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First Report on the Species Composition of the Nike Fish (*Gobiidae*) in the Coastal Waters of Bolaang Mongondow Raya, North Sulawesi, Indonesia

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ABSTRACT

The Nike fish (family *Gobiidae*) are ecologically and economically significant components of the coastal ecosystems in Bolaang Mongondow Raya, North Sulawesi Province. However, morphological identification of Nike fish species presents challenges due to high similarities in physical characteristics among species. This study aimed to accurately identify Nike fish species and evaluate their genetic diversity using a molecular approach, thereby providing insights into species composition and potential cryptic diversity in the region. The study was carried out between February and March 2025 at the Laboratory of Fisheries and Marine Science, UNSRAT Manado, and the Indonesian Biodiversity Laboratory (BIONESIA), Bali. A molecular identification technique was employed using DNA barcoding targeting the cytochrome oxidase subunit I (COI) gene. Specimens collected from each sampling site were labeled as BO1, BO2, BO3 (Bolaang Mongondow District) and BMU1, BMU2, BMU3 (North Bolaang Mongondow). The COI gene fragments obtained were 655 base pairs in length. BLAST analysis of the sequences revealed that the six samples belong to three species: *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, and *Awaous ocellaris*, with similarity levels ranging from 99.55 to 100%. These findings confirm the presence of at least three genetically distinct Nike fish species in the coastal waters of Bolaang Mongondow Raya. The results contribute to a better understanding of local goby biodiversity and highlight the importance of incorporating molecular tools into fisheries management and conservation strategies for sustaining this valuable resource.

INTRODUCTION

Bolaang Mongondow Raya, which includes Bolaang Mongondow Regency and North Bolaang Mongondow Regency in North Sulawesi Province, is a region known for its significant fisheries potential. One of the most notable seasonal catches in this area is the Gobiidae species locally referred to as "nike," which appears in large numbers during the new moon phase. According to **Sahami (2019)**, this lunar-associated phenomenon has long been observed and utilized by local fishers based on traditional ecological knowledge.

Nike holds both cultural and nutritional significance. Nutritional analysis reveals that it contains 79.76% water, 16.89% protein, 0.30% carbohydrates, and 0.76% fat. It is also rich in essential amino acids such as leucine (1.153%) and lysine (0.843%), as well as beneficial fatty acids, including docosahexaenoic acid (DHA) at 14.81%, oleic acid at 8.50%, and eicosapentaenoic acid (EPA) at 2.22%. In addition, nike is a good source of important minerals like magnesium and calcium, which are crucial for bone development and the prevention of anemia **(Arisanti, 2017)**.

As a renewable biological resource, nike—like other fishery species—can regenerate its population through reproduction if harvest levels are managed sustainably **(Nikijuluw, 2002; Imran & Yamao, 2014)**. Therefore, effective resource management requires a comprehensive understanding that integrates biological, social, economic, and technological factors **(Syakila, 2009)**.

Several studies on nike have been conducted in other regions, such as Gorontalo Bay **(Sahami *et al.*, 2019a; 2020; 2024)**, the estuaries of the Lako Akelamo and Tiabo Rivers in Galela Bay **(Kader *et al.*, 2023)**, Lake Tondano **(Pangemanan *et al.*, 2020a)**, and the Poigar River Estuary **(Pangemanan *et al.*, 2020b; 2024)**. However, these studies are geographically limited and do not cover the Bolaang Mongondow Raya region, where Nike fish is also heavily exploited.

Despite high exploitation levels, there is currently no comprehensive published data on species composition, catch volume, or the spatial-temporal occurrence of nike in Bolaang Mongondow Raya. Preliminary field observations and interviews with local fishers indicate that key information on catch timing, location, and quantity remains undocumented. Moreover, no studies have yet applied DNA barcoding in this region. Given the morphological similarity among nike individuals, visual identification is often unreliable. DNA barcoding offers a precise and reliable method for species-level identification and is increasingly used in fishery and biodiversity research.

This study hypothesizes that the Nike fish in Bolaang Mongondow Raya consists of more than one species within the Gobiidae family, which can be genetically distinguished using DNA barcoding techniques. The findings are expected to fill critical data gaps and to provide a scientific foundation for the sustainable management of this valuable fishery resource.

MATERIALS AND METHODS

1. Specimen collection and handling

This study was conducted using a survey method and a simple random sampling technique, where each specimen had an equal chance of being selected. This approach aimed to obtain a representative and easily applicable sample **(Notoatmodjo, 2002)**. The Nike fish samples were collected once during their emergence period in each research location (Fig. 1). The study areas were located in two regions: Bolaang Mongondow Regency and North Bolaang Mongondow Regency, North Sulawesi Province. Sampling was conducted during the peak occurrence of the nike fish, which coincides with the new moon phase, in February and March 2025. This time frame was selected to reflect the seasonal emergence pattern of the nike and to enhance the ecological relevance of the data collected.

Collected specimens were placed in cool boxes with crushed ice and subsequently frozen to prevent tissue degradation before being sent to the Indonesian Biodiversity Laboratory (BIONESIA) in Bali. A total of 25 individuals were randomly selected from each location based on their melanophore patterns. Each sample was placed in a plastic container for melanophore pattern identification, morphological observation, and molecular phylogenetic analysis, resulting in six containers in total.

Melanophore patterns were analyzed using images captured with a Canon 7D camera equipped with an EF 100 mm f/2 BL Image Stabilizer USM macro lens. Morphometric analysis was performed on a total of 150 individuals at the Fisheries Science and Marine Science Laboratory, Faculty of Fisheries and Marine Science, UNSRAT Manado.

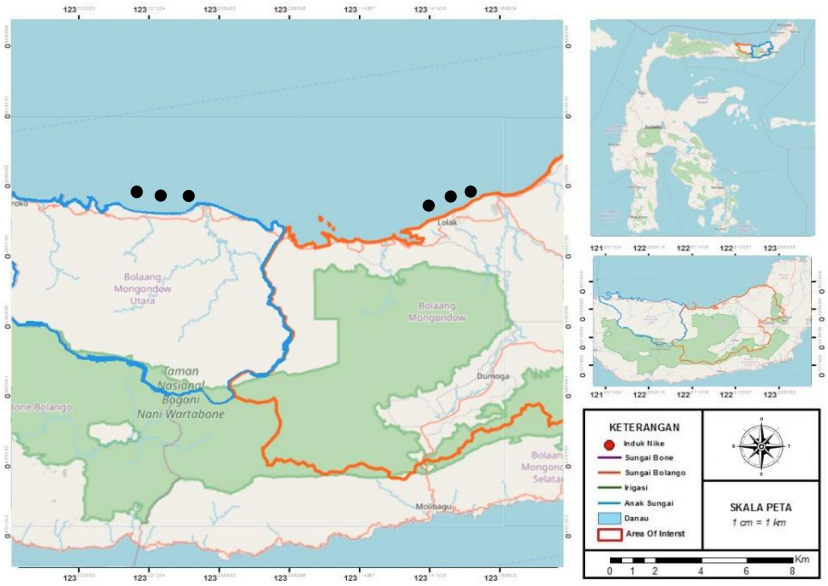

Fig. 1. Map of sample collection locations, indicated by black dots along the coastal waters of Bolaang Mongondow and North Bolaang Mongondow

2. Molecular analysis and DNA barcoding

For molecular analysis using the DNA barcoding approach, five individuals from each sampling site were selected based on similar pigmentation/melanophore patterns. These samples were preserved in 70% ethanol, resulting in a total of 30 individuals. All molecular analyses were conducted at the Indonesian Biodiversity Laboratory (BIONESIA) in Bali. Samples were labeled as BO1, BO2, and BO3 (Bolaang Mongondow Distrck) and BMU1, BMU2, and BMU3 (North Bolaang Mongondow). The mitochondrial cytochrome oxidase I (COI) gene was selected as the genetic marker for species identification in this study. COI is widely recognized as the standard barcode region for animals due to its appropriate mutation rate, which enables accurate species-level discrimination, particularly among closely related taxa. Furthermore, COI has extensive reference data available in global databases such as GenBank and BOLD, facilitating reliable sequence matching and identification. Compared to other mitochondrial markers such as 16S rRNA, which is more conserved, or cytochrome b (cytb), which may show high intraspecific variation, COI offers an optimal balance of universality and resolution for fish molecular taxonomy.

3. DNA barcoding approach

DNA barcoding is now widely used as a global biological identification system for animals. Taxonomic ambiguities are still frequently found in several fish genera/species, and accurate identification is crucial for management and trade. Utilizing DNA sequence variation among species as a DNA-based approach for taxonomic diagnosis can be used to identify fish and resolve taxonomic ambiguities, including the discovery of new or cryptic species **(Hebert *et al.*, 2003)**. This barcoding system relies on sequence variation within a specific gene region, namely a portion of the mitochondrial cytochrome oxidase I (COI) gene. New products and specimens can be identified by comparing their DNA barcode sequences with an established reference library **(Taufani *et al*., 2023)**. According to this method, the COI sequences obtained from morphologically identified species can serve as DNA barcode references for those species. Based on sequence similarity, these reference barcodes can then be used to identify previously unidentified specimens **(Gangan & Pavan-Kumar, 2019)**. The Nike fish (Gobiidae) samples, previously stored in frozen condition, were then used for DNA extraction. This process began with the collection of white muscle tissue as the primary material. DNA extraction was conducted using the Chelex method, with the following steps:

- Approximately 10 grams of the Nike fish muscle tissue were placed into a 1.5ml microtube containing 10% Chelex solution.
- The mixture of tissue and Chelex was heated at 95°C for 45 minutes. After heating, the mixture was homogenized using a vortex mixer for 20 minutes to ensure thorough mixing.
- The solution from the previous step was centrifuged at 2000 rpm for 10 minutes.
- The resulting supernatant was then collected and stored at -4°C **(Pringgenis & Susilowati, 2016; Taufani *et al.*, 2023)**. The quality and concentration of the extracted DNA were analyzed using a nanodrop spectrophotometer, with reference to the A260/280 absorbance ratio, which should ideally fall between 1.8 and 2.0.

For molecular analysis, the cytochrome oxidase I (COI) gene marker was used, targeting a gene fragment of approximately 600–700 base pairs (bp) in length **(Fahmi *et al.*, 2020)**. DNA amplification was conducted using PCR, following the protocol of the BIONESIA laboratory. The primers used were Fish F1 and Fish R1. The term "Fish" indicates that these primers are specifically designed for fish DNA amplification. “F1” refers to the first forward primer, which binds to one strand of the DNA and extends in the 3’ direction. The nucleotide sequence of the primer is read from the 5’ to the 3’ end, with each letter representing a nitrogenous base: T = Thymine, C = Cytosine, A = Adenine, and G = Guanine. “R1” refers to the reverse primer, which binds to the complementary DNA strand and extends in the opposite direction from Fish F1, also from 5’ to 3’ **(Ward *et al.*,**

2005). During the genetic amplification stage, the PCR reaction mixture (commonly referred to as the PCR cocktail) was composed of several components: 12.5µL of GoTaq® Green Master Mix (Promega), 1µL each of the Fish F1 and Fish R1 primers, 1µL of DNA template, and 9.5µL of nuclease-free water, resulting in a total reaction volume of 25µL. PCR amplification was carried out using an Applied Biosystems™ 2720 Thermal Cycler. The temperature settings followed these steps: an initial denaturation at 94°C for 3 minutes, followed by 38 cycles of denaturation, annealing, and extension at 94°C, 50°C, and 72°C respectively, with each step lasting 30 seconds, 30 seconds, and 60 seconds. The process concluded with a final extension step at 72°C for 2 minutes, in accordance with the BIONESIA laboratory protocol. The resulting PCR products were then analyzed using 1% agarose gel electrophoresis with GelRed® staining to detect the presence of DNA bands. Samples that produced positive results, indicated by the appearance of fluorescent bands on the gel, proceeded to the DNA sequencing stage. The sequencing method used was Sanger sequencing with a single-pass approach, and the analysis was conducted at PT. Genetika Science, Jakarta.

4. BLAST homology and phylogenetic tree

After the sequencing process was completed, the data in Ab1 file format were analyzed using computer software. The obtained DNA base sequences were then edited and aligned using the ClustalW method in MEGA XI software. At this stage, the forward and reverse primer amplification results were merged to form a single consensus sequence. Each base was manually checked to ensure data quality. If any low-quality data were found, the PCR and sequencing processes were repeated to obtain better results. Once valid sequences were obtained, the next step was to compare the sequences with data available in the GenBank database of the National Center for Biotechnology Information (NCBI) using sequence homology matching tools. Species with the highest similarity scores were selected as references for further analysis and phylogenetic tree construction. The identity values and sequence match data were recorded for each sample. In addition to the BLAST analysis, the data were also analyzed through the construction of a phylogenetic tree to examine the evolutionary relationships among individuals or species and to confirm the species identification results from the BLAST analysis. The tree was constructed using the Neighbor-Joining (NJ) method with 1000 bootstrap replications, following the method described by **Ayuningrum *et al*. (2019)**, and generated using MEGA XI software.

RESULTS AND DISCUSSION

1. Morphological identification of Gobi fish

The Nike fish (*Gobiidae*) are generally small, scaleless fish commonly found in coastal areas and river estuaries, with body lengths ranging from 2 to 4cm. Their

morphology is characterized by a rounded caudal fin that is not connected to the anal fin, a single dorsal fin, and the absence of scales. According to **Andriyani (2018)**, the Nike fish have a fusiform (torpedo-shaped), transparent body with brown stripes along the sides. They possess a terminal mouth position and have pectoral, caudal, pelvic, and anal fins. The morphological features of nike fish collected from the coastal areas of Bolaang Mongondow Raya showed general similarities with the typical characteristics of this species. Based on morphological identification (Fig. 2), the specimens from these locations did not exhibit significant differences compared to previously established morphological descriptions.

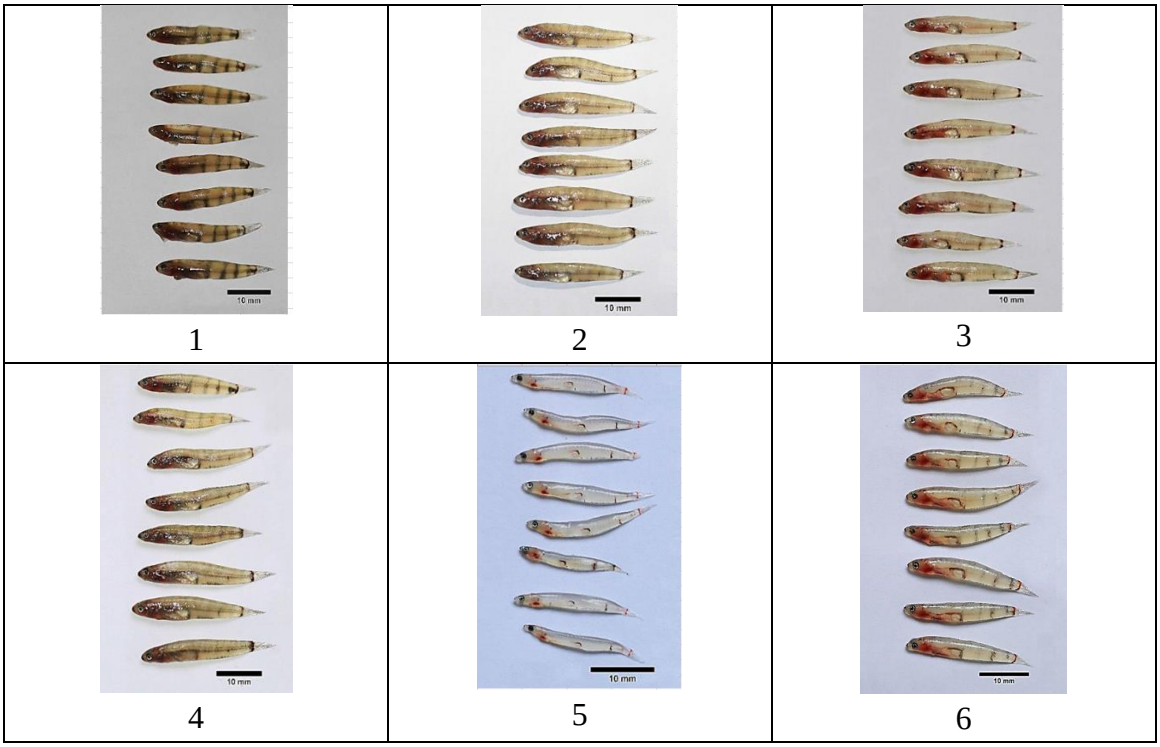

Fig. 2. Morphological identification of the Nike fish (Gobiidae) samples from the coastal waters of Bolaang Mongondow Raya. (1: BO1, 2: BO2, 3: BO3, 4: BMU1, 5: BMU2, 6: BMU3)

2. DNA barcoding approach for Nike fish

Molecular identification using DNA barcoding was conducted by amplifying the mitochondrial COI gene using Fish F1 and Fish R1 primers. Gel electrophoresis confirmed the successful amplification of a 655 bp fragment for all six samples (Fig. 3), which were subsequently sequenced.

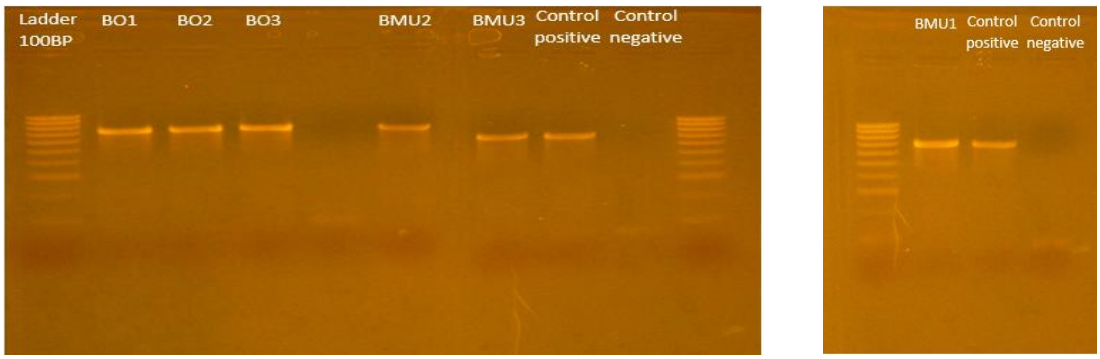

Fig. 3. Agarose gel electrophoresis results showing COI gene bands (655 bp) from the Nike fish (Gobiidae) samples in Bolaang Mongondow Raya

BLAST alignment of the resulting sequences revealed high similarity (99.55–100%) to known Gobiidae species in GenBank, indicating accurate species identification (Table 1). These include *Sicyopterus cynocephalus*, *Sicyopterus pugnans,* and *Awaous ocellaris*.

Table 1. BLAST results of six Nike fish (Gobiidae) samples using Fish F1/Fish R1 primers and COI gene sequences

Field ID	Lab ID	Species	bp	Gene	Accession Number	Query Cover (%)	Identity (%)
BO1	BIOSUB314.001	*Sicyopterus pugnans*	655	COI	MT227831.1	100	100
BO2	BIOSUB314.002	*Sicyopterus cynocephalus*	655		MT706640.1	100	99.85
BO3	BIOSUB314.003	*Sicyopterus cynocephalus*	655		MT706640.1	100	99.55
BMU1	BIOSUB314.004	*Sicyopterus cynocephalus*	655		MT706640.1	100	99.70
BMU2	BIOSUB314.005	*Awaous ocellaris*	655		OQ152445.1	100	99.85
BMU3	BIOSUB314.006	*Sicyopterus pugnans*	655		MT227831.1	100	100

The DNA sequence similarity levels of the analyzed Nike fish (Gobi) samples showed high results ranging from 99.55 to 100% when compared to reference data available in GenBank. This indicates that the specimens have a strong genetic similarity with previously identified species. **Sofro (1994)** stated that individuals from populations with close kinship relationships usually exhibit high similarity in both morphological and genetic aspects, where environmental factors also influence this similarity. On the other hand, genetic mutations are one of the main causes of genetic diversity and play an important role in the process of new species formation. The phylogenetic relationship analysis of Nike fish (*Gobi*) was carried out using the neighbor-joining method with 1000 bootstrap replications to investigate the evolutionary history and genetic relationships among specimens. Specimens with similar DNA sequences were grouped into the same phylogenetic branch, forming a clade. Based on the phylogenetic tree (Fig, 4), the six local

samples were distributed into two major sub-clades rather than clustering strictly by their geographic collection sites. Specifically, BMU1 (*BIOSUB314 004*) showed a close relationship with BO2 (*BIOSUB314 002*) and BO3 (*BIOSUB314 003*), forming a distinct clade along with the reference species *Sicyopterus cynocephalus* (MT706640.1), supported by a high bootstrap value of 99%. BMU3 (*BIOSUB314 006*) and BO1 (*BIOSUB314 001*) were grouped with *Sicyopterus pugnans* reference sequences (MT227831.1 and KJ202204.1) with a strong bootstrap value of 100%, indicating clear species-level similarity. Interestingly, BMU2 (*BIOSUB314 005*) did not cluster with the other local samples but was instead grouped with *Awaous ocellaris* (OQ152445.1) with 96% bootstrap support, suggesting potential species-level divergence or misidentification.

These results indicate that although the nike fish samples are morphologically similar, genetically they may belong to distinct species or lineages. This pattern supports the presence of cryptic diversity among nike fish populations and highlights the value of mtDNA analysis, particularly COI gene sequencing, in revealing population structure, kinship patterns, and possible evolutionary isolation, as also emphasized by **Avise (1994)**. A comparison of six *cytochrome oxidase subunit I* (COI) gene sequences from nike fish samples collected from the coastal waters of Bolaang Mongondow Raya with eight nike fish sequences available in GenBank resulted in a phylogenetic tree. This tree was constructed using the neighbor-joining (NJ) method with 1000 bootstrap replications, using the MEGA XI software (Fig. 4). Based on the analysis, the six local samples did not cluster according to their geographic migration locations but were instead distributed into two major sub-clades (Fig. 4).

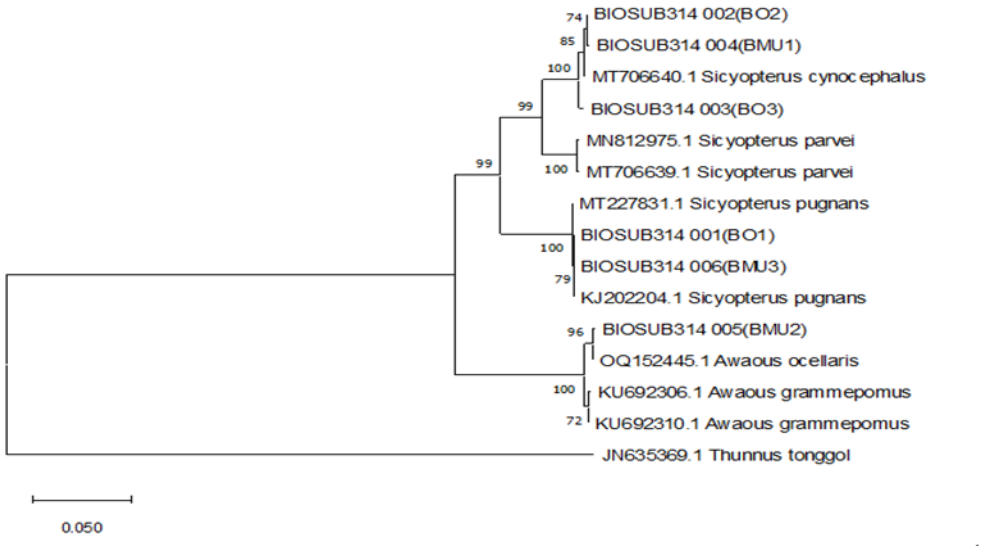

Fig. 4. Phylogenetic tree of three Nike fish species identified based on electrophoresis analysis using FISH-F1/FISH-R1 primers

There are two major groups of Nike fish (Gobiidae) identified in this study, namely the genus *Sicyopterus* and the genus *Awaous*. The *Sicyopterus* group is further divided into subgroups including *S. cynocephalus*, *S. pugnans*, and other related species. To improve the robustness of the phylogenetic analysis and to address the possibility of closely related species not detected by BLAST, additional reference sequences from related *Sicyopterus* species such as *Sicyopterus longifilis*, *Sicyopterus lagocephalus*, and other previously reported Nike fish sequences available in GenBank were included in the analysis. This broader dataset revealed that the *Sicyopterus* genus consists of several distinct populations and clarified the relationships among local samples and known species. For instance, although sequence BO3 differed from BO2 and BMU1 and was placed on a different branch, all clustered within the clade related to *Sicyopterus parvey*. The Nike fish from the genus *Sicyopterus*, represented by sequences BO1 and BMU3, were identified as *S. pugnans* and found in the waters of Bolaang Mongondow and Bolaang Mongondow Utara. The other group consisted of the genus *Awaous*, with sequence BMU2 from Bolaang Mongondow Utara identified as *A. ocellaris*. This comprehensive analysis supports the genetic diversity within the nike fish populations and highlights the importance of including diverse reference sequences to avoid potential misidentification due to BLAST limitations.

It is important to note, however, that the phylogenetic analysis in this study used a limited number of reference sequences from GenBank. Other species such as *Sicyopterus longifilis*, *Sicyopterus lagocephalus*, and previously reported Nike fish species available in GenBank were not included. Incorporating these sequences in future analyses may provide a more complete picture and help resolve potential misidentifications due to BLAST limitations, as BLAST results are not always definitive and can be affected by incomplete or erroneous database entries. Overall, in the coastal waters of Bolaang Mongondow Raya, particularly in the regions of Bolaang Mongondow Regency and Bolaang Mongondow Utara Regency, three different nike fish species were identified, as presented in Table (1). The most dominant species based on occurrence was *Sicyopterus cynocephalus*, which was found in three out of six samples and across all sampling locations. In contrast, *Sicyopterus pugnans* and *Awaous ocellaris* were each found in one to two samples, indicating lower relative frequency. Preliminary observations also suggest a possible relationship between substrate type and species distribution: *S. pugnans* and *A. ocellaris* tended to occur in areas with sandy or muddy substrates, whereas *S. cynocephalus* was more broadly distributed across various habitat types. Further habitat analysis is recommended to confirm these patterns.

Our findings are consistent with previous studies conducted in other regions of Sulawesi, particularly Gorontalo Bay, where *Sicyopterus pugnans* and *S. cynocephalus* were also identified as dominant species within nike fish assemblages **(Sahami *et al.*, 2019)**. Subsequent research by **Sahami *et al*. (2020)** uncovered even greater cryptic

diversity in the same area, identifying up to six species, including *S. longifilis*, *S. parvei*, and *S. lagocephalus*. These results suggest that the occurrence of multi-species Nike fish communities may be a widespread phenomenon in northern Sulawesi. Moreover, studies on larval dispersal patterns in the Bone River estuary **(Olii *et al*., 2017)** demonstrate that amphidromous gobies undergo seasonal migrations driven by hydrological cycles, allowing for interpopulation mixing across geographically separate coastal areas. Together, these comparative insights strengthen the evidence for cryptic diversity and highlight the complex dispersal and evolutionary dynamics of nike fish populations. These patterns are reflected in our phylogenetic and DNA barcoding results from the coastal waters of Bolaang Mongondow Raya.

The coastal waters of North Bolaang Mongondow showed the highest species richness of the Nike fish (*Gobiidae*) among all surveyed sites, with three species identified: *Sicyopterus cynocephalus*, *Sicyopus pugnans*, and *Awaous ocellaris*. The close proximity of sampling locations allows for easier genetic exchange and interaction among species. According to **Rizal *et al.* (2015)**, the proximity of these habitats may facilitate gene flow and interspecific interactions. However, genetic differences observed among individuals suggest that geographic proximity alone does not fully explain the observed patterns. ARLINDO plays a crucial role in larval transport, yet its influence is modulated by regional oceanographic conditions. **Atmadipoera and Mubaraq (2016)** reported that ARLINDO in the Sulawesi Sea exhibits multilayer current structures and significant seasonal variability due to monsoonal wind forcing. **Atmadipoera *et al.* (2015)** also highlighted spatial heterogeneity of current velocity and direction in the Makassar Strait, a key channel of ARLINDO, which can shape larval trajectories and retention zones.

Furthermore, mesoscale eddies, internal waves, and salinity-driven stratification documented in this region **(Atmadipoera, 2017, 2018)** can act as oceanographic barriers or facilitators depending on their strength and persistence. These physical features, combined with the complex coastal geomorphology, may limit gene flow across short distances or isolate certain populations during critical larval stages. The interaction between hydrodynamic factors and biological traits may thus contribute to the observed genetic differentiation of nike fish populations. Similar mechanisms of isolation by oceanography have been reported in amphidromous goby populations in other tropical island systems **(Crandall *et al.*, 2010)**. Therefore, integrating oceanographic parameters such as current dynamics, salinity gradients, and larval behavior is essential for a comprehensive understanding of population connectivity. Future efforts should involve seascape genetics approaches to guide sustainable management of the Nike fisheries in North Sulawesi and surrounding waters.

This phenomenon allows for genetic exchange between populations of the Nike fish in the waters of Bolaang Mongondow Raya and other regions, as the fish move along ocean

currents in search of food sources. According to **Tomascik *et al*. (1997)**, water masses from the Indonesian Throughflow (ARLINDO) can mix with local waters along its path through Indonesia, causing interactions between two different oceanic water systems. As part of a major ocean current route, this coastal area is also known to be rich in essential nutrients that support the development of phytoplankton, which are a key component of the marine food chain in this productive ecosystem. Given its high ecological productivity, this region should be prioritized for ecosystem-based resource management to ensure that fisheries activities remain sustainable and do not disrupt the food web or lead to overexploitation. Conservation strategies, such as seasonal closures or marine protected areas, may be necessary to preserve the ecological integrity and long-term availability of marine resources.

CONCLUSION

Based on DNA barcoding analysis, three Nike fish species were identified in the coastal waters of Bolaang Mongondow Raya: *Sicyopterus cynocephalus*, *Sicyopterus pugnans*, and *Awaous ocellaris*, with similarity values between 99.55% and 100%. This finding enhances the taxonomic resolution of *gobiid* diversity in a region characterized by dynamic coastal oceanography. From a conservation standpoint, recognizing these species supports targeted protection efforts, especially for populations that may be less abundant or ecologically sensitive. Moreover, accurate species identification is essential for sustainable fisheries management, enabling species-specific monitoring, regulation of harvesting, and informed policy development. Future research should integrate continuous genetic monitoring and ecological assessments to guide ecosystem-based management strategies that promote both biodiversity preservation and long-term fisheries productivity in the region.

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