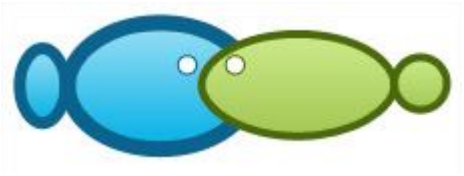

Exploration of adult phase of Nike fish to maintain its sustainability in Gorontalo Bay waters, Indonesia

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Abstract. Nike is an amphidromous goby species that migrates from the Gorontalo Bay waters to the Bone Bolango River and metamorphoses into adult goby. This fish is very popular, and it becomes people's favorite as delicious food and turns into an important economic commodity in Gorontalo. Therefore, the exploration of Nike fish's adult phase as an essential phase in the availability of Nike in the Gorontalo Bay waters needs to be done. This study aims to determine the types of goby as the adult phase of Nike fish in the Bone Bolango River waters based on genetic analysis of the mitochondrial DNA COI gene. Sampling was carried out in April and June 2020. The captured samples are then grouped according to their morphological similarity (shape, color, and body pattern). Each of the two samples from each morphological group was preserved in a 95% ethanol solution for genetic identification needs. This study succeeded in finding the adult phase of six species from a total of nine species of a composer for Nike fish schools in Gorontalo; *Sicyopterus longifilis, S. lagocephalus, Belobranchus belobranchus, B. segura, Bunaka gyrinoides*, and *Stiphodon semoni*. *S. longifilis* and *S. lagocephalus* species are fish with the local name Busalo; *B. segura*, *B. belobranchus*, and *B. gyrinoides* are locally named Hundala; and *S. semoni* is locally named Timudu'o. The sustainability of Nike's resources can be done with proper management of this adult phase of Nike fish in the river, such as not overfishing goby or releasing goby that are carrying eggs into the river.

Key Words: amphidromous, Busalo, COI gene, gobi, Hundala, Nike, Timudu'o.

Introduction. Nike fish is a typical commodity found in Gorontalo which has important economic value and is commonly favored by the local community as foodstuff due to its good taste and distinction. It belongs to an amphidromous goby group in the postlarva to juvenile stage. Amphidromy is a diadromous pattern of life, the adult spawn in freshwater and their larvae drift downstream into the sea; larvae develop in the marine environment then migrate back into rivers to grow and spawn (Keith et al 2008; Yamasaki et al 2011; Taillebois et al 2012; Iida et al 2017). In tropical rivers, amphidromous fish species are major contributors to the diversity of ecosystems (Ellien et al 2014; Lejeune et al 2016).

Olii et al (2017) and Pasisingi & Abdullah (2018) report that Nike fish in the Gorontalo Bay waters will firstly appear in the sea and then move closer to the river mouth over time until they eventually disappear. Nike's high fishing effort should be balanced with resources and habitat ecology management to ensure its sustainability in the future. Exploring the adult phase of fish is one effort that can be conducted to understand the fish biology and ecology. Thus, the management and conservation plans in preserving stocks and biodiversity can be carried out properly. Many studies recently focused on Nike fish (Usman 2016; Olii et al 2017; Pasisingi & Abdullah 2018; Olii et al 2019; Nurjirana et al 2019; Sahami et al 2019a, b; Pasisingi et al 2020b; Sahami et al 2020). Whereas adult goby as a potential Nike fish parent is also a commodity that plays an important role in the availability of Nike in the Gorontalo Bay waters.

The DNA barcode method in species identification is used to overcome the limitations of species identification morphologically, including phenotypic plasticity, specific life stages of identification cues, small size, often cryptic ecology, and the possible emergence of new species (Viswambharan et al 2013; Linh et al 2018). The cytochrome oxidase subunit I (COI) gene is the fastest and most reliable gene used as a

barcoding marker to identify and discriminate against species that are very closely related to various animal phyla (Hebert et al 2004; Hubert et al 2008; Viswambharan et al 2013; Linh et al 2018). This study aims to determine the types of gobies as the adult phase of Nike fish in the Bone Bolango River waters based on genetic analysis of the mitochondrial DNA COI gene.

Material and Method

Sampling. Goby fish sampling was carried out in the Bone Bolango River (Figure 1) which was suspected to be an adult phase area of the Nike fish. Sampling was carried out in April and June 2020 by using a hand net and modified electric fishing (12V, 10A) with a radius and depth of less than 1 m. The samples obtained were filled in a labeled zip-lock plastic, then, it was stored and given some ice in a cool box. Sample sorting was carried out at the Hydrology and Biometrics Laboratory of the Faculty of Fisheries and Marine Sciences, Gorontalo State University by grouping caught gobies based on their morphological similarity (shape, color, and body pattern). In total, there were 14 groups of caught fish. Two samples from each morphological group were preserved in a 95% ethanol solution for genetic identification needs.

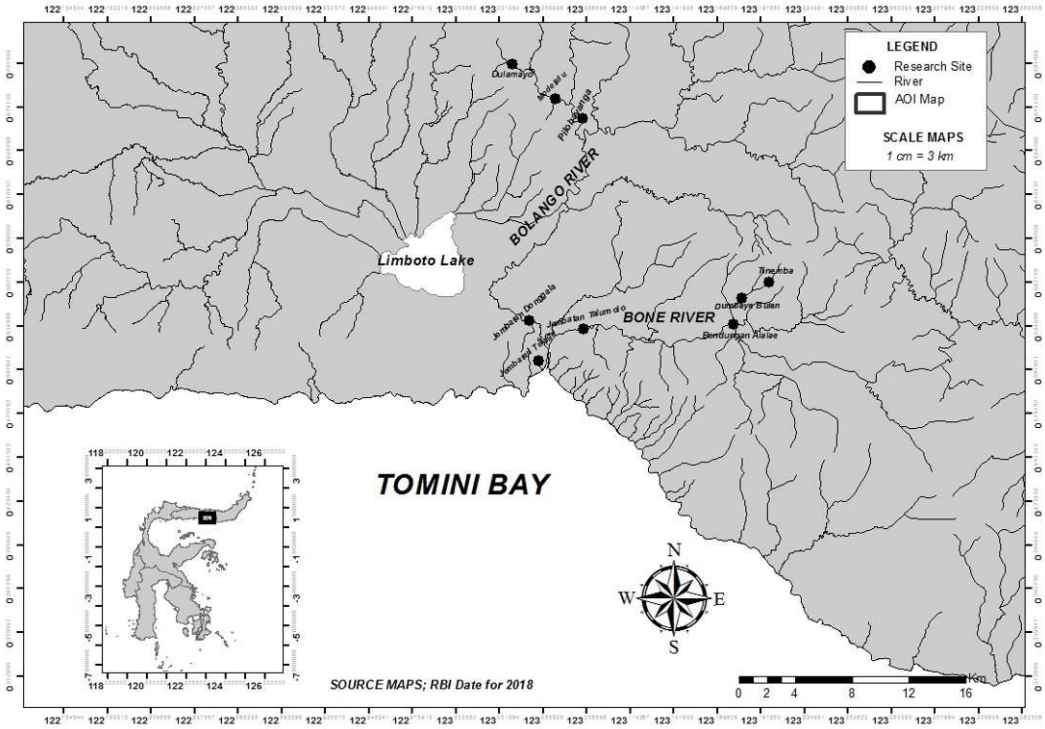

Figure 1. Map of the research location.

DNA extraction, ***PCR amplification***, ***and sequencing***. Molecular analysis was carried out in the Bionesia Laboratory, Denpasar, through several stages, including a collection of fish tissue, DNA isolation, DNA Polymerase Chain Reaction (PCR), electrophoresis, and DNA sequencing. Isolation of DNA samples was carried out using Qiagen Tissue and Blood Extraction kits following the protocol kit. The mitochondrial DNA Cytochrome Oxidase subunit I (COI) gene was chosen because the resolution of the COI gene at the intraspecific level is better than other core genes. Therefore, it is best to be used to identify species to the intraspecific level (Strüder-Kypke & Lynn 2010).

The mitochondrial DNA COI gene was further amplified using a forward primer FishF1 5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3' and reverse primer FishR1 5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3' (Ward et al 2005). Three samples, namely B5, B6, and H7 were amplified using a pair of forwarding primer FF2d 5'-TTC TCC ACC AAC CAC AAR GAY ATY GG-3' and reverse primer FR1d 5'-CAC CTC AGG GTG TCC GAA RAA YCA RAA-3' (Ivanova et al 2007). The PCR profiles used were predenaturation at 94°C for

five minutes, denaturation at 94°C for 30 seconds, primary annealing at 50°C for 30 seconds, elongation at 72°C for 30 seconds, and final elongation at 72°C for seven minutes. This PCR process lasted for 38 cycles. DNA samples that have been amplified and electrophoresed would be sequenced afterward.

Data analysis. The samples that had been amplified by the PCR method were then sequenced by using the Dideoxy Sanger Termination Method through PT Genetika Science Indonesia. Nucleotide sequences from DNA sequencing that have been processed and carried out by CONTIG were then matched with data available in the GenBank database (www.ncbi.nlm.nih.gov) through the BLAST (Basic Local Alignment Search Tool). The phylogenetic tree was arranged by aligning the identified DNA sequence samples with DNA samples of composer's species for Nike fish schools that were available in the GenBank database (Acc. Num. MN069305.1; MN069306.1; MN069307.1; MN069308.1; MT227827.1; MT227828.1; MT227829.1; MT706639.1; MT706640.1; MT706641.1; MT706720.1; MT706721.1; MT706722.1; MT706723.1; MT706724.1; MT706725.1; MT706726.1; and MT706791.1). The phylogenetic tree was created using the Maximum Likelihood 1000 bootstrap method in MEGA X software (Kumar et al 2018).

Results and Discussion. The local community and general public believe that Nike is a fish with maximum body size; thus juvenile size has become a parent size for Nike fish. Whereas Nike fish as an amphidromous species migrates from the Gorontalo Bay waters to the Bone Bolango River and metamorphoses into an adult goby. The results of the molecular analysis indicate that Nike fish schools are composed by nine species so far; *Sicyopterus pugnans*, *S. longifilis*, *S. cynocephalus*, *S. lagocephalus*, *S. parvei*, *Belobranchus segura*, *B. belobranchus*, *Bunaka gyrinoides*, and *Stiphodon semoni* (Sahami et al 2019b; Sahami et al 2020). The high diversity of species of a composer for Nike fish schools indicates the potential high diversity of goby species in the Bone Bolango River.

Based on its morphological characters, gobies in the Bone Bolango River generally have a torpedo (fusiform) body shape. They have two separate dorsal fins, pectoral fins, pelvic fins, anal fin, and rounded caudal fin. In general, the goby species can be grouped into three large groups known locally as Hundala, Busalo, and Timudu'o. The special characteristics that distinguish between Hundala and Busalo fish are the pelvic fins of Hundala fish are separated and not connected by a membrane and the pelvic fins of Busalo fish are combined to form something like suction discs. In contrast, Timudu'o fish are identified as small gobies found in river waters and have pelvic fins that joined like Busalo fish but with a different morphological appearance.

After editing and cutting sequences, 651 bp of mitochondrial DNA COI were obtained for all the goby samples. A total of 14 groups of goby species were confirmed as adult phases of Nike fish based on the results of COI mitochondrial DNA BLAST. Five of which were Busalo (B1-B5), seven groups were Hundala fish species (H1-H7), and two groups were Timudu'o fish species (T1-T2) as shown in Table 1.

Table 1 shows goby in the Bone Bolango River consisting of four genera and six species; *Sicyopterus longifilis*, *S. lagocephalus*, *Belobranchus belobranchus*, *B. segura*, *Bunaka gyrinoides*, and *Stiphodon semoni*. This species' identification results indicate that the Hundala fish are part of the Eleotridae family, and Busalo and Timudu'o fish are the fish group of the Gobiidae family. The same genetic identity in two or more different morphological groups can be influenced by the environment and can also occur due to sexual dichromatism. Sexual dichromatism usually occurs in freshwater Gobioid fish, in which males have a brighter color than females (Keith 2003). *S. semoni* is one species that shows the phenomenon of sexual dichromatism in which males have a blackish gray body color with greenish staining on the head to the chest area no longer after being captured (T1). On the other hand, females tend to have a yellowish-brown color, and they have transverse lines from the tip of snout to the base of the caudal fin (T2) as described by Maeda & Tan (2013).

Table 1

BLAST results of the mitochondrial COI DNA of adult phases of species of composers for Nike schools

No	Sample codes	Species	NCBI accession number	Query cover (%)	Per. identity (%)
1	B1	*Sicyopterus longifilis*	MK496959.1	100	99.69
2	B2	*Sicyopterus lagocephalus*	KF668859.1	100	99.39
3	B3	*Sicyopterus lagocephalus*	KF668859.1	100	99.54
4	B4	*Sicyopterus longifilis*	NC_044142.1	99	99.56
5	B5	*Sicyopterus longifilis*	NC_044142.1	99	99.71
6	H1	*Belobranchus belobranchus*	KU692346.1	99	99.39
7	H2	*Belobranchus segura*	MN069308.1	99	99.69
8	H3	*Belobranchus segura*	MN069308.1	99	99.69
9	H4	*Belobranchus belobranchus*	MT706791.1	99	99.08
10	H5	*Belobranchus belobranchus*	MT706791.1	99	99.23
11	H6	*Belobranchus segura*	MN069308.1	99	99.69
12	H7	*Bunaka gyrinoides*	MN069307.1	100	99.85
13	T1	*Stiphodon semoni*	MT706724.1	95	99.70
14	T2	*Stiphodon semoni*	MT706724.1	92	99.70

Other species that also show the phenomenon of sexual dichromatism are *S. longifilis* and *S. lagocephalus*. *S. longifilis* males (B1) are green with particular features. They have vertical stripes that sometimes extend to the abdomen, the black pectoral fins with white ribbons on edge, and slightly orange with red and blue lines at the dorsal and ventral tips of the caudal fin. On another side, the female fish are brown and duller (B4 and B5). At the same time, the similar condition is also shown by *S. lagocephalus* species, in which male fish has a very attractive color that is bluish to greenish with a reddish-orange caudal fin (B3). In contrast, the female fish are brownish to grayish and have an orange ribbon on the edge of the pectoral fin, anal fin, and the dorsal and ventral sides of the caudal fin (B2).

Sexual dichromatism is not found in *B. segura* and *B. belobranchus* species, as reported by Keith et al (2012). Both of these species are goby sleepers with the elongated body with anterior parts forming cylindrical and posterior compressed, and having brownish to mahogany body colors. The distinctive characteristic between them is the presence of darker and thicker color that extends from chest to the base of caudal fin along the lateral line in *B. segura* species. Whereas the *B. gyrinoides* species found in one morphological characteristic in this study. This species is also goby sleepers with a typical large body, a similar body shape with *Belobranchus* species, a blackish-brown body color, and having fins with blackish-brown spots and blackish-brown vertical stripes on the caudal fin (Huang & Chen 2008). The relationships between adult species of Nike fish and the species of a composer for Nike fish schools are illustrated through the phylogenetic tree in Figure 2.

The phylogenetic tree (Figure 2) clearly shows the kinship between the species of a composer for Nike fish schools and goby fish which have been reported and found in this study. The whole phylogenetic tree forms two monophyletic clades as family clades. The first monophyletic clade is the Gobiidae Family clade consisting of *S. lagocephalus*, *S. longifilis*, and *Stiphodon semoni*. The second monophyletic clade belongs to Eleotridae family which includes *B. gyrinoides*, *B. segura* and *B. belobranchus*. From nine species of a composer for Nike fish schools, three of them (*S. cynocephalus*, *S. pugnans*, and *S. parvei*) have not been found yet in the adult phase in this study. However, the genetic distance between species found in Bone Bolango River shown in Table 2 will strengthen the results of the phylogenetic tree formed in Figure 2.

Eleotridae is one of the families in the Gobioidei Order known as a sleeper species with prey-waiting characteristics. They have a large blunted head, a torpedo body shape, rounded caudal fins, protruding mandible, protruding pelvic fins that are not connected by membranes, and not bright body color, namely light brown to dark brown (Murdy & Hoese 2002). Eleotrid species usually prefer water habitats where have small flow and they usually stay in river banks. These morphological characteristics have been found in Hundala fish and confirmed through its genetic analysis as species of Eleotridae Family.

Unlike the Eleotridae fish, the species in Gobiidae Family are mostly active swimmers and are very fond of currents. In Reunion Island, adult species of *S. lagocephalus* are known to be rheophilic and like clear habitats with good oxygen levels (Keith 2003). Gobiidae is widely distributed in the sea, estuary, and freshwater. Gobiid species generally have pelvic fins that combined to form something like sucking discs, both partially and wholly. This modification allows quick access to the river's headwaters by attaching itself to the substrate when the species is against the current (Taillebois et al 2014). Also, this species' body color varies greatly from the drab to various bright patterns (Murdy & Hoese 2002; Thacker & Roje 2011). The morphological characteristics are found in Busola and Timudu'o fish, which also confirmed through genetic analysis as species in Gobiidae Family.

Therefore, it has been genetically confirmed that six goby species in the adult phase are the species of a composer for Nike fish schools in the Gorontalo Bay waters. *S. longifilis* species with its female morphological characteristics have been reported by Olii et al (2019) as the adult stage of Nike fish. Besides, *B. belobranchus* species with its morphological features of H1 has also been found by Pasisingi et al (2020a) and confirmed as an adult phase of Nike fish through this study. The discovery of the adult phase of Nike fish has been new and important information for the local community in Gorontalo so that they can actively contribute to the availability of Nike fish resources sustainably in the future. It can be carried out by not overfishing gobies or releasing gobies, which carrying eggs into the river. This discovery also refutes the local community's opinions regarding Nike fish only live in sea waters, they cannot increase their size, and they tend to disappear on the surface.

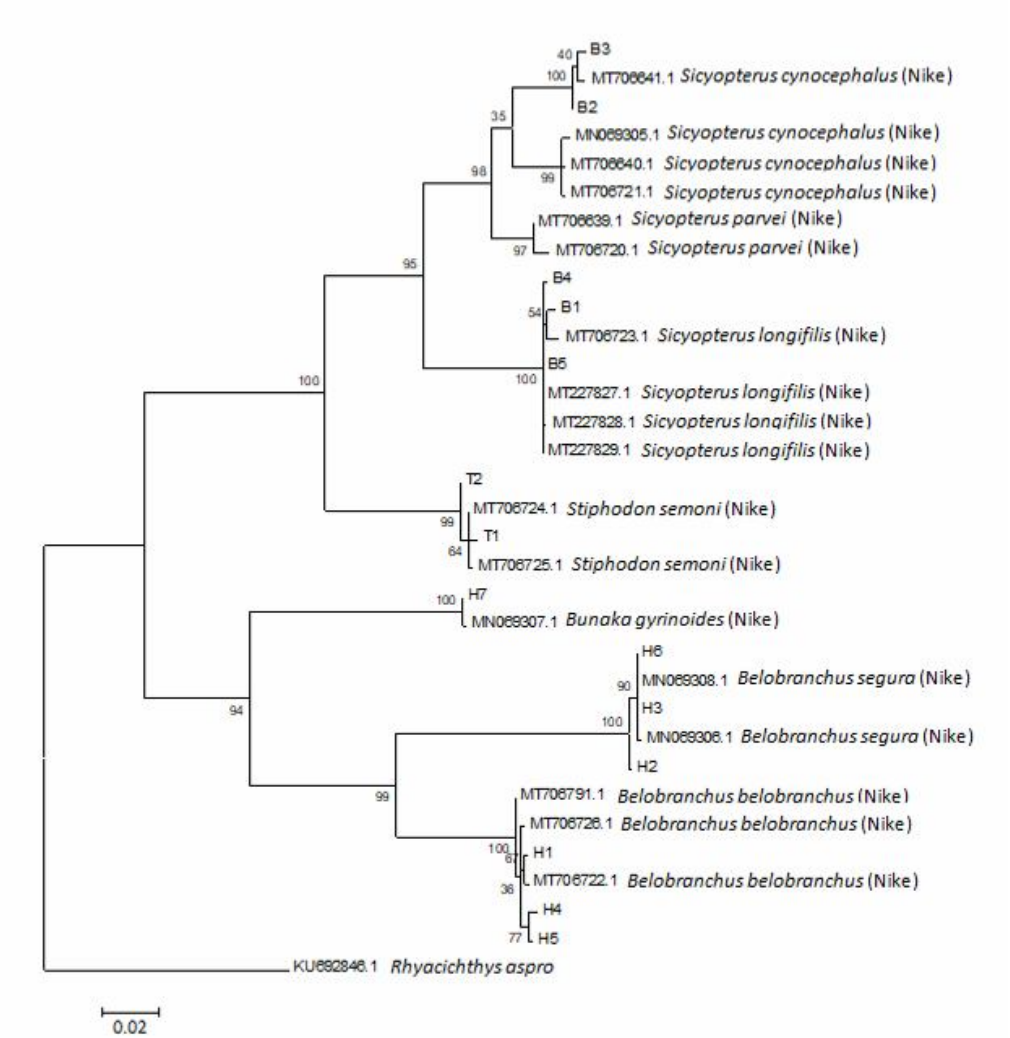

Figure 2. Phylogenetic tree of adult fish goby and species of a composer for Nike fish schools in the Gorontalo Bay waters.

Table 2

The genetic distance of goby adult phases of species of composers for Nike fish schools

	B1	*B2*	*B3*	*B4*	*B5*	*H1*	*H2*	*H3*	*H4*	*H5*	*H6*	*H7*	*T1*	*T2*
B1														
B2	0.10													
B3	0.10	0.00												
B4	0.01	0.09	0.10											
B5	0.01	0.09	0.09	0.00										
H1	0.22	0.20	0.21	0.22	0.22									
H2	0.24	0.23	0.23	0.24	0.24	0.13								
H3	0.25	0.23	0.23	0.24	0.24	0.13	0.00							
H4	0.23	0.21	0.21	0.22	0.22	0.01	0.14	0.14						
H5	0.22	0.20	0.21	0.22	0.22	0.01	0.13	0.13	0.00					
H6	0.25	0.23	0.23	0.24	0.24	0.13	0.00	0.00	0.14	0.13				
H7	0.20	0.22	0.22	0.20	0.20	0.17	0.19	0.19	0.17	0.17	0.19			
T1	0.12	0.12	0.12	0.12	0.12	0.21	0.22	0.23	0.21	0.21	0.23	0.20		
T2	0.12	0.12	0.12	0.12	0.12	0.21	0.23	0.23	0.22	0.21	0.23	0.19	0.01	

Conclusions. This research has confirmed that Nike is an amphidromous species which supported by the discovery of the adult phase of six species composers of Nike fish assemblages; *Sicyopterus longifilis, S. lagocephalus, Belobranchus belobranchus, B. segura, Bunaka gyrinoides*, and *Stiphodon semoni* in Bone Bolango River based on the DNA COI mitochondria analysis. The sustainability of Nike's resources can be done with proper management of the adult phase of Nike fish in the river, such as not overfishing goby or releasing goby that carries eggs into the river.

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