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Molecular Approach to Identify Gobioid Fishes, “Nike” and “Hundala” (Local Name), from Gorontalo Waters, Indonesia

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Abstract: Data of the species scientific name is required to study biodiversity and conservation of local fish species. Nike and hundala are the local names for fish found in Gorontalo whose scientific names are unknown and recognized by local people as two different species of fish. This study aims to identify the genetic and species of nike and hundala fish. Nike and hundala specimens were collected using a fish net from the estuary of Gorontalo Bay and Bone River, Gorontalo, Indonesia in April 2018. Molecular analysis of fish over sequencing methods shows that nike and hundala at Gorontalo waters are alleged as *Scyopterus longifilis*.

Keywords: Gorontalo, Local Fish Hundala, Nike, *Scyopterus longifilis*, *Scyopterus pagmans*

Introduction
Nike and hundala are the local names of fish found in the waters of Gorontalo, Indonesia where the scientific name of these two types of fish is not known. Biological and ecological information on both types of these species is also not yet available. These two types of fish are endemic in Gorontalo and have not been determined scientifically. A comprehensive scientific study of nike and hundala fish in Gorontalo waters is required to facilitate further research, especially those related to biocology in supporting the management and conservation of fish species in the world.
Nike is the local name of small fish found in the waters of the sea and estuary of Bone River, Gorontalo, Indonesia (Olli *et al.*, 2017). Nike fish in Gorontalo waters will only appear on a few days at the end of the lunar phase (Pasinggi and Abdallah, 2018) and move from the sea towards the estuary and disappear and then reappear in the final few days of the next phase of the month and at the beginning of the new month. Nike fish is thought to have transformed into adult fish during the time of movement to reach the river so that its form is no longer recognized.
Morphologically, hundala fish exhibit features that similar to nike. This led to the notion that the hundala fish was an adult nike that had reached the river to spawn. However, this hypothesis has not been scientifically proven. Therefore, it is necessary to study the comparison of nike and hundala. This study aims to identify the species of nike and hundala fish with a molecular approach.

Materials and Methods
Sampling
Nike and hundala specimens were collected from the estuary of Gorontalo Bay (N 00°30.305' and E 123°03.739') and Bone River (N 00°31.347' and E 123°04.358') in Gorontalo waters, Indonesia (Fig. 1) in April 2018. Samples were caught using a fish net.
Molecular Analysis
Genomic DNA was isolated from the muscle tissue for sequencing method using the *Genomic DNA mini kit (plano)*, Geneaid. Sequencing target was the cytochrome c oxidase subunit I (COI) gene due to DNA barcoding based on a fragment of the COI gene in the mitochondrial genome is widely applied in species identification and biodiversity studies (Binggeng *et al.*, 2018). The COI gene is recommended as the standard barcoding marker for most animals (Renwick *et al.*, 2018). Based on COI barcoding, high rates of species-level identification are well documented in many animal species, for instance, 95.27% for numerous northwestern pacific mollusks (Sun *et al.*, 2016) and 98% for marine fishes (Ward, 2009). In addition, many studies have shown that intraspecific variations of COI barcodes is generally pretty small and clearly discriminable from interspecific variation (Hubert *et al.*, 2008; Steink *et al.*, 2009; Ward *et al.*, 2009; Bucklin *et al.*, 2011).

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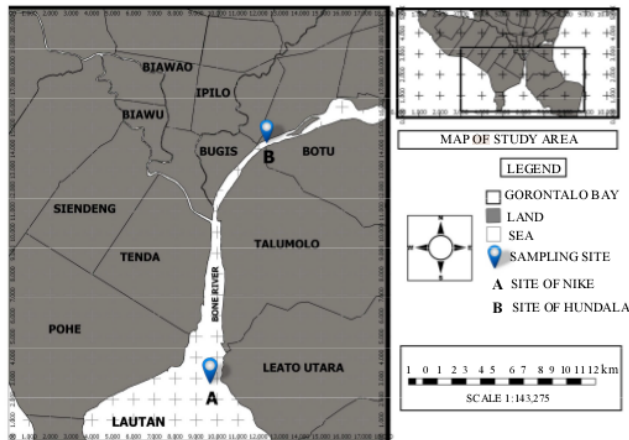


Fig. 1: Sampling sites of nike and hundala in Gorontalo Waters

The COI gene was amplified with primers Fish BCL: 5' TCAACYAATCAYAAAGATATYGGCAC-3' and Fish BCH: 5'- ACTTCYGGGTGRCCRAARAATCA-3' (Baldwin *et al.*, 2008) in a 50- μ L volume with 18 μ L ddH₂O, 2.5 μ L of Fish BCL (10 μ M), 2.5 μ L of Fish BCH (10 μ M), 1.0 unit of DMSO, 25 μ L of Go Taq Green PCR Mix 2X and 2 μ L of DNA template. The PCR conditions were pre-denaturation at 80°C for 10 s and initial denaturation at 94°C for 5 min, followed by 40 cycles of 30 s at 94°C, 30 s at 50°C, 45 s at 72°C and the final extension at 72°C for 7 min. The PCR products were visualized on 1.5% agarose gel.

The sequencing process was carried out using the Sanger Termination Dideoxy Method. The sequencing process was carried out by 1st Base, Malaysia, through PT. Genetic Science. The nucleotide sequence from DNA sequencing was carried out by CONTIG using the BioEdit application with manual editing and pruning during the alignment process. The Alignment data obtained was then matched with the data available on the Genbank, namely Barcode of Life Data (BOLD) system and National Center for Biotechnology Information (NCBI).

A phylogenetic tree was arranged by aligning DNA sequences of nike and hundala fish identified with several DNA sequences of species in the same family. The tree was made using the Maximum Likelihood Method with 1000 bootstraps. DNA sequences and phylogenetic tree compilation are aligned using MEGA 6.0 application with default settings.

Results

Genetic Identification of Nike and Hundala Fish

Genetic identification carried out to confirm genetics of nike and hundala fish in Bone River, Gorontalo.

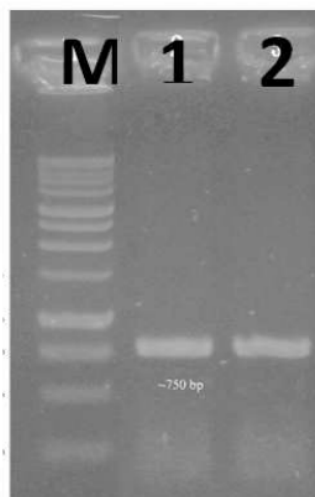


Fig. 2: Electrophoresis results of nike and hundala DNA (M= DNA ladder 100bp; 1= Hundala; 2= Nike)

Electrophoresis of mitochondrial DNA COI genes of nike and hundala fish is shown in Fig. 2, with amplicon lengths of ~ 600-700 bp.

CONTIG results of nucleotide sequencing of nike and hundala using Bioedit software were subsequently identified based on the BOLD system database on the www.boldsystems.org website, by selecting the database Species Level Barcode Records (3,170,080 Sequences/192,274 Species/77,605 Interim Species). The results of genetic identification comparison of nike and hundala to fish sequences in BOLD and NCBI database are presented in Table 1 and 2 respectively.

Nike and hundala are thought to be the same species as *Sicyopterus longifilis*. This is as based on BOLD database (Table 1) they have the highest similarity to *Sicyopterus longifilis* with the number of 100% and 99.68% consecutively. Whereas, according to NCBI database, the highest similarity value of merely 99% is indicated by nike and hundala fish as *Sicyopterus pugnans* (Table 2).

The phylogenetic tree also aligns some of the genetics of fish in the same genus available in BOLD which are also found in other areas (Fig. 3). Nike and hundala species have a very close relationship with the species *S. pugnans* caught in French, Polynesia.

Table 1: The comparison of nike and hundala sequences with BOLD fish database

No	Specimens	Species	Similarity	Data Status
1	Nike	<i>Sicyopterus longifilis</i>	100%	Private
2		<i>S. longifilis</i>	100%	Private
3		<i>S. longifilis</i>	100%	Private
4		<i>S. longifilis</i>	100%	Private
5		<i>S. longifilis</i>	100%	Private
1	Hundala	<i>S. longifilis</i>	99.68%	Private
2		<i>S. longifilis</i>	99.52%	Private
3		<i>S. longifilis</i>	99.52%	Private
4		<i>S. longifilis</i>	99.52%	Private
5		<i>S. longifilis</i>	99.52%	Private

Table 2: The comparison of nike and hundala sequences with NCBI fish database

No	Specimens	Species	Accession Number	Identity
1	Nike	<i>Sicyopterus pugnans</i>	KJ202204.1	99%
2		<i>S. pugnans</i>	KF668861.1	96%
3		<i>S. pugnans</i>	HQ639045.1	95%
4		<i>S. pugnans</i>	HQ639044.1	95%
5		<i>S. pugnans</i>	JQ432155.1	95%
1	Hundala	<i>S. pugnans</i>	KJ202204.1	99%
2		<i>S. pugnans</i>	KF668861.1	96%
3		<i>S. pugnans</i>	HQ639045.1	95%
4		<i>S. pugnans</i>	HQ639044.1	95%
5		<i>S. pugnans</i>	JQ432155.1	96%

Due to *S. longifilis* sequence data in BOLD database is still private and has not been permitted to be accessed, the study used fish sequences available in NCBI database to display nike and hundala fish relations to the other gobiidae (Fig. 4).

Figure 4 shows that nike and hundala fish are obviously separated from *S. pugnans*. On the other hand, nike and hundala share the highest similarity to each other. Unfortunately, the location of *S. longifilis* among nike and hundala fish cannot be displayed on the phylogenetic tree since this species is not available in NCBI database. In this case, although a more comprehensive analysis is needed to convince that they are characterized as the same fish, this allegation is still possible due to their nucleotide variation occurs only three times with base sequences of 97, 391 and 454 from the edited sequence along 650bp. As a result, the small p-distance of 0.0046 between the two samples supports that these two samples were the same species.

Morphological Characteristics of Nike and Hundala Fish

Nike and hundala are considered fish that have different stages of life. Nike is a juvenile while hundala is an adult stadium (Fig. 5), yet in this study, no detail morphological identification of nike and hundala species was carried out.

The morphological characteristic of nike is shown by the small body size which only reaches 3 cm and the body color is still transparent. The body has no scales, incomplete fins and an undeveloped mouth.

Hundala morphology showed a long body size reaching 6.5 cm compared to nike. Hundala has the dark body color and there were grayish spots on the dorsal part. Its body was scaly and the fin was complete with the blonde caudal fin color, flatted head shape, and the inferior mouth has developed perfectly.

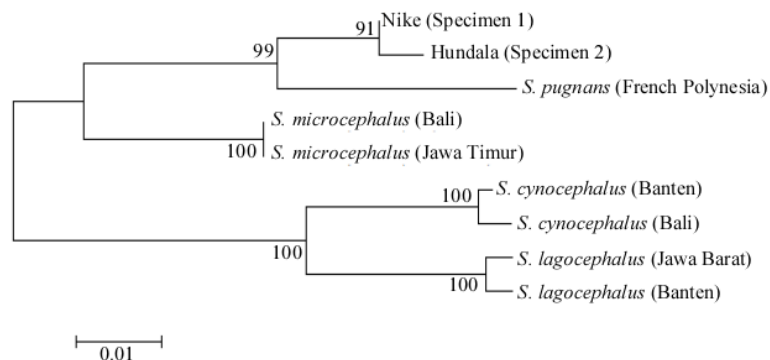


Fig. 3: Phylogenetic trees of nike and hundala compared to species found from other areas available in BOLD

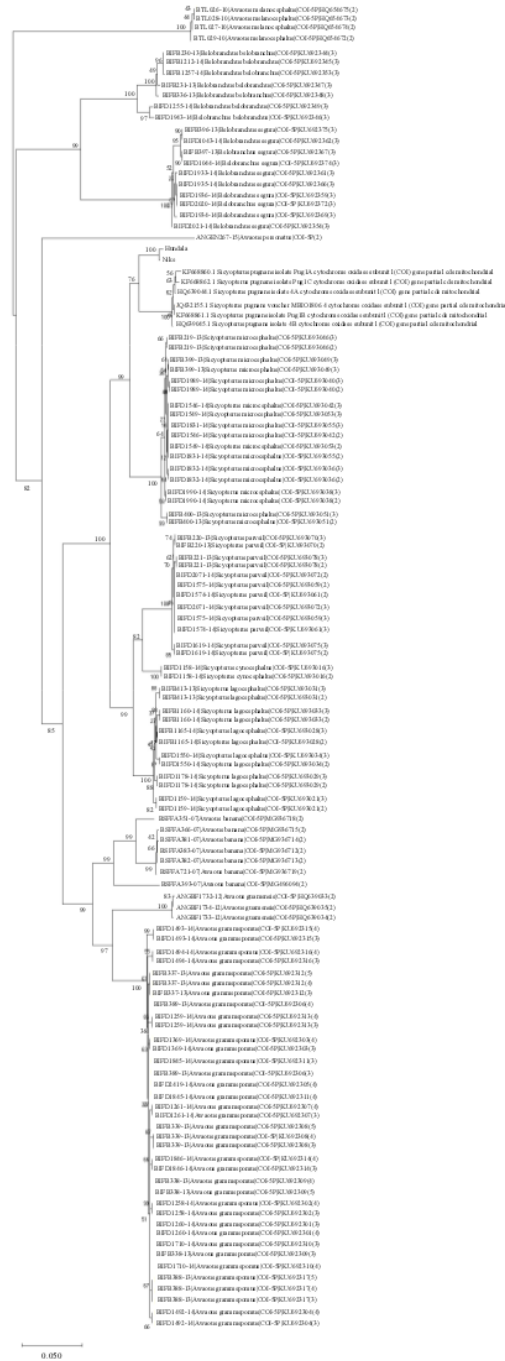


Fig. 4: Phylogenetic tree of nike and hundala compared to some gobiidae species available in NCBI



Fig. 5: Specimens of (a) nike and (b) hundala from Gorontalo Waters

Discussion

¹ This study shows that nike and hundala fish in Gorontalo waters are apparently as *Sicyopterus longifilis* species which is a freshwater fish type from the gobioid group based on their highest sequence similarity to *S. longifilis* in BOLD system. The species of *S. longifilis* originates from the Sicydiinae family (Wang *et al.*, 2001). Lord *et al.* (2010) stated that fresh water of the Pacific region is inhabited by Gobiidae (Sicydiinae) species with a specific life cycle adapted to the insular amphidromous called as the environment (Keith, 2003). In this life cycle, newly hatched fish drift downstream to the ocean, recruit back to stream mouths as juveniles and then migrate upstream to live as adults (Luton *et al.*, 2005).

The results of this study indicate that nike fish is the local designation of the Gorontalo community for juvenile phase, while the adult is hundala. Both are alleged as *Sicyopterus longifilis*. Keith *et al.* (2008) found that the morphological transformation of *Sicyopterus lagocephalus* between hatching and the adult phase is composed of two larval stages (L1, L2). In an adult stage, coloration is typical of sexual dimorphism. The mouth is inferior. The caudal fin distal border is rounded (% C superior to 100%). Adults are found in the lower, medium and upper courses of the river. Yamasaki *et al.* (2007) described briefly the developmental stages of another Sicydiine Gobiidae, a *Stiphodon*, using the terms "pelagic larvae", "settled larvae", "juvenile" and "adult".

Although scientific studies of *S. longifilis* species in Gorontalo waters are not yet available, Keith *et al.* (2011) described five new species of *Sicyopterus*, freshwater gobies, are described in Papua New Guinea and Papua Province, Indonesia. They differ from other species belonging to the genus by the combination of characters including the upper lip morphology, the second dorsal fin, the scales in the lateral, pre-dorsal, transverse back, transverse forward and zigzag series, sexual dimorphism and live colors.

Conclusion

From this study, we come to the conclusion that nike and hundala fish supposedly as *Sicyopterus longifilis* which are considered as species available in the Bone River and the estuary of Gorontalo Bay. Furthermore, a more comprehensive analysis of other divergent gene sequences is needed to convince this species characterization.

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Author's Contributions

Abdul Hafidz Olii: Coordinated the implementation of research work, conducted research, compiled the literary review, analyzed and interpreted the study findings, drew conclusions, contributed manuscript preparation and revisions ⁷

Femy M. Sahami: Designed the research plan, organized the study, conducted research, and contributed to result analysis and the writing manuscript.

Sri Nuryatin Hamzah: Conducted research, compiled the literary review, analyzed and interpreted the results, contributed manuscript revisions.

Nuralim Pasisingi: Conducted research, contributed manuscript revisions

Ethics

¹ This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and there are no ethical issues involved

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