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RESEARCH ARTICLE

**What species make up the Nike fish assemblages at the macrotidal estuary in Gorontalo Bay, Indonesia? [version 1; peer review: awaiting peer review]**

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**Open Peer Review**

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**Abstract**

**Background:** No study has documented the species composition of Nike fish (fam. Cobitidae) schools. The aim of this study is to document the species composition of the Nike fish schooling.

**Methods:** All samples were collected randomly from fisher's catch during the fishing season on 5th–11th October 2018 at macrotidal area in Leato. Then, all specimens were identified morphologically by melanophore pattern differences. Subsequently, all identified-samples by melanophores pattern differences were sent to the genetic laboratory for identification.

**Results:** The morphological results show there are five individuals with a different melanophores pattern. On the contrary, the genetic results only show four species from those five individuals. They are *Sicyopterus pugnans*, *S. cynocephalus*, *Belobranchius segara*, and *Bunaka gymnooides*.

**Conclusions:** Our findings show that there are only four species that compose the Nike fish schooling in Gorontalo Bay. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchius segara*, and *Bunaka gymnooides*.

**Keywords:** Nike-fish, Gorontalo, melanophores pattern, genetic, morphology

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Author roles: Sahami FM: Methodology; Kepel RC: Visualization; Olii AH: Writing – Original Draft Preparation; Pratik SB: Writing – Review & Editing

**Competing interests:** No competing interests were disclosed.

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*by* Femy M. Sahami

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## Abstract

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## Introduction

Estuaries are a crucial habitat for biota and small fish, in particular juveniles of commercially relevant species. They are considered as the most productive and dynamic ecosystem in the world (Cantera & Blanco, 2001; Lahjie *et al.*, 2019; McHugh, 1967; Sreekanth *et al.*, 2017). They also perform the most crucial role in the population dynamic for a lot of invertebrate and fish species. These ecosystems also significantly contribute to provide some ecological services such as nursery ground, feeding ground and breeding habitats for both freshwater and marine species (Beck *et al.*, 2001; McLusky & Elliott, 2004; Sun *et al.*, 2019). The most well-known species that occupy the seas and estuary area in Gorontalo Bay is Nike fish.

Nike (pronounced nee-K) is a local name for transparent juvenile of unknown fish. These fish are approximately 2–4 cm in length; they appear seasonally and fished at estuary waters around the Gorontalo Bay. These juvenile fish has been fished and marketed traditionally for a long time. They are preferable for consumption by the local people than other fisheries products. As a consequence, fishing activity has increased over time to supply local demand for Nike (Wolok *et al.*, 2019).

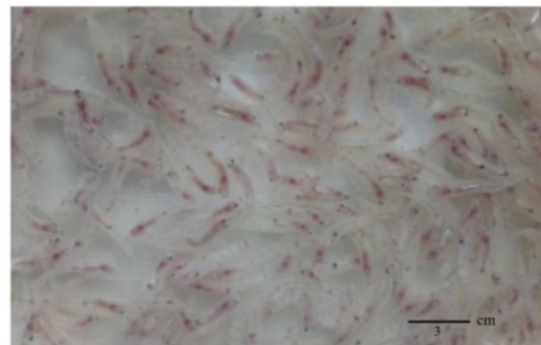
However, the impact of fishing activities is unknown. A recent paper concerning Nike only reports the seasonal appearance during the fishing season (Pasingi & Abdullah, 2018), total length and morphometric measurements (Zakaria, 2018), nutrition content (Liputo *et al.*, 2013), and mercury contamination of these fish (Salam *et al.*, 2016). To our knowledge, no studies have documented the species diversity that composed the schooling of Nike. Although, Yamasaki *et al.* (2011) have

reported that species in juvenile form can be determined by its melanophores pattern and genetic determination.

The objective of the present study is to address this lack of knowledge by identifying the fish species that composed a Nike fish schooling. This information is very urgent and required for fisheries management. Therefore, we aimed to identify the species that composed the schooling of Nike fish in Gorontalo Bay by melanophores pattern and genetic identification.

## Methods

This study was conducted in October 2018 at Leato (0°30'0.58"N, 123°3'55.42"E), Gorontalo Bay, Indonesia (Figure 2). Approximately 100 g of the Nike-Fish Assemblages (Figure 1) were collected randomly from the fishermen's catch at fishing grounds



**Figure 1.** Nike fish assemblages.



**Figure 2.** Study site. The red dot indicates the position of fishing ground where the samples were collected from fishermen.

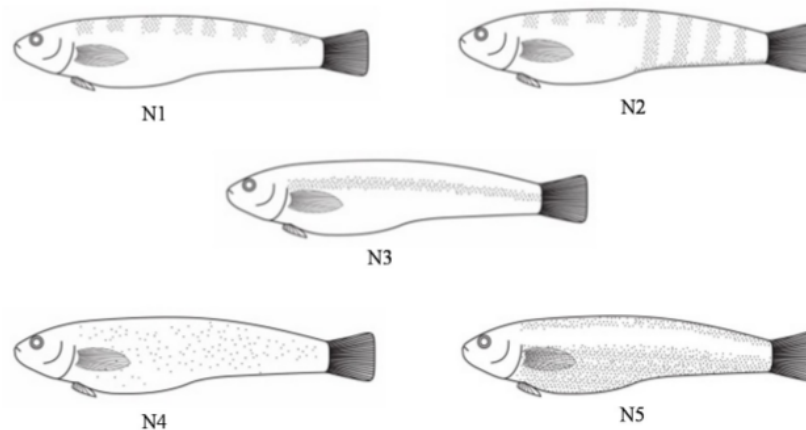
during the catch-season (on October 5<sup>th</sup>–11<sup>th</sup>). All samples were transported using a cool-box to the lab for measurement. Immediately after collection, all samples were identified visually according to [Yamasaki et al. \(2011\)](#), and the specimens with different melanophore patterns were separated according to their melanophore display. We assumed that those separated individuals were different on species.

Then, we selected one individual from each group and labeled these as N1, N2, N3, N4, N5, for genetic identification. Images of the selected samples were captured using Canon EOS 100d with 58 mm pro Digital Wide Converter 0.45X Lens and subsequently converted to black and white using CorelDraw Graphic Suite 2019.

After selection, all of the individuals with different melanophores were preserved with ethanol 70% in a separate bottle and sent to the Genetics Laboratory at Manokwari for genetic identification by Sanger sequencing. The DNA cytochrome oxidase subunit I (CO1) of the sample was isolated with a Geneaid™ DNA Isolation Kit. Editing, and proofreading of sequences, and construction of the phylogenetic tree was generated with [MEGA 5.0](#) software.

## Results

Five unspecified individuals of Nike-fish were identified morphologically by melanophore differences, as shown in [Figure 3](#). N1 was revealed as *Sicyopterus pugnans*; N2 as *Sicyopterus cynocephalus*; N3 and N5 as *Belobranchus segura*; and N4 as *Bunaka gyrinoides*. The specimens with melanophore differences of each group is shown in [Figure 4](#).



**Figure 3.** Nike fish with different melanophore patterns.

## Melanophores pattern

Nike-fish schools consist of various species with the same body-shape, but different melanophore displays. Moreover, from 100 g (~145 individuals) of the total specimens that we identified, only five individuals with different melanophore patterns were identified ([Figure 3](#)).

## Genetic identification

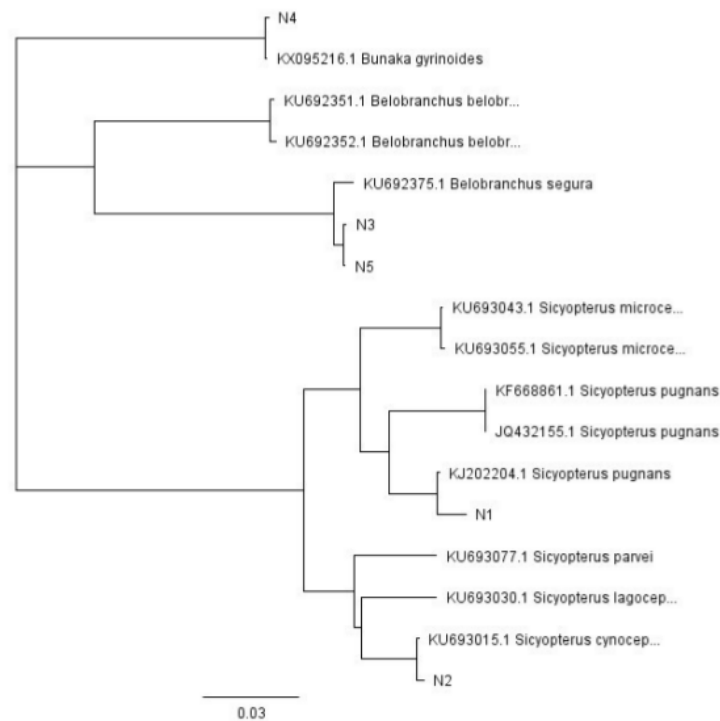
[Figure 3](#) shows the genetic identification among the individuals (species). The outcomes of genetic identification for N3 and N5 shows that both samples are the same species: *Belobranchus segura*.

## Discussion

Although the melanophore patterns in N3 and N5 are different, their genetics are identical, meaning they are the same species (*Belobranchus segura*). This dissimilarity might be affected by the changes of melanophore during the development of the larvae. [Valade et al. \(2009\)](#) report that such melanophores change on *Sicyopterus langocephalus* during the larvae stage. These changes could represent a problem for morphological identification. We can not count the species by morphological differences. Therefore, for the next examination we strongly recommended determining the species composition of the Nike fish schools by genetic rather than morphological identification because for that reason.

## Conclusion

Our findings show that there are four species that compose Nike fish schooling. They are *Sicyopterus pugnans*, *Sicyopterus cynocephalus*, *Belobranchus segura*, and *Bunaka gyrinoides*.



**Figure 4.** Phylogenetic tree of individuals with different melanophore patterns.

#### Data availability

##### Underlying data

Group N1, *Sicyopterus pugnans* isolate N1\_LEATO\_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number [MN065178](#).

Group N2, *Sicyopterus cynocephalus* isolate N2\_LEATO\_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number [MN069305](#).

Group N3, *Belobranchus segura* isolate N3\_LEATO\_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number [MN069306](#).

Group N4, *Bunaka gyrinoides* isolate N4\_LEATO\_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number [MN069307](#).

Group N5, *Belobranchus segura* isolate N5\_LEATO\_1 cytochrome oxidase subunit I (COI) gene, partial cds; mitochondrial. GenBank accession number [MN069308](#).

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