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Are *Awaous ocellaris* and *Belobranchus belobranchus* the two species of Nike fish schools ?

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ABSTRACT

Investigating goby fish is vital to perform an integrated and comprehensive study in order to maintain the roles of the fish, thus providing balanced ecosystem functions and services, as well as contributing to fish biodiversity. Local societies simply recognize fish species by their local names, which are not common. This condition, in turn, causes hitches in conducting further studies. Nike, the name of a local fish, refers to the schools of goby fish larvae whose adult phase has not been fully confirmed. This study aimed to reveal the species that categorizes as nike fish through tracing adult goby inhabiting freshwater. Two fish samples, i.e., Unknown 01 and Unknown 02, were taken from two sites in Bone River, Gorontalo, Indonesia. These samples were captured purposively using a hand net by considering the morphological similarity between the two target samples and the general characteristic of goby. Furthermore, the samples were analyzed genetically through the PCR sequencing method using the Mitochondrial Cytochrome Oxidase Subunit 1 (CO1) gene. Based on the NCBI database, Unknown 01 had the highest similarity to *Belobranchus belobranchus* (99.54%), while Unknown 02 was identical with *Awaous ocellaris* (100%). Unknown 01 and Unknown 02, compared to the BOLD database, the similarity level, had the highest percentage of similarity with *B. belobranchus* (99.85%) and *A. ocellaris* (100%), respectively. Therefore, *A. ocellaris* and *B. belobranchus* were strongly alleged as two species making up the goby schools in the adult stadia that reach freshwater during their migration.

Introduction

Goby fish plays a role in ecosystem functions, services, and it contributes to ichthyofauna biodiversity. This fish has a wide geographical distribution and a high number of species. Several goby species considered to be endemic (Munoz-Arroyo *et al.*, 2020; Nitta and Nagasawa, 2020; Patimar *et al.*, 2020), while some are recognized as invasive species (Holmes *et al.*, 2019; Higgs *et al.*, 2020; Liang *et al.*, 2020; Qin *et al.*, 2020). Conservation challenges currently face the role and status of goby fish as the data are inadequate. Sadeghi *et al.* (2017) stated that due to their cryptic nature, small size, and lack of immediate economic importance, the information regarding gobies are insufficient. To date, along with the present study, additional goby species continue to be updated and recorded in particular waters (Larson *et al.*, 2020; Suzuki *et al.*, 2020). Gobies are famous as amphidromous species. According to Kaiser (2020), there is a goby species classified as partially amphidromous. Therefore, investigating goby fish is vital to performing a thorough study to prevent the species from being extinct.

Information regarding local goby fish species in Gorontalo, Indonesia, has not provided, while the exploitation of the resources continues. Goby species distributed in Gorontalo waters vary as the aquatic area in the province is as part of the tropical zone with its high biodiversity. Miesen *et al.* (2016) stated that Gobiidae was dominating ichthyofauna in the inland waters of Sulawesi. Nonetheless, local societies merely recognize fish species by its local names, which are not common, and it causes hitches

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in conducting further scientific studies. Initially, nike fish was referred to as the endemic fish of Gorontalo due to its unique characteristics, i.e., appearing in a group only at the beginning of the new moon period (Pasisingi and Abdullah, 2018). At present, the number of species of nike schools is unknown. Hence, the tracing of nike fish speciation is currently being studied continually through meristic, morphometric, and genetic approaches. The previous studies (Yusuf, 2011; Salam *et al.*, 2016; Olii *et al.*, 2017) regards nike fish as *Awaous melanocephalus*. Additionally, Olii *et al.* (2019) find that nike fish as *Sicyopterus longifilis*. Olii *et al.* (2019) has discovered a nike species *S. longifilis* in Gorontalo Sea in its larval stage as well as in Bone River in the adult phase. Recently, Sahami *et al.* (2019) reveals that nike fish is made up of *S. pugnans*, *S. cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*.

Recent research shows that nike fish migrates from seawater to freshwater, and vice versa. However, there has been no specific scientific study revealing that nike fish migrates for reproductive purposes. Pasisingi *et al.* (2020) considers nike fish as the schools of amphidromous goby fish larvae found in Gorontalo Waters, Indonesia migrating from Gorontalo Bay area to Bone River that matures in the freshwater. However, limitations in tracking the migration of the fish larvae as they move along the waters cause doubt in concluding that all nike fish have managed to reach river water. On the other hand, all adult goby inhabiting Bone River has not been recorded. Therefore, the study result will support the hypothesis that the goby species found in Bone River is the adult of species composing nike schools. With the assumption that the goby found in Bone River are nike that grow and mature in the freshwater, this study aims to reveal the species that make up nike fish through tracing adult goby dwelling freshwater.

Materials and Methods

Study Site

The sampling was conducted in April 2018, at the beginning of the Hijr month, with the assumption that nike fish migration area had entered freshwater in this period. The samples were taken from Bone River, Gorontalo at Site 1 (N 00°31.347'; E 123°04.358') and Site 2 (N 00°30.305'; E 123°03.739') (Figure 1).

Sampling technique

The determination of sampling points was assisted by the local community who were familiar with the general morphology of the fish that they frequently find in the river. Two samples of adult goby fish, i.e., Unknown 01 and Unknown 02, were captured purposively from the waters using a hand net. This process took into account the morphological similarity between the two target samples, generally fish with large heads and tapered bodies. All samples were then stored in 70% alcohol for genetic analysis.

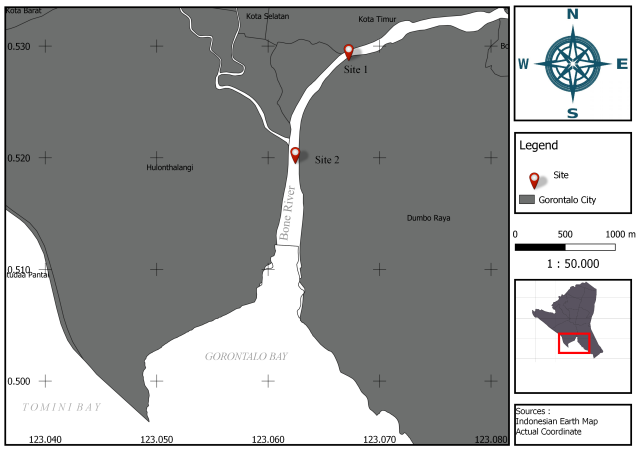

Figure 1. Fish sampling location (unknown 01 specimen taken from Site 1, and unknown 02 specimen taken from Site 2)

Genetic sample analysis

Isolation of the DNA genome was carried out using a Genomic DNA Mini Kit (Tissue) (Geneaid) isolation kit following the standard product protocols. The samples were analyzed genetically through the sequencing method using the CO1 gene. Polymerase Chain Reaction (PCR) analysis used primer pairs as (forward) Fish BCL: 5'-TCAACYAATCAYAAAGATATYGGCAC-3' and (Reverse) Fish BCH: 5'-ACTTCYGGGTGRCCRAARAATCA-3' [2]. The thermocycler temperature profiles were predenaturation at 94 °C for 5 minutes, denaturation at 94 °C for 30 seconds, annealing at 50 °C for 30 seconds, and elongation at 72 °C for 45 seconds. The PCR amplification process was repeated in 40 cycles; it ended with a final elongation at 72 °C for 7 minutes. Moreover, the PCR results were electrophoresed, and the visualization of the DNA band was performed using a UV transilluminator. The sequencing was processed using the Dideoxy Sanger Termination Method at 1st Base Malaysia through PT Genetika Science Indonesia.

Data Analysis

Sequencing data editing and checking were performed using MEGA5 software. The CONTIG data were matched with the genetic data available in the NCBI and BOLD databases. Phylogenetic trees

were created using MEGA5 software with the Maximum Likelihood method to see the kinship between species.

Results

Based on NCBI database, Unknown 01 has the highest similarity to *Belobranchus belobranchus* (Figure 1a, 1c), while Unknown 02 has that of *Awaous ocellaris* (Figure 1b, 1d). Likewise, the similarity level of Unknown 01 and Unknown 02 has the highest percentage with *B. belobranchus* and *A. ocellaris,* respectively, compared to BOLD database (Table 1). The phylogenetic tree aligns the two goby fish samples with several genetic fish available in NCBI (Figure 2) and BOLD databases (Figure 3). The trees specify the Unknown 01 species as *B. belobranchus* and Unknown 02 as *A. ocellaris*.

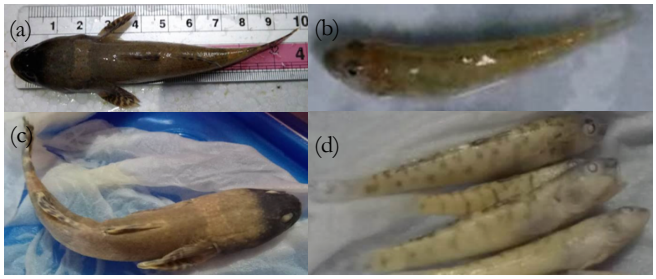

Figure 1. (a) Fresh *Belobrachus belobranchus*, (b) Fresh *Awaous ocellaris*, (c) Preserved *B. belobranchus*, (d) Preserved *A. ocellaris*

Table 1. The comparison of the result sequences based on NCBI and BOLD databases

Specimens	Species	Similarity (%)	
		NCBI	BOLD
Unknown 01	*Belobranchus belobranchus*	99.54	99.85
Unknown 02	*Awaous ocellaris*	100	100

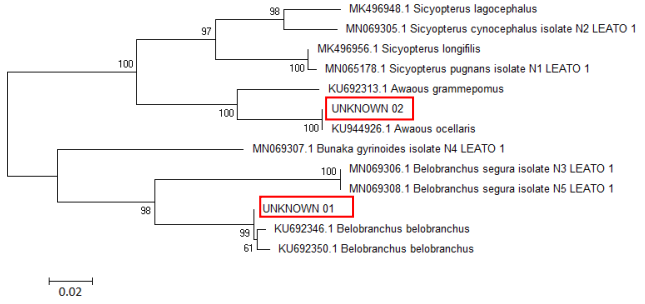

Figure 2. Phylogenetic tree of the adult goby fish samples compared to some Gobiidae species available in NCBI database

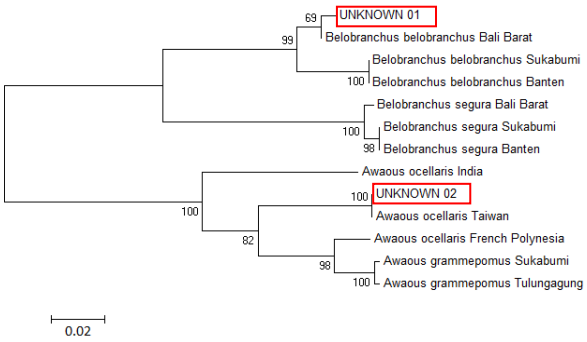

Figure 3. Phylogenetic tree of the adult goby fish samples compared to some Gobiidae species available in BOLD database

Discussion

Investigation of other goby fish as an adult nike fish is needed to ease further scientific studies of this fish for sustainability management purposes. Studies regarding the discovery of several new species of goby fish in Indonesia (Keith *et al.*, 2011; Keith *et al.*, 2012; Keith, Hadiaty, Busson, *et al.*, 2014; Keith, Hadiaty, Hubert, *et al.*, 2014; Keith *et al.*, 2015) as well as in other areas (Keith *et al.*, 2011; Mennesson *et al.*, 2016; Keith *et al.*, 2019) indicate that goby fish distribution is not only broad but also holds potential as high species diversity. Gani *et al.* (2019) mentions that exploration of the species diversity of freshwater fishes, in particular gobies, contributes to the knowledge on the species richness of Indonesian ichthyofauna. The study has the potential to discover and to improve awareness regarding the dispersal of new and known species.

Gobies have been variously grouped into two to nine families, some with included subfamilies. However, most existing taxonomies are not phylogenetic, and few cladistics hypotheses of relationships among goby groups have been advanced (Thacker, 2003). Morphologically, two fundamental differences between Gobiidae and Eleotridae are the shape of the pelvic fins and the relative length of the base of the dorsal fin (Nurjirana *et al.*, 2019). To date, scientific data show that nike fish is composed of six species from Gobiidae dan Eleotridae families. The results of this study reveal that two adult gobies species found in Bone River belong to Gobiidae and Eleotridae families. In other words, the adult species found in Bone River, are strongly alleged as other species of a compiler for the schools of nike fish in Gorontalo Bay. It is convincing since adult gobies from this investigation have a similar genus as that found from the prior studies.

The classification of the family Gobiidae has been revised from time to time with the evidence from molecular data (Laskar *et al.,* 2017). *A. ocellaris* is one of the species of the Gobiidae family, which is scientifically recorded generally as a species inhabiting Indo-Pacific waters. *A. ocellaris* is part of the goby fry fish group living in Northern Luzon, Philippines (Asis *et al.,* 2013) as well as inhabiting coastal wetlands, Hainan Island, South China Sea (Xiong *et al.*, 2018), and has been reported in the freshwaters of Andaman and Nicobar Islands, India (Praveenraj *et al.,* 2017). Additionally, *A. ocellaris,* as the species associated with differences in Iligan River environmental gradients (Requieron and Demayo, 2015), becomes one of three major species found in the seasonal stream in the South Andaman Islands, India (Kumar *et al.,* 2016). This Gobiidae is recorded as the native species in the Prafi River system, West Papua, Indonesia (Manangkalangi *et al.*, 2020).

Eleotris species (Teleostei: Eleotridae) are one of the most common fish in Indo-Pacific estuaries and insular freshwater streams that have an amphidromous life cycle, i.e., the adults grow, feed and reproduce in rivers; meanwhile, the larvae have a marine dispersal phase (Mennesson *et al.*, 2019). *B. belobranchus* belongs to this family. The lack of distribution and biological data of *B. belobranchus* potentially threatens the existence of the species. *B. belobranchus* as Eleotridae fish is also recorded in Danao, Cebu, Philippines (Ken *et al.*, 2011), Sukamade River, East Java (Rukmana *et al.,* 2014), Efate Island, Vanuatu (Bouffandeau *et al.*, 2019), and Biak River, Central Sulawesi (Gani *et al.,* 2019). *B. belobranchus* is one of the freshwater indigenous goby populations inhabiting the whole stretch of Mandulog River in Iligan City. This species is under the threats of water pollution, unregulated extraction of resources, and sprawling urbanization (Vedra *et al.*, 2013). The constituent species of nike schools from this fish genus is *B. segura.* Eleotridae family develops many endemic genera in the lower to the middle reaches of rivers since *B. segura* and *B. belobrancha* are the two known species of the genera *Belobranchus* in the Pacific area and the Andaman Islands. These sites are located in the remote Eastern Islands of the Indian Ocean. The present study revealed that both the species also present (Rajan and Sreeraj, 2014).

Both goby species found in this study are required for future investigations of its life cycle, migratory pattern, dan species abundance in both saline and freshwater. Also, tracking nike fish species from other goby families should be conceived since uncontrolled nike fishing have the potential to integrate the diversity of species making up the fish schools. These data indirectly illustrate that the existence and richness of goby fish species in Gorontalo waters are threatened. Therefore, species protection and conservation must be implemented immediately.

Conclusions

Nike schools are not only composed of six species of gobies. Based on a genetic investigation using the CO1 gene markers, *Awaous ocellaris* and *Belobranchus belobranchus* are strongly alleged as two species making up the goby schools in the adult stadia that reach freshwater along their migration life cycle.

Declarations of interest

No competing interests were disclosed

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