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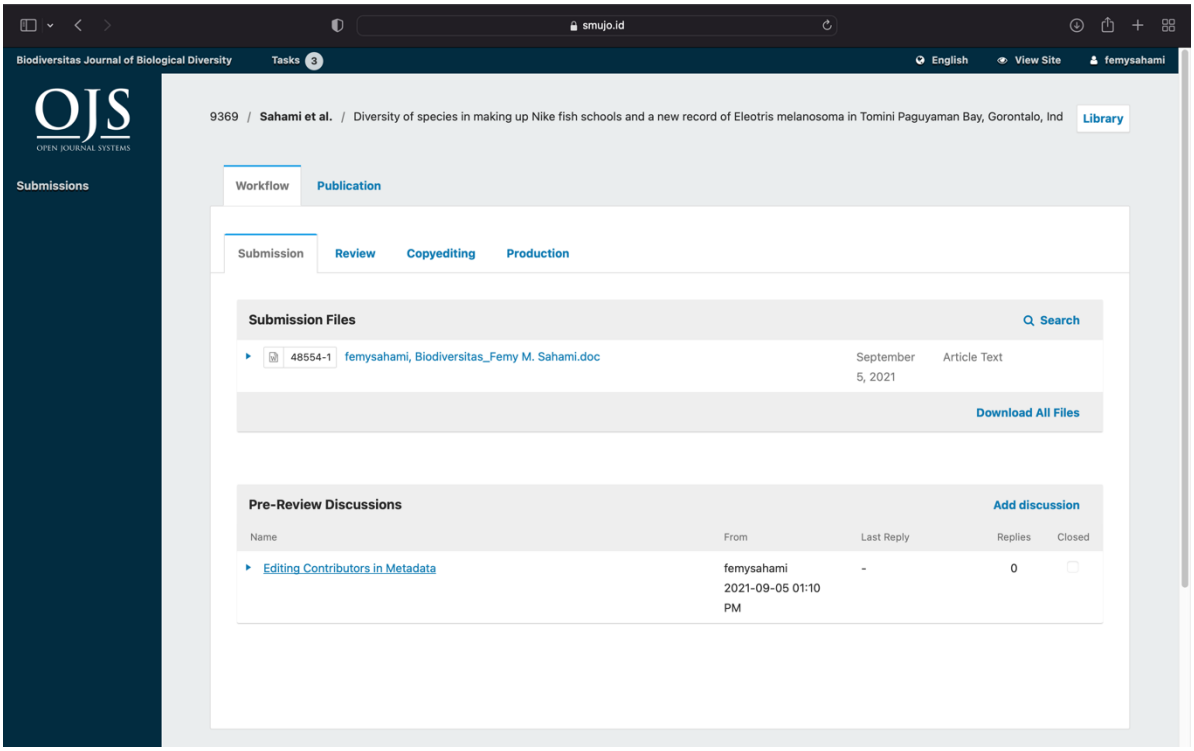

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Diversity of Species Making Up Nike Fish Schools and a New Record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia

Author(s) name:

Femy M. Sahami, Sitty Ainsyah Habibie

Address

(Fill in your institution's name and address, your personal cellular phone and email)

Faculty of Fisheries and Marine Sciences, Gorontalo State University,
Jl. Jend. Sudirman No. 6, City of Gorontalo, Gorontalo, 96128, Indonesia
Hp. : +62-81340374949, email: femysahami@ung.ac.id

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This research has reported that the Nike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. The *E. melanosoma* was the first species of *Eleotris* to be genetically and morphologically confirmed as a species constituting the Nike fish school. These results further complement the diversity data of Nike fish compilers in Gorontalo waters. In addition, this study also found a typical species dominance trend every day during the recruitment process of Nike fish back to freshwater, i.e., *S. longifilis* (52.00 %) on the first day, *Belobranchus segura* (63.27 %) on the second day, and *Stiphodon semoni* (83.43 %) on the third day.

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Femy M. Sahami

Diversity of Species Making Up Nike Fish Schools and a New Record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia

FEMY M. SAHAMI[1♥], SITTY AINSYAH HABIBIE[1]
[1]Faculty of Fisheries and Marine Sciences, Gorontalo State University. Jl. Jend. Sudirman No. 6, Gorontalo, 96128, Gorontalo, Indonesia.
Tel.: +62-435-821125, Fax.: +62-435-821752. ♥email: femysahami@ung.ac.id

Manuscript received: 5 September 2021. Revision accepted: 2021

Abstract. Nike fish is an essential economic commodity in Gorontalo with a distinctive and memorable taste. Several estuaries in the waters of Tomini Bay are reported as a primary location for Nike fishing in Gorontalo, one of them is Paguyaman Bay. Tomini Paguyaman Bay is an estuary of the Paguyaman River whose watershed crosses three regencies, i.e., Gorontalo, Boalemo, and Pohuwato. Observation of the species making up the Nike fish schools is crucial in its contribution to fish biodiversity. The present study aims to determine the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species that have never been reported for their distribution in Gorontalo waters. A total of 1,773 samples of Nike fish were caught in the sea to the estuary of Paguyaman Bay on one period of their appearance on April 8–10, 2021. The species were further grouped based on the similarity of melanophore patterns and analyzed morphometrically. The molecular identification was performed on species with new melanophore patterns. The results indicated that the Nike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. The identification of *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay is a new record of the distribution of this species in the waters of Gorontalo. Based on the species composition, Nike fish schools in the waters of Paguyaman Bay showed a typical species dominance trend during the recruitment process back to freshwater, i.e., *S. longifilis* (52.00 %) on the first day, *Belobranchus segura* (63.27 %) on the second day, and *Stiphodon semoni* (83.43 %) on the third day.

Keywords: *Belobranchus*, *Eleotris*, Sicydiinae, *Sicyopterus*, *Stiphodon*.

Abbreviations: Basic Local Alignment Search Tools (BLAST), Cytochrome Oxidase I (COI), Deoxyribonucleic acid (DNA), Polymerase Chain Reaction (PCR)

Running title: Diversity of Nike fish schools in Paguyaman Bay

INTRODUCTION

Tomini Bay is the largest bay in Indonesia which has direct contact with 14 districts or cities in three provinces in Sulawesi, such as North Sulawesi, Central Sulawesi, and Gorontalo. One of the essential fishery commodities from Tomini Gorontalo Bay is Nike fish. They consist of amphidromous gobi schools in the pelagic to juvenile larval stages in the recruitment process from marine waters to freshwaters. Their embryos that hatch will be carried by rivers to the sea and develop as pelagic larvae before being recruited back into rivers and grow into adults and spawn (Thuesen et al. 2011; Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The diversity of species making up Nike fish schools (postlarva gobi) in Gorontalo waters is one of the studies that need to be continuously observed and conducted, considering the potential economic value of Nike fish as a food commodity in Gorontalo is quite high (Wolok et al. 2019). About 1,120 species from 30 genera in the Gobi group have been described to date. The gobies group plays a vital role in ichthyofauna diversity with a wide distribution area and a high number of species. This group of fish is dominantly found in eastern Indonesia, with a fairly high diversity of species (Tweedley 2013; Miesen et al. 2016; Hadiaty and Sauri 2017; Nurjirana et al. 2020).

There are, geographically, several rivers in Gorontalo which disembogue the sea waters of Tomini Bay. Some of the river estuaries are important locations for Nike fish catching in Gorontalo, including Gorontalo Bay and Paguyaman Bay (Salam et al. 2016). Nike fish in Gorontalo Bay has been studied by many researchers (Olii et al. 2017; Olii et al. 2019; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020a; Sahami et al. 2020) due to its close location and significant productivity. Meanwhile, scientific data on Nike fisheries in the Paguyaman Bay estuary has not been widely observed. Paguyaman Bay is part of the Tomini Bay areas located on the border of Girisa Village, Paguyaman District, Boalemo Regency with Bilato Village, Boliyohuto District, Gorontalo Regency. This bay is the estuary of the Paguyaman River, whose watershed crosses three regencies, i.e., Gorontalo, Boalemo, and Pohuwato. The presence of the Paguyaman watershed as the second-largest watershed in Gorontalo and the direct contact with three administrative regencies make the location of the Paguyaman Bay estuary interesting to observe.

Demographic information on fish, such as life history at early stages, recruitment, migration patterns, and other biological characteristics, are crucial data for managing fish populations (Habibie et al. 2015). Besides, information on the identification and composition of species is also an essential part of management efforts, and thus, the management can be carried out effectively. The types of species making up the Nike fish schools in the waters of Gorontalo City Bay are

reported consisting of nine species to date, i.e., *Sicyopterus pugnans, S. longifilis, S. lagocephalus, S. cynocephalus, S. parvei, Bunaka Gyrinoides, Belobranchus segura, B. Belobranchus,* and *Stiphodon semoni* (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). The present study aims to find out the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species that have never been reported for their distribution in the waters of Gorontalo Bay. The data are then expected to be significant as scientific information for the basis of Nike fish resource management in Paguyaman Bay and to support attempts to optimize Nike fishery potential in the Gorontalo waters.

MATERIALS AND METHODS

Sampling

The sampling of Nike fish in Paguyaman Bay (N 00º31'1.10" and E 122º39'0.73") (Figure 1) was performed for three days in one appearance period on April 8–10, 2021. A total of 150 grams of Nike fish samples were taken from the catch of fishermen using nets every day. The samples were brought to the Hydrobioecology and Biometrics Laboratory, Faculty of Fisheries and Marine Sciences, Gorontalo State University for analysis. The samples were then identified for its species by referring to Sahami et al. (2019b) and Sahami et al. (2020) and counted for its number. The samples were then photographed for morphometric analysis. If the number of samples were large, then the sample for morphometric analysis would only be limited to 50 individuals. The species with new melanophores were also photographed, sketched, morphologically described, and molecularly analyzed.

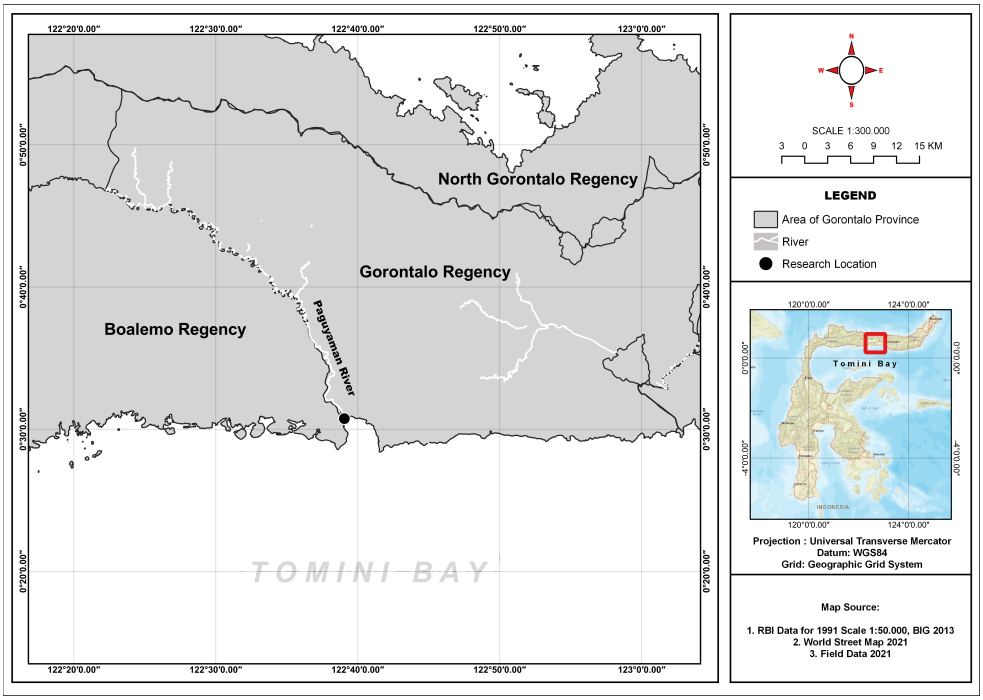

Figure 1. Research location map

Morphometric Characters

Ten morphometric characters of Nike fish referring to Sahami et al. (2020) (Figure 2 and Table 1) were measured using the imageJ application with an accuracy of 0.001 cm.

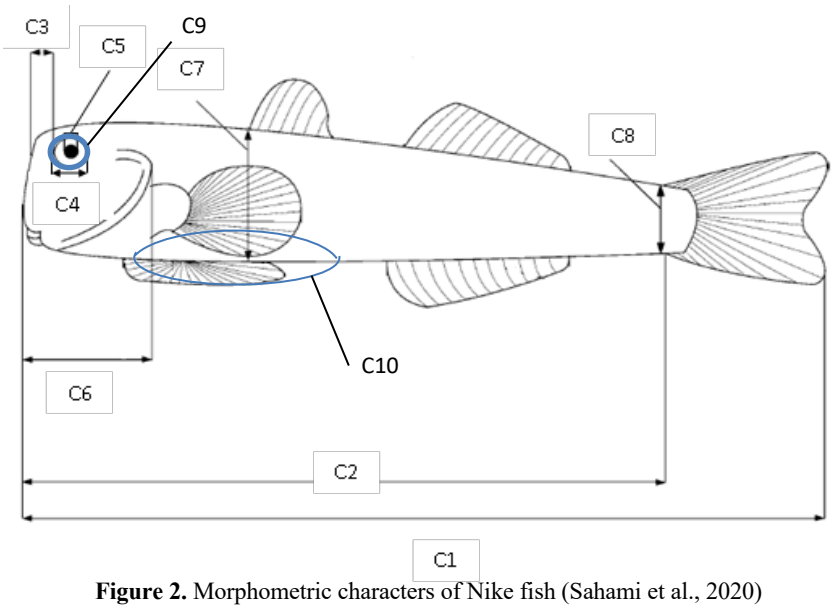

Figure 2. Morphometric characters of Nike fish (Sahami et al., 2020)

Table 1. Morphometric characters of Nike fish (Sahami et al. 2020)

No	Morphometric Characters	No	Morphometric Characters
C1	*Total Length* (TL)	C6	*Head Length (HL)*
C2	*Standard Length* (SL)	C7	*Body Depth (BD)*
C3	*Preorbital Length* (PL)	C8	*Peduncle Depth (PD)*
C4	*Eye Diameter (ED)*	C9	*Eye Area (EA)*
C5	*Eye Lens diameter (EL)*	C10	*Yolk Sac area (YS)*

Each measured morphometric character data was then standardized following the allometric formula according to Elliott et al. (1995) as follows:

$$M_{adj} = M\ (L_s/L_0)^b$$

M_{adj} is the standardized morphometric data, M is the measured morphometric data, L_0 is the total length of fish, L_s is the average total length, and parameter *b* is the slope of log linear curve M to log L_0 of all data.

DNA Extraction, PCR Amplification, and Sequencing

The target gene for molecular analysis is the mitochondrial DNA Cytochrome Oxidase Subunit 1 (COI) gene. COI gene had better resolution at the intraspecific level than the other core genes (Bellagamba et al. 2015; Hubert et al. 2015; Rodrigues et al. 2017; Bingpeng et al. 2018; Roesma et al. 2018, Yulianto et al. 2020). In addition, mitochondrial COI genes are also widely and reliably used in identifying species in the gobi group (Jeon et al. 2012; Viswambharan et al. 2013; Laskar et al. 2016; Lejeune et al. 2016; Wang et al. 2017; Linh et al. 2018; Olii et al. 2019; Roesma et al. 2020; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020b; Sahami et al. 2020). Following the protocol kit, the DNA sample of fish tissue was isolated using Qiagen Tissue and Blood Extraction kits. The mitochondrial DNA COI gene was further amplified using the primer pair FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). Samples were amplified at pre-denaturation temperature of 94 ºC for five minutes, denaturation at 94 ºC for 30 seconds, annealing at 50 ºC for 30 seconds, extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 7 minutes. This Polymerase Chain Reaction (PCR) process lasted for 38 cycles.

Data Analysis

The DNA samples that had been amplified and electrophoresed were then sequenced to obtain their nucleotide sequences using the Dideoxy Sanger Termination Method through PT Genetika Science Indonesia. The nucleotide sequences resulting from DNA sequencing that had been processed and carried out contig. After that, the results were matched with the data available in the GenBank database (www.ncbi.nlm.nih.gov) through the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was arranged by aligning the DNA sequences of identified samples with some DNA of gobies (accession number KF489573, KU232392, KU692483, KU692484, and KU692490) and the species of Nike fish schools in Gorontalo Bay (accession number MN069306, MN069307, MN069308, MT706639, MT706640, MT706641, MT706720, MT706721, MT706722, MT706723, MT706724, MT706725, MT706726, and MT706791) available in the GenBank database using the Maximum Likelihood 1000 bootstrap method on MEGAX software (Kumar

et al. 2018). The results of genetic identification were further confirmed morphologically, referring to Maeda and Tachihara (2005). Furthermore, the Discriminant Function Analysis (DFA) (Landau and Everit 2004) was performed to find out the main distinctive characters among species by using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

New Records of Species that Makes Up the Nike Fish Schools

The appearance of Nike fish in Paguyaman Bay does not have a regular rhythm like in Gorontalo Bay, where the periodization occurs almost every month. In 2021, Nike fish in Paguyaman Bay first appeared in April with a 3-day appearance period, namely April 8–10, 2021. A new melanophore pattern of species that make up the Nike fish school was found on the third day of its appearance. Mitochondrial COI gene sequencing results showed that the species with the new melanophore pattern was identified as *Eleotris melanosoma* (Figure 3) based on the NCBI database. The nucleotide arrangement of *E. melanosoma* had been entered in the NCBI database with accession number MZ401475.

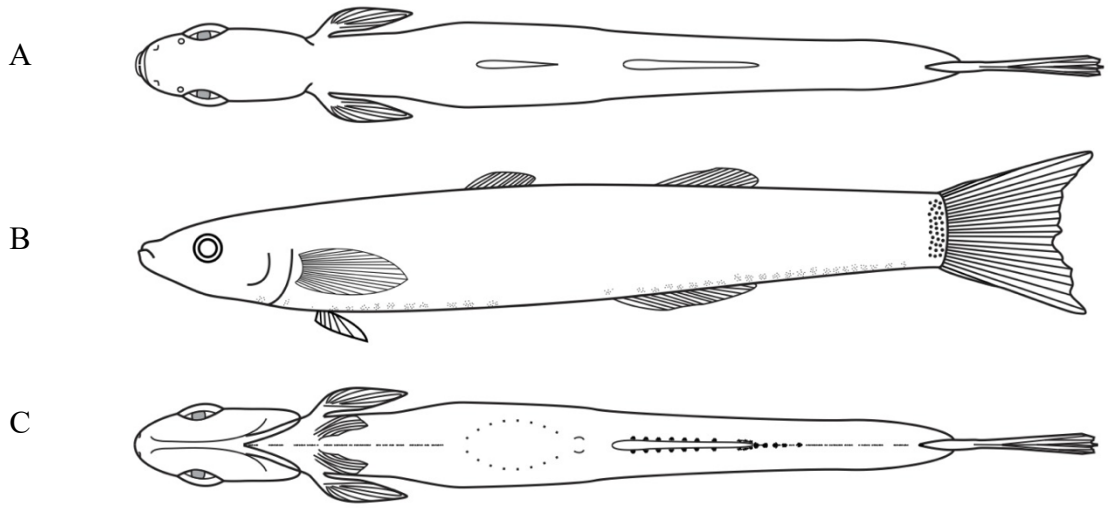

Figure 3. Pelagic larvae of *Eleotris melanosoma* in Paguyaman Bay. A. Dorsal view; B. Lateral view; C. Ventral view.

The caught species of *E. melanosoma* had a standard length of 19.05–20.04 mm with an average standard length of 19.55 mm. This species has a transparent body, an elongated and compressed body shape, has no scales, body fins are not perfect yet, pelvic fins are separated, and the caudal fin tends to form emarginate. There are no melanophore points distributed from head to body, and few melanophore points accumulate at the base of the caudal fin. This morphological feature is in line with the morphology of the pelagic larvae of *E. melanosoma* reported by Maeda and Tachihara (2005).

The results of the alignment of the DNA sequences of *E. melanosoma* with the DNA sequences of the species that make up the Nike fish school in Gorontalo Bay and several *Eleotris* species available in the NCBI Genbank are displayed through a phylogenetic tree (Figure 4). The analysis results show that *E. melanosoma* is very closely related to *Eleotris fusca* and is in the same monophyletic clade as *Belobranchus segura*, *B. belobranchus*, and *Bunaka gyrinoides*. The similarity of the *clades* in these five species is because they are members of the *Eleotridae* family, although it is clear that there are sub-clades based on the similarity of the genus in this clade. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species *Stiphodon semoni, Sicyopterus longifilis, S. lagocephalus, S. parvei,* and *S. cynocephalus.* The second monophyletic clade also clearly groups species in the genus *Sicyopterus* in the same sub-clade and separate from the genus *Stiphodon*.

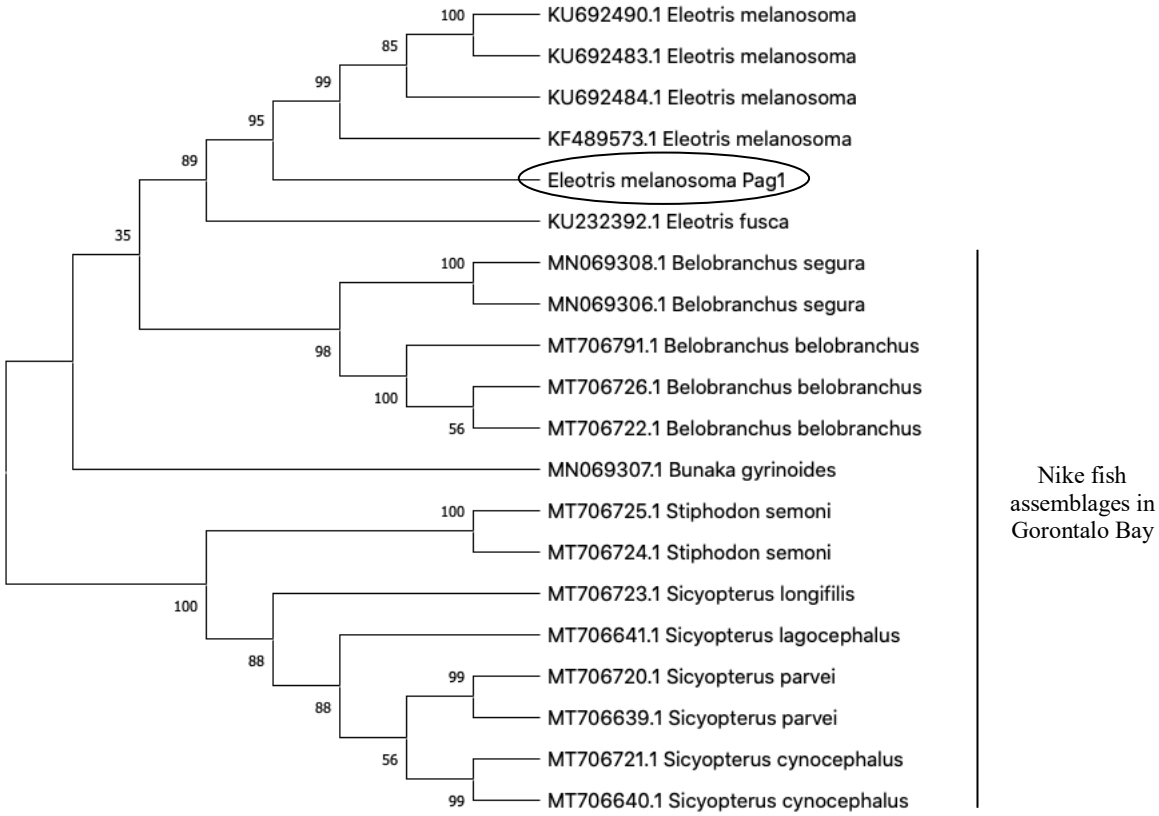

Figure 4. Phylogenetic tree of *Eleotris melanosoma* with several species of gobies and composers of Nike fish in Gorontalo Bay waters available in the NCBI database

Composition of Species

A total of 1,773 specimens were found as composers making up Nike fish schools in Paguyaman Bay for three days of appearance in April 2021. Two families, i.e., Gobiidae and Eleotridae, were found to make up Nike fish schools. The Gobiidae family consists of four species, i.e., *Sicyopterus longifilis, S. parvei, S. cynocephalus,* and *Stiphodon semoni*. Meanwhile, the Eleotridae family consists of three species, i.e., *Belobranchus segura, B. belobranchus*, and *Eleotris melanosoma*. Among the seven species, three species from the family Gobiidae, i.e., *S. longifilis, S. parvei*, and *Stiphodon semoni* were found as composers making up the Nike fish schools during three days of their emergence. The type of species that was consistently found in considerable numbers was S. *longifilis.* Species of *B. segura* were only found on the second day, while *B. belobranchus* and *E. melanosoma* were only found on the third day of appearance of Nike fish. The most abundant species found were *S. semoni* (34.07 %), while the least were *S. parvei* and *B. segura* with 0.003 % each. Based on the species composition, the tendency of species dominance was different each day. On the first day, the school of Nike fish was dominated by *S. longifilis* (52.00 %), the second day was dominated by *Belobranchus segura* (63.27 %), and the third day was dominated by *Stiphodon semoni* with a composition value of 83.43 % (Figure 5).

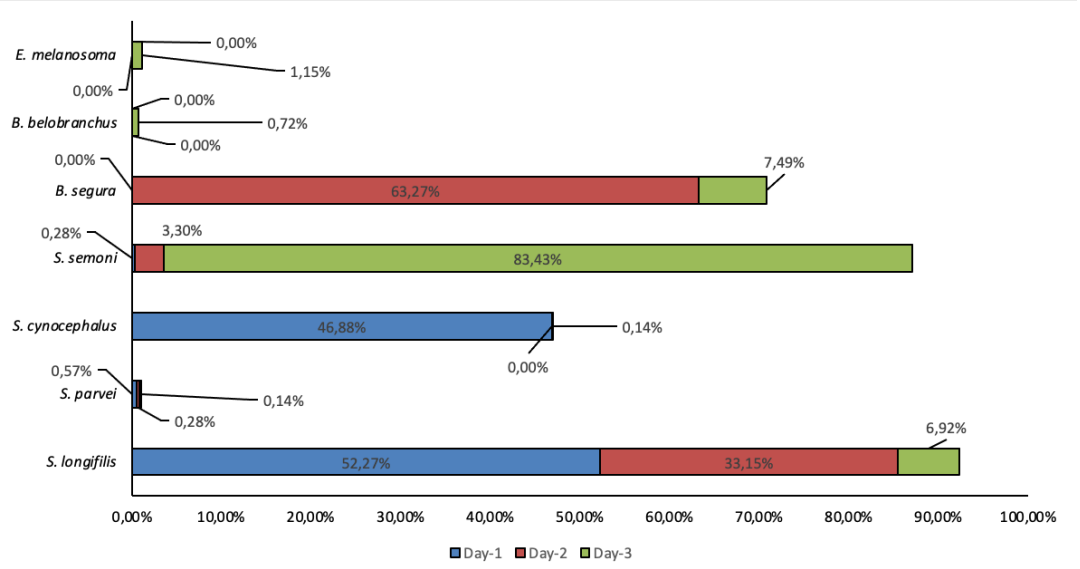

Figure 5. Nike fish schools composition in Paguyaman Bay waters

Size Distribution and Morphometric Characters

The total length of Nike fish that had been caught, in general, ranged from 1,523 to 3,572 cm (Table 2). The species found with the smallest size was *Stiphodon semoni* (Gobiidae), while the largest was *S. parvei* (Gobiidae). The species with the broadest size range was *S. cynocephalus* (1,150 cm), while the species with the smallest size range was *B. belobranchus* (0,113 cm).

Table 2. Size Range of Each Species Making Up Nike Fish Schools in Paguyaman Bay Waters

No	Species	Mean of Size (cm)	Range of Size (cm)
1	*Sicyopterus longifilis*	2.666	2.262–3.044
2	*Sicyopterus parvei*	3.036	2.831–3.403
3	*Sicyopterus cynocephalus*	2.775	2.422–3.572
4	*Stiphodon semoni*	2.137	1.523–2.586
5	*Belobranchus segura*	2.311	2.005–2.638
6	*Belobranchus belobranchus*	2.347	2.290–2.403
7	*Eleotris melanosoma*	2.377	2.304–2.448

The morphometric characters can be used in taxonomy as early identification in fisheries science (Sara et al. 2016). The summary of data on the measurement results of morphometric characters that have been standardized following the allometric formula Elliott et al. (1995) is presented in Table 3. Furthermore, the results of the analysis on the discriminant function are shown in Figure 6. Two discriminant functions, respectively, can explain 67.3 % and 24.5 % of total variance of morphometric characters. Based on the results of the analysis, the main distinctive character of the Nike population in Paguyaman Bay was the C7 character (body depth). Figure 6 shows the tendency for the formation of groupings of Nike fish samples in Paguyaman Bay. *Sicyopterus longifilis, S. cynocephalus, S. Parvei*, and *S. semoni* indicated a tendency to form overlapping and close points since the four species are members of the Gobiidae family. Furthermore, *Belobranchus segura* and *Belobranchus belobranchus* have adjacent points because these two species are members of the same genus, the genus *Belobranchus*. The *Eleotris melanosoma*, although they have been distributed in separate areas, are quite close to the *B. belobranchus* species because these two species are members of the Eleotridae family.

Table 3. Morphometric Character Data of Nike Fish Schools in Paguyaman Bay Waters

Species	Unit of Characters (cm)							
	SL	PL	ED	EL	HL	BD	PD	
S. longifilis	2.054 ± 0.05	0.111 ± 0.02	0.136 ± 0.02	0.060 ± 0.01	0.518 ± 0.04	0.381 ± 0.03	0.208 ± 0.02	0.0
S. parvei	2.086 ± 0.03	0.128 ± 0.03	0.133 ± 0.02	0.062 ± 0.01	0.535 ± 0.03	0.376 ± 0.01	0.205 ± 0.01	0.0
S. cynocephalus	2.043 ± 0.05	0.114 ± 0.02	0.133 ± 0.02	0.057 ± 0.01	0.521 ± 0.05	0.375 ± 0.03	0.203 ± 0.02	0.0
S. semoni	2.062 ± 0.05	0.104 ± 0.02	0.129 ± 0.02	0.056 ± 0.01	0.489 ± 0.04	0.343 ± 0.04	0.188 ± 0.03	0.0
B. segura	2.094 ± 0.04	0.126 ± 0.02	0.129 ± 0.02	0.061 ± 0.01	0.556 ± 0.05	0.392 ± 0.03	0.221 ± 0.02	0.0
B. belobranchus	2.051 ± 0.05	0.140 ± 0.03	0.128 ± 0.02	0.067 ± 0.01	0.579 ± 0.03	0.325 ± 0.01	0.213 ± 0.01	0.0
E. melanosoma	2.033 ± 0.02	0.123 ± 0.01	0.129 ± 0.00	0.058 ± 0.01	0.535 ± 0.04	0.280 ± 0.02	0.208 ± 0.01	0.0

Notes: SL= *Standard Length; PL = Preorbital Length; ED = Eye Diameter; EL = Eye Lens diameter; HL = Head Length; BD = Body Depth; PD =*
YS = Yolk Sac area

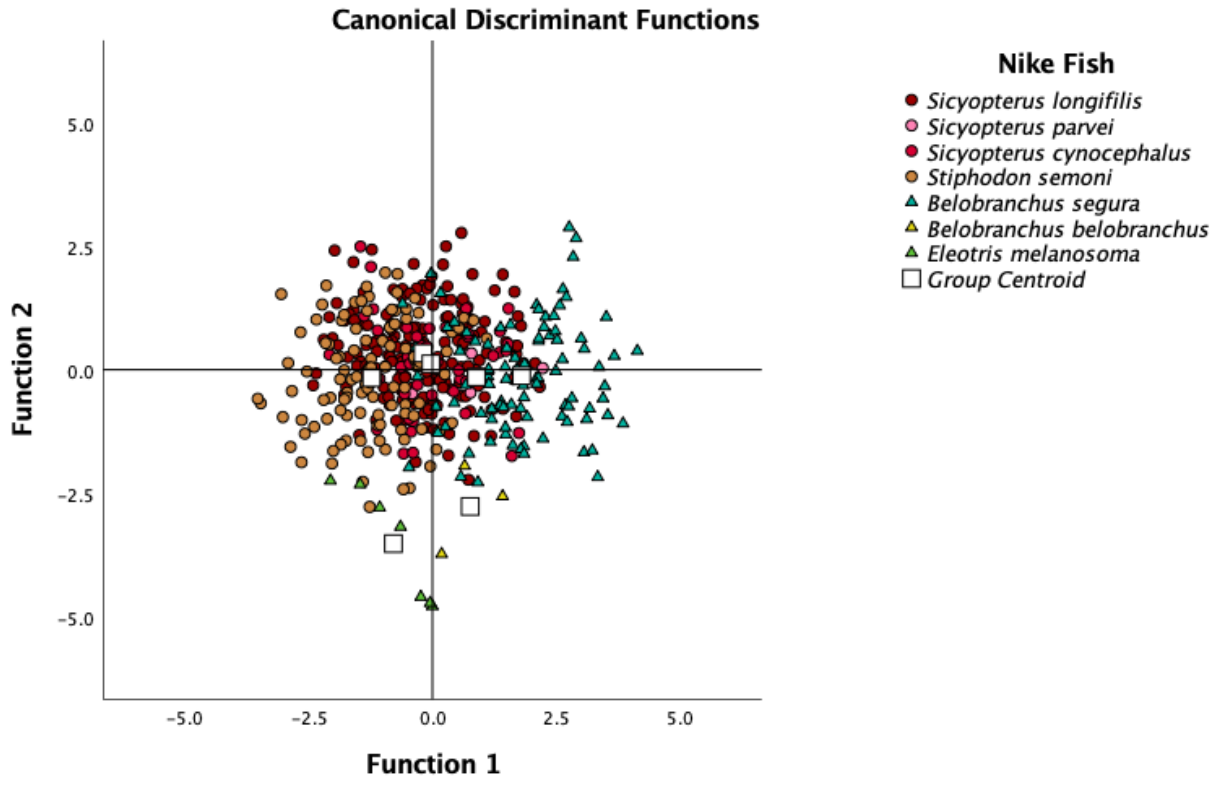

Figure 6. Diagram of the canonical discriminant function of Nike fish in Paguyaman Bay waters

Discussion

The types of species making up the Nike fish schools in Paguyaman Bay only consist of seven species, fewer than those making up the Nike fish schools in Gorontalo Bay, which has been reported to consist of nine species to date (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Two species of gobies, *S. pugnans* and *Bunaka gyrinoides*, were not found as the composers of Nike fish schools in Paguyaman Bay. However, the present study reported a new record of the distribution of *E. melanosoma*. Identifying *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay based on molecular and morphological analysis is a new record of the distribution of this species in Gorontalo waters. A total of eight species of *E. melanosoma* were caught as the composers of the Nike fish schools in Paguyaman Bay when they began to approach the river estuary on the third day of their appearance.

The species *E. melanosoma* is one of three main species of *Eleotris* reported to be distributed in the Indo-Pacific waters. The other two species are *E. fusca* and *E. acanthopoma* (Maeda et al. 2011; Mennesson et al. 2019; Subchan et al. 2020). Additional *Eleotris* species may continue to be recorded as more research focuses on this research topic in more Indo-Pacific locations. Maeda and Tachihara (2005) reported that the pelagic and newly settled larvae of *E. fusca* had a larger body size than *E. melanosoma* and *E. acanthopoma*. *Eleotris* pelagic larvae migrate to river areas by utilizing the rising tides at night. *E. melanosoma* and *E. acanthopoma* settle at the upper end of the tidal affected area, while *E. fusca* migrates upstream of freshwater against river currents.

Based on the size, the species of *E. melanosoma* caught in Paguyaman Bay (average standard length 19.55 mm) should have been in the newly settled larval stage based on research Maeda and Tachihara (2005) in the Teima River estuary area, Okinawa Island with visible body pigmentation increasingly numerous and completely pigmented caudal fin with no distal border. However, the morphology of *E. melanosoma* species in Paguyaman Bay still characterizes the morphology of the pelagic larval stage, with the most striking feature that it does not have melanophore dots on its sides. This difference can occur due to differences in the aquatic habitat of the fishing area (Habibie et al. 2018). In addition, the fairly stable temperature throughout the year in the tropics area has no significant effect on the growth rate of fish (Habibie et al. 2015). The pelagic to juvenile larval stages of *E. melanosoma* species also recorded in the estuary area of the Teima River, Okinawa Island (Maeda and Tachihara 2005) and Sri Lankan waters estuary (Batuwita et al. 2017). Meanwhile, the adult species was found in the fresh waters of Japan (Maeda et al. 2011); rivers on the Buton and Kabaena Islands (Tweedley et al. 2013); Opak River,

Yogyakarta (Djumanto et al. 2013); freshwater of Sri Lanka (Batuwita et al. 2017); freshwater of West Sulawesi (Nurjirana et al. 2020); and Sanenrejo and Wonoasri River Resorts (Subchan et al. 2020).

Among amphidromous fish, the Eleotridae and Gobiidae (Teleostei: Gobioidei) are the two most common families found in estuaries and freshwaters of the Indo-Pacific region. Eleotrid species most commonly inhabit the lower and middle parts of freshwater streams with the characteristic of sitting and waiting for prey (Mennesson et al. 2015; Mennesson and Keith 2020). *Eleotris* pelagic larvae morphological features are transparent and compressed bodies, conspicuous swim bladder, and emarginate caudal fin (Maeda and Tachihara 2005). While the adult species has a large blunt head, torpedo-shaped body, rounded caudal fin, prominent lower jaw, fin ventral discrete, has no lateral line on the sides of the body, and has an inconspicuous body-color, mostly light brown or dark brown or olive with some metallic sheen (Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species in the Gobiidae family are active swimmers. This species has pelvic fins that are fused and form a sucking disc that can be used to attach themselves to the substrate while actively swimming against currents upstream (Keith 2003; Taillebois et al. 2014). The genus *Sicyopterus* uses its mouth as a secondary sucking disc that allows this genus to quickly access the headwaters above the waterfall (Keith 2003).

Like the Nike fish in Gorontalo Bay, the Nike fish in Paguyaman Bay is also an amphidromous species. As an amphidromous species, adults hatch in rivers, larvae then flow downstream (sea) and grow in coastal or offshore marine habitats, then recruitment occurs into rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively drift with the river currents shortly after hatching at dusk and reach the estuary area in the middle of the night (Maeda and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so the larvae are carried downstream with lots of yolks and the risk of starvation is minimized (McDowall 2009). One of the strategies that has been developed is spawning in river areas that are quite close to the estuary to shorten the larval drift time in freshwater environments (Lagarde et al. 2017).

The period of Nike fish appearance in groups in the bay area and moving into the estuary is pre-colonization. In this phase, gobies' postlarvae will swarm and choose to swim near the shoreline, where the flow is weaker and can even be reversed by tides or waves (Keith et al. 2008). Schooling is a gobies strategy to avoid predators and find food (Keith 2003). The diversity of species and sizes of species making up the fish schools is strongly influenced by the characteristics of the species, season, and hatching time. Taillebois et al. (2012) explained that the duration of pelagic larvae is not the only factor in determining species distribution. Still, it is most likely the result of interactions between larval behavior, environment, currents, and substrate preferences. Apart from these factors, fish larvae found in larger sizes are very likely to be the result of earlier hatching compared to smaller fish larvae (Mennesson et al. 2015). The rainy season will help the newly hatched larvae drift faster to the sea (Keith 2003).

The discriminant analysis determines the main distinctive character among populations (Landau and Everit 2004). The present study confirmed that the main distinctive character among Nike fish populations in Paguyaman Bay is body depth, in contrast to the main distinctive character among Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The distinction in the main distinctive characters between the two different locations could be found since the gobies species develop various morphological specificities as adaptations to their environment (Gani et al. 2019; Roesma et al. 2020). Thus, different backgrounds could affect the morphological characters of species in that environment.

Based on their species composition, the Nike fish schools in the Paguyaman Bay waters indicated a typical species dominance trend every day during the recruitment back to freshwaters. On the first day of their appearance in Paguyaman Bay, the Nike fish schools consisted of species in the Gobiidae family and was dominated by *S. longifilis* (52,00 %). Furthermore, the species composition was dominated by *Belobranchus segura* (63,27 %) on the second day and *Stiphodon semoni* (83,43%) on the third day when the schools began to enter the river estuary. Nevertheless, further study is needed to elucidate these results. In general, gobies are relatively easy to adapt to almost all types of aquatic habitats, although with varying degrees of abundance (Gani et al. 2019).

Finally, the present study has reported the diversity of species making up the Nike fish schools in Paguyaman Bay and a new record of *E. melanosoma* species as the first species of the genus *Eleotris* making up the Nike fish schools in Paguyaman Bay in specific and Gorontalo waters in general. Exploration of adult species and their distribution in the freshwaters of the Paguyaman River, which is thought to be the habitat of adult Nike in Paguyaman Bay, must be performed to ensure the sustainability of Nike fish resources in the future. Although the adult species of Nike fish are not economic value commodity, the postlarva stage of the species when it is about to be recruited back into the river is often targeted by fishermen for human consumption (Ellien et al. 2016; Roesma et al. 2020), including Nike fish in Gorontalo waters. Species identification needs to be done continuously to prevent the loss of certain fish species along with commodity fishing. This research indicates that many types of species that make up the Nike fish school may be found again distributed in Gorontalo waters if a more extensive study is carried out in the future, especially in river estuarine locations that have never been studied before.

ACKNOWLEDGEMENTS

The authors would like to thank the Institute of Research and Community Service (LPPM) of the Gorontalo State University, which funded this research through the Penelitian Dasar scheme for the 2021 Fiscal Year. The thanks are also conveyed to Thomas Tammu and Adistya for technical assistance during the study and all parties who contributed to the implementation of the research.

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(*COMMENT REVIEWER* A)

[biodiv] Editor Decision

2021-11-11 11:22 PM

Femy M. Sahami:

We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity of species making up nike fish schools and a new record of Eleotris melanosoma in Tomini Paguyaman Bay, Gorontalo, Indonesia".

Our decision is: Revisions Required

Reviewer A:

Dear Authors,

Many thanks for sending this interesting paper over for review. The paper describes the sampling of Nike fish composition, using a mixture of both physical identification, and genetic analysis. The work has some considerable merit as a study as it helps to identify local biodiversity.

However, there are some limitations to the paper. This means that revisions are required if the paper is to be published. My biggest concern is the limited number of dates of observation, as this reduces the reliability of the results. While this in itself does not prevent publication, the limitations need to be acknowledged fully. I have provided specific feedback comments on the word document version of the file. Additionally, please consider the following comments when making your revisions:

1. Missing methods. Some parts of the methods seem to be missing. For example, what times did fishing take place, and what net types and styles were used? How many fish were captured, and what part of the fish was sampled for genetic analysis? Were samples pooled or sampled individually?
2. You have only sampled the Nike fish during a very brief window of time (April 8-10). It is therefore possible that fish school composition might change hugely between seasons (e.g. March or May). This might also explain why different species were more common on specific days. You need to discuss this limitation fully in the discussion, as otherwise your results might be misleading.
3. Proof read. While I have made some suggestions for wording, the work would benefit from a full proof read. Watch out for use of commas instead of full stops in numbers as they change the meaning!
   With these changes, the manuscript should be in a better position for acceptance.

Recommendation: Revisions Required

Diversity of species making up nike fish schools and a new record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia

Abstract. Several estuaries in the waters of Tomini Bay are reported as the primary location for Nike fishing in Gorontalo; one of which is Paguyaman Bay. However, scientific information on Nike fish in the Tomini Bay waters is currently limited in Gorontalo Bay. The present study aims to determine the diversity of species making up the Nike fish schools in Paguyaman Bay and show the types of constituent species whose distribution in Gorontalo waters has never been reported. A total of 1,773 samples of Nike fish were collected from the sea to the estuary of Paguyaman Bay in one period of their emergence on April 8–10, 2021. The species were grouped based on the melanophore patterns similarity and then analyzed morphometrically. The molecular identification of COI mitochondrial DNA was performed on species with different morphological appearances from those found in Gorontalo Bay. The Discriminant Function Analysis (DFA) indicated that the main distinguishing character of morphometrics is body depth. The morphological results suggested that the Nike fish schools in Paguyaman Bay consisted of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. The first finding of *E. melanosoma* as a species making up the Nike fish schools in Paguyaman Bay was a new variant of the distribution of this species in Gorontalo waters and confirmed using morphology and molecular analysis. Further, based on the species composition, Nike fish schools in the waters of Paguyaman Bay show a typical species dominance trend during the recruitment process returning to freshwater, i.e., *S. longifilis* (52.00 %) on the first day, *Belobranchus segura* (63.27 %) on the second day, and *Stiphodon semoni* (83.43 %) on the third day.

Keywords: *Belobranchus*, *Eleotris*, Sicydiinae, *Sicyopterus*, *Stiphodon*.

Abbreviations: Basic Local Alignment Search Tools (BLAST), Cytochrome Oxidase I (COI), Deoxyribonucleic acid (DNA), Polymerase Chain Reaction (PCR)

Running title: Diversity of Nike fish schools in Paguyaman Bay

INTRODUCTION

Tomini Bay is Indonesia's largest bay intersecting related with 14 regencies in three provinces of Sulawesi, namely North Sulawesi, Central Sulawesi, and Gorontalo. Nike fish is among the essential fishery commodities in Tomini Gorontalo Bay communities. It consists of amphidromous gobi schools in the pelagic to juvenile larval stages in the recruitment process from marine waters to freshwaters. Their hatched embryos are carried by the river flow to the sea and develop as pelagic larvae before being recruited, returning to rivers and growing as adults and spawn (Thuesen et al. 2011; Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The diversity of species making up Nike fish schools (postlarva gobi) in Gorontalo waters is one of the important studies that should be conducted continuously, considering the high economic value potential as a food commodity in Gorontalo with an R/C ratio of 2.68 (economically feasible) (Wolok et al. 2019). As of today, approximately 1,120 species from 30 genera in the gobi group are described. The gobi group plays a vital role in ichthyofauna diversity with a wide area distribution beside a high number of species. These fish groups are dominantly found in the eastern part of Indonesia, with high species diversity (Tweedley 2013; Miesen et al. 2016; Hadiaty and Sauri 2017; Nurjirana et al. 2020).

Geographically, there are several rivers in Gorontalo which empty into Tomini Bay. Some of these river estuaries are important locations for Nike fish catching in Gorontalo, including Gorontalo Bay and Paguyaman Bay (Salam et al. 2016). Numerous studies have reported Nike fish in Gorontalo Bay (Olii et al. 2017; Olii et al. 2019; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020a; Sahami et al. 2020). Meanwhile, scientific data on Nike fisheries in the Paguyaman Bay estuary are not widely observed. Paguyaman Bay is part of the Tomini Bay areas located on the border of Girisa Village, Paguyaman District, Boalemo Regency with Bilato Village, Boliyohuto District, Gorontalo Regency. The bay is

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the estuary of the Paguyaman River, whose watershed flows across three regencies, i.e., Gorontalo, Boalemo, and Pohuwato. The presence of the Paguyaman watershed as the second-largest watershed in Gorontalo and directly intersecting with three administrative regencies draws the interest to observe the Paguyaman Bay estuary.

The demographic information, such as life history at early stages, recruitment, migration patterns, and other biological characteristics, is crucial for managing fish populations (Habibie et al. 2015). Information about the identification and composition of species is also essential in the management process. Hence, the management can be carried out effectively. As currently reported, the types of species making up the Nike fish schools in the waters of Gorontalo Bay consist of nine species i.e., *Sicyopterus pugnans, S. longifilis, S. lagocephalus, S. cynocephalus, S. parvei, Bunaka Gyrinoides, Belobranchus segura, B. Belobranchus,* and *Stiphodon semoni* (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Although the goby is widely distributed in tropical Indo-Pacific waters (Keith et al. 2015; Lord et al. 2019), the diversity of species in each water location needs to be examined further. Therefore, the present study aims to find out the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species whose distribution in the waters of Gorontalo Bay has never been reported. The present work is also expected to provide significant scientific information for Nike fish resource management in Paguyaman Bay and to support and optimize Nike fisheries in the Gorontalo waters.

MATERIALS AND METHODS

Sampling

The samples of Nike fish were collected in Paguyaman Bay (N 00º31'1.10" and E 122º39'0.73") (Figure 1) for three days in one period of emergence on April 8–10, 2021. A total of 150 grams of Nike fish samples were taken from the fishermen by using fishing nets. The samples were then brought to the Hydrobioecology and Biometrics Laboratory, Faculty of Fisheries and Marine Sciences, Gorontalo State University, for analysis. The number of samples were calculated and then identified, referring to Sahami et al. (2019b) and Sahami et al. (2020) methods. The samples were photographed for morphometric analysis. After this, five individuals of the new variant discovery were taken and placed in a sample bottle with 95 % ethanol solution for genetic analysis.

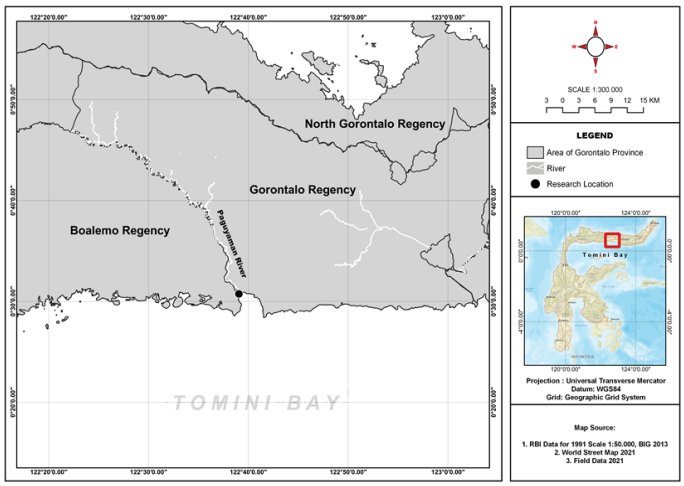

Figure 1. Research site map

Morphometric characters

Ten morphometric characters of Nike fish referring to Sahami et al. (2020) (Figure 2 and Table 1) were measured using the imageJ application with an accuracy of 0.001 cm.

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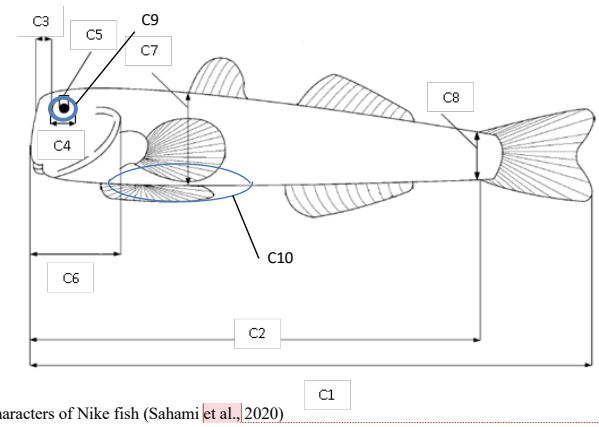

Figure 2. Morphometric characters of Nike fish (Sahami et al., 2020)

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Table 1. Morphometric characters of Nike fish (Sahami et al. 2020)

No	Morphometric Characters	No	Morphometric Characters
C1	*Total Length* (TL)	C6	*Head Length (HL)*
C2	*Standard Length* (SL)	C7	*Body Depth (BD)*
C3	*Preorbital Length* (PL)	C8	*Peduncle Depth (PD)*
C4	*Eye Diameter (ED)*	C9	*Eye Area (EA)*
C5	*Eye Lens diameter (EL)*	C10	*Yolk Sac area (YS)*

Each measured morphometric character datum was then standardized following the allometric formula according to Elliott et al. (1995) as follows:

$M_{adj} = M\ (L_s/L_0)^b$

M_{adj} is the standardized morphometric data, M is the measured morphometric data, L_0 is the total length of fish, L_s is the average total length, parameter *b* is the slope of log-linear curve M to log L_0 of all data.

DNA extraction, PCR amplification, and sequencing

The target gene for molecular analysis was the mitochondrial DNA Cytochrome Oxidase Subunit 1 (COI) gene. COI gene is the best resolution of the intraspecific level than other core genes (Bellagamba et al. 2015; Hubert et al. 2015; Rodrigues et al. 2017; Bingpeng et al. 2018; Roesma et al. 2018, Yulianto et al. 2020). In addition, mitochondrial COI genes are also widely and reliably utilized to identifying species in the gobi group (Jeon et al. 2012; Viswambharan et al. 2013; Laskar et al. 2016; Lejeune et al. 2016; Wang et al. 2017; Linh et al. 2018; Olii et al. 2019; Roesma et al. 2020; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020b; Sahami et al. 2020). Following the protocol kit, the 20 grams of fish meat tissues was isolated using Qiagen Tissue and Blood Extraction kits for genetic analysis. The mitochondrial DNA COI gene was further amplified using the primer pair FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). Samples were amplified at pre-denaturation temperature of 94 ºC for five minutes, denaturation at 94 ºC for 30 seconds, annealing at 50 ºC for 30 seconds, extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 7 minutes. This Polymerase Chain Reaction (PCR) process lasted for 38 cycles.

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Data analysis

The DNA samples were amplified and electrophoresed, and then the gene was sequenced using the Dideoxy Sanger Termination Method. Contig was done for the nucleotide sequences. After that, the results were matched with the data available in the GenBank database (www.ncbi.nlm.nih.gov) through the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was arranged by aligning the DNA sequences of identified samples with some DNA of gobies (accession number KF489573, KU232392, KU692483, KU692484, and KU692490) and the species of Nike fish schools in Gorontalo Bay (accession number MN069306, MN069307, MN069308, MT706639, MT706640, MT706641, MT706720, MT706721, MT706722, MT706723, MT706724, MT706725, MT706726, and MT706791) available in the GenBank database using the Maximum Likelihood 1,000 bootstrap method on MEGAX software (Kumar et al. 2018). The results of genetic identification were further confirmed morphologically, referring to Maeda and Tachihara (2005).

Furthermore, the Discriminant Function Analysis (DFA) (Landau and Everit 2004) was performed to determine the main distinctive characters among species using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

New variant of species that makes up the Nike fish schools

The emergence of Nike fish in Paguyaman Bay does not have a regular pattern as in Gorontalo Bay, which the periodization occurs almost every month. In 2021, Nike fish in Paguyaman Bay first appeared in April with a 3-day emergence period (April 8–10, 2021). Based on the NCBI database referring to the mitochondrial COI gene sequencing, a new melanophore pattern species was identified as *Eleotris melanosoma* (Figure 3). The nucleotide sequence of *E. melanosoma* was listed in the NCBI database with accession number MZ401475.

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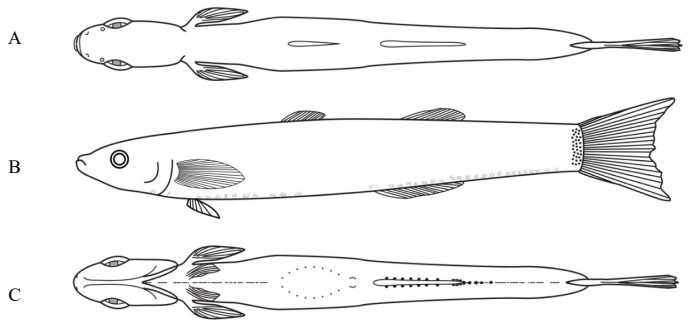

Figure 3. Pelagic larvae of *Eleotris melanosoma* in Paguyaman Bay. A. Dorsal view; B. Lateral view; C. Ventral view.

The caught species of *E. melanosoma* have a standard length of 19.05–20.04 mm with an average standard length of 19.55 mm. These species have a transparent body, an elongated and compressed body shape, and no scales. In addition, the body fins are not perfect yet, pelvic fins are separated, and the caudal fin tends to form emarginate. There are no melanophore spots distributed from head to body, and few melanophore spots accumulated at the base of the caudal fin. These morphological features correspond to the morphology of the pelagic larvae of *E. melanosoma* as reported by Maeda and Tachihara (2005).

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The results of the alignment of the DNA sequences of *E. melanosoma* with the DNA sequences of the species that make up the Nike fish school in Gorontalo Bay and several *Eleotris* species available in the NCBI Genbank are displayed through a phylogenetic tree (Figure 4). The analysis results show that *E. melanosoma* is closely related to *Eleotris fusca* and is in the same monophyletic clade as *Belobranchus segura*, *B. belobranchus*, and *Bunaka gyrinoides*. The similarity of the *clades* in these five species is because they are members of the *Eleotridae* family, although it is clear that there are sub-clades based on the similarity of the genus in this clade. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species *Stiphodon semoni, Sicyopterus longifilis, S. lagocephalus, S. parvei,* and *S. cynocephalus.* The second monophyletic clade also groups the species in the genus *Sicyopterus* in the same sub-clade and separated from the genus *Stiphodon*.

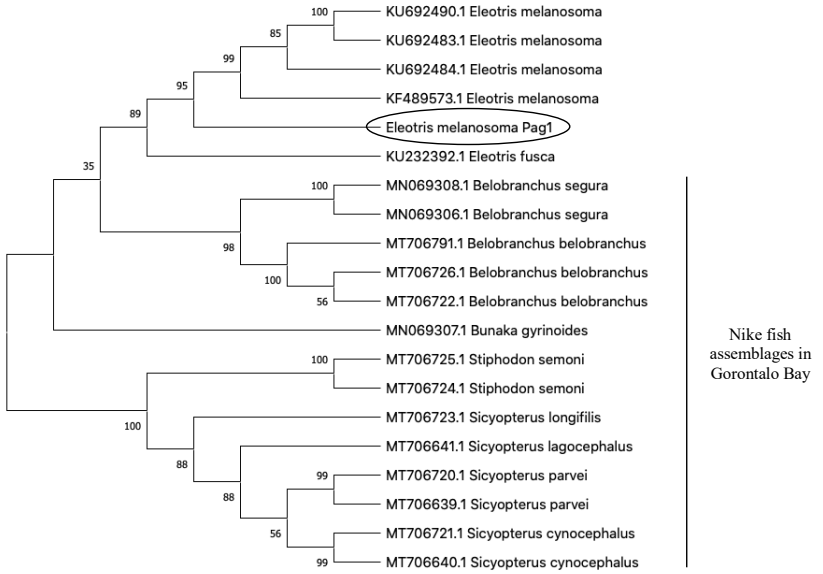

Figure 4. Phylogenetic tree of *Eleotris melanosoma* with several species of gobies and composers of Nike fish in Gorontalo Bay waters available in the NCBI database

Composition of species

A total of 1,773 specimens were observed as constituents of Nike fish schools in Paguyaman Bay for three days of emergence in April 2021. Two families, i.e., Gobiidae and Eleotridae, were recorded to make up Nike fish schools. The Gobiidae family consists of four species, i.e., *Sicyopterus longifilis, S. parvei, S. cynocephalus,* and *Stiphodon semoni*. Meanwhile, the Eleotridae family consists of three species, namely *Belobranchus segura, B. belobranchus*, and *Eleotris melanosoma*. Among seven species, three species in family Gobiidae, i.e., *S. longifilis, S. parvei*, and *Stiphodon semoni* were observed as species that made up Nike fish schools during their emergence. The species was consistently observed in considerable numbers i.e. S. *longifilis*, whereas *B. segura* was observed only on the second day. On the third day of emergence, *B. belobranchus* and *E. melanosoma* were observed. *S. semoni* is the most abundant species observed (34.07 %), meanwhile *S. parvei* and *B. segura* are at least 0.003 %. Based on the species composition, species dominance was different each day. On the first, second, and thir day, the school of Nike fish were dominated by *S. longifilis*, *Belobranchus segura*, and *Stiphodon semoni* with a composition value of (52.00 %), 63.27 %, and 83.43 %, respectively (Figure 5).

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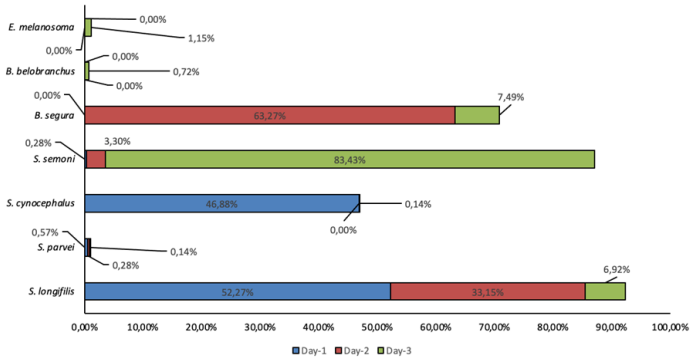

Figure 5. Nike fish schools composition in Paguyaman Bay waters

Size distribution and morphometric characters

The total length of the collected Nike fish ranged from 1,523 to 3,572 cm (Table 2). The *Stiphodon semoni* (Gobiidae) and *S. parvei* (Gobiidae) species have the smallest and biggest size. The species with the widest size range is *S. cynocephalus* (2.422–3.572 cm), and the species with the smallest size range is *B. belobranchus* (2.290–2.403 cm).

Table 2. Size range of each species making up Nike fish schools in Paguyaman Bay waters

No	Species	Mean of Size (cm)	Range of Size (cm)
1	*Sicyopterus longifilis*	2.666	2.262–3.044
2	*Sicyopterus parvei*	3.036	2.831–3.403
3	*Sicyopterus cynocephalus*	2.775	2.422–3.572
4	*Stiphodon semoni*	2.137	1.523–2.586
5	*Belobranchus segura*	2.311	2.005–2.638
6	*Belobranchus belobranchus*	2.347	2.290–2.403
7	*Eleotris melanosoma*	2.377	2.304–2.448

The morphometric characters can be used in taxonomy as early identification in fisheries science (Sara et al. 2016). The summary morphometric characters data standardized following the allometric formula Elliott et al. (1995) is presented in Table 3. Moreover, the analysis of discriminant function is presented in Figure 6. Two discriminant functions are able to explain 67.3 % and 24.5 % of the total variance of morphometric characters. Based on this work, the C7 character (body depth) is the main distinctive character of the Nike population in Paguyaman Bay. The tendency of Nike fish formation in Paguyaman Bay is clearly shown by a group of centroid in Figure 6. The species of *Sicyopterus longifilis, S. cynocephalus, S. Parvei*, and *S. semoni* tend to overlap and adjacent since four species are members of the Gobiidae family. Furthermore, *Belobranchus segura* and *Belobranchus belobranchus* are the most related spots because both species are from the same genus of *Belobranchus*. The *Eleotris melanosoma*, although was distributed in separate areas, is close to *B. belobranchus* because both species are members of the Eleotridae family. In general, species identification through canonical discriminant diagrams (Figure 6) is in line with the phylogenetic tree shown in Figure 4.

Table 3. Morphometric character data of Nike fish schools in Paguyaman Bay waters

Species	Unit of Characters (Mean (cm) ± SD)								
	SL	PL	ED	EL	HL	BD	PD	EA	YS
S. longifilis	2.054 ± 0.05	0.111 ± 0.02	0.136 ± 0.02	0.060 ± 0.01	0.518 ± 0.04	0.381 ± 0.03	0.208 ± 0.02	0.013 ± 0.00	0.026 ± 0.00
S. parvei	2.086 ± 0.03	0.128 ± 0.03	0.133 ± 0.02	0.062 ± 0.01	0.535 ± 0.03	0.376 ± 0.01	0.205 ± 0.01	0.012 ± 0.00	0.031 ± 0.01
S. cynocephalus	2.043 ± 0.05	0.114 ± 0.02	0.133 ± 0.02	0.057 ± 0.01	0.521 ± 0.05	0.375 ± 0.03	0.203 ± 0.02	0.012 ± 0.00	0.029 ± 0.01
S. semoni	2.062 ± 0.05	0.104 ± 0.02	0.129 ± 0.02	0.056 ± 0.01	0.489 ± 0.04	0.343 ± 0.04	0.188 ± 0.03	0.012 ± 0.00	0.020 ± 0.01
B. segura	2.094 ± 0.04	0.126 ± 0.02	0.129 ± 0.02	0.061 ± 0.01	0.556 ± 0.05	0.392 ± 0.03	0.221 ± 0.02	0.012 ± 0.00	0.035 ± 0.01
B. belobranchus	2.051 ± 0.05	0.140 ± 0.03	0.128 ± 0.02	0.067 ± 0.01	0.579 ± 0.03	0.325 ± 0.01	0.213 ± 0.01	0.015 ± 0.00	0.021 ± 0.00
E. melanosoma	2.033 ± 0.02	0.123 ± 0.01	0.129 ± 0.00	0.058 ± 0.01	0.535 ± 0.04	0.280 ± 0.02	0.208 ± 0.01	0.012 ± 0.00	0.013 ± 0.00

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1,523 cm is a very big fish!

Commented [JB28]: Mean size (cm)

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Notes: SL= *Standard Length; PL = Preorbital Length; ED = Eye Diameter; EL = Eye Lens diameter; HL = Head Length; BD = Body Depth; PD = Peduncle Depth; EA = Eye Area; YS = Yolk Sac area*

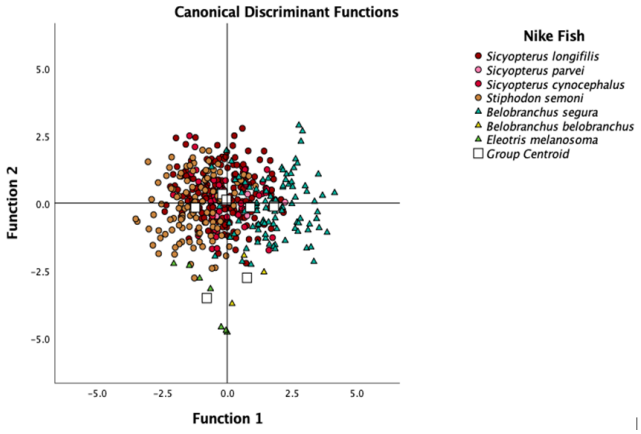

Figure 6. Diagram of the canonical discriminant function of Nike fish in Paguyaman Bay waters

Discussion

The species that make up the Nike fish schools in Paguyaman Bay consist of seven species, less than nine species reported in Gorontalo Bay (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Two species of gobies are *S. pugnans* and *Bunaka gyrinoides* are not observed as the constituents of Nike fish schools in Paguyaman Bay. However, the present study reports a new record of the distribution of *E. melanosoma*. Identifying *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay based on molecular and morphological analysis is a new record of the distribution of this species in Gorontalo waters. A total of eight species of *E. melanosoma* were caught as the constituents of the Nike fish schools in Paguyaman Bay when they began to approach the river estuary on the third day of their emergence.

The *E. melanosoma* species is one of the three main species of *Eleotris* reported to be distributed in the Indo-Pacific waters. Two of the other species are *E. fusca* and *E. acanthopoma* (Maeda et al. 2011; Mennesson et al. 2019; Subchan et al. 2020). The other *Eleotris* species can continue to be recorded when more studies focus on this topic in more locations in Indo-Pacific waters. As reported by Maeda and Tachihara (2005), the pelagic and newly settled larvae of *E. fusca* show a larger body size than *E. melanosoma* and *E. acanthopoma*. *Eleotris* pelagic larvae migrate to river areas by utilizing the rising tides at night. *E. melanosoma* and *E. acanthopoma* settle at the upper end of the tidal affected area, meanwhile *E. fusca* migrates to freshwater upstream against river water flow currents.

In terms of the size, the species of *E. melanosoma* caught in Paguyaman Bay (average standard length 19.55 mm) should in the newly settled larval stage based on research Maeda and Tachihara (2005) in the Teima River estuary area, Okinawa Island, with increasingly numerous visible body pigmentation and completely pigmented caudal fin with no distal border. However, the morphology of *E. melanosoma* species in the site area still characterizes the morphology of the pelagic larval stage, with the most conspicuous feature and no melanophore spots on its sides. Such a difference in feature is due to the difference in the aquatic habitat area (Habibie et al. 2018). Besides, the tropical area's stable temperature throughout the year has no significant effect on the fish rate growth (Habibie et al. 2015). The pelagic to juvenile larval stages of *E. melanosoma* species were also recorded in the estuary area of the Teima River, Okinawa Island (Maeda and Tachihara 2005) and Sri Lankan waters estuary (Batuwita et al. 2017). Meanwhile, the adult species were found in the fresh waters of Japan (Maeda et al. 2011); rivers on the Buton and Kabaena Islands (Tweedley et al. 2013); Opak River, Yogyakarta (Djumanto et al. 2013); freshwater of Sri Lanka (Batuwita et al. 2017); freshwater of West Sulawesi (Nurjirana et al. 2020); and Sanenrejo and Wonoasri River Resorts (Subchan et al. 2020).

Among amphidromous fish, the Eleotridae and Gobiidae (Teleostei: Gobioidei) are the most common families discovered in estuaries and freshwaters of the Indo-Pacific area. The Eleotrid species mostly inhabits the lower and middle parts of freshwater flows characterized by waiting for prey (Mennesson et al. 2015; Mennesson and Keith 2020). *Eleotris* pelagic larvae morphological features are transparent and compressed bodies, conspicuous swim bladder, and emarginate caudal fin (Maeda and Tachihara 2005). On the other hand, the adult species has a large blunt head, torpedo-shaped body, rounded caudal fin, prominent lower jaw, discrete fin ventral, has no lateral line on the sides of the body, and

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inconspicuous body color, mostly light brown, dark brown, or olive with some dazzled metallic (Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species of the Gobiidae family are active swimmers. They have pelvic fins that are fused and form a sucking disc to attach themselves to the substrate while actively swimming against currents towards the upstream (Keith 2003; Taillebois et al. 2014). The genus *Sicyopterus* uses its mouth as a secondary sucking disc that allows this genus to quickly access the upstream of the waterfall (Keith 2003).

The Nike fish both in Paguyaman Bay and Gorontalo Bay is an amphidromous species. As an amphidromous species, the adults are hatch in rivers, then larvae flow downstream (sea) and grow in coastal or offshore marine habitats, then recruitment occurs in rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively follow the river water currents after hatching in the afternoon and reach the estuary in the middle of the night (Maeda and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so that the larvae are carried downstream with lots of yolks, and the risk of starvation is minimized (McDowall 2009). One of the strategies employed is spawning in river areas near the estuary to shorten the larval drift time in freshwater environments (Lagarde et al. 2017).

The emergence period of Nike fish schools in the bay and moving into the estuary is pre-colonization phase. In this phase, gobies' post-larvae will swarm and prefer to swim near the shoreline because the water flowing is slower and can even be reversed by tides or waves (Keith et al. 2008). Making schools is a gobies strategy to avoid predators besides finding food (Keith 2003). The species' diversity and sizes making up the fish schools is strongly influenced by the characteristics of the species, season, and hatching time. According to Taillebois et al. (2012), the duration of the pelagic larvae is not the only factor determining species distribution; It is most likely interactions between larval behavior, environment, currents, and substrate preferences. Apart from these factors mentioned previously, the larger fish larvae possibly resultes from earlier hatching compared to smaller fish larvae (Mennesson et al. 2015). In addition, the rainy season will support the newly hatched larvae drifting into the sea faster (Keith 2003).

According to Landau and Everit (2004), the discriminant analysis determines the main distinctive character among populations. The present study confirmed that the main distinguishing characteristic among Nike fish populations in Paguyaman Bay is body depth. It is contrary to the main distinctive character of Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The difference in the main distinguishing characters between two locations since the gobies species are able to develop various morphological specificities as adaptations strategy for their environment (Gani et al. 2019; Roesma et al. 2020). Thus, the different backgrounds are able to affect the morphological characters of species in their environment. Populations in different environments are likely to have different population structures. Differences in population structure will influence population size, which can be observed through differences in morphometric and meristic characters (Aisyah and Syarif 2018).

Based on their species composition, the Nike fish schools in Paguyaman Bay waters indicate a trend of typical species dominance every day during the recruitment back to freshwaters. On the first day of their emergence in Paguyaman Bay, the Nike fish schools were composed of species in the Gobiidae family and dominated by *S. longifilis* (52,00 %). Furthermore, the species were dominated by *Belobranchus segura* (63,27 %) on the second day and *Stiphodon semoni* (83,43%) on the third day when the fish schools started to enter the river estuary. Nevertheless, further studies are required to explain these phenomena. In general, the gobies are adapt mostly to aquatic habitats, although with various abundance degrees (Gani et al. 2019).

In conclusion, the Nike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. Species with the new melanophore pattern is *E. melanosoma*, confirmed through morphological and molecular analysis. A new variant of *E. melanosoma* species as the first species of the genus *Eleotris* composes the Nike fish schools in Paguyaman Bay and Gorontalo waters in general. Exploration of adult species and their distribution in the freshwaters of the Paguyaman River assumed to be the habitat of adult Nike fish should be performed to ensure the sustainability of Nike fish resources in the future. Although the adult species of Nike fish is an unvalued economic commodity, unfortunately the post larva stage when it is recruiting back into the river is often targeted by fishermen for consumption (Ellien et al. 2016; Roesma et al. 2020), including in Gorontalo waters. Identification of these species must be carried out continuously to prevent the loss of these fish species while catching along with commodity fishing. This research indicates many types of species constituents in Gorontalo waters may be collected when an extensive study is conducted later in the future, specifically in river estuarine locations that are not explored.

ACKNOWLEDGEMENTS

The authors express our deep gratitude to the Institute of Research and Community Service (LPPM) of the Gorontalo State University that has funded this research through the scheme of Penelitian Dasar (Basic Research) for the 2021 Fiscal Year. We also would like to thank Thomas Tammu, Adistya, and all parties for their technical assistance and contribution during the study.

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(*COMMENT REVIEWER* B)

Diversity of species making up nike fish schools and a new record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia

Abstract. Nike fish is an essential economic commodity in Gorontalo with a distinctive and memorable taste. Several estuaries in the waters of Tomini Bay are reported as a primary location for Nike fishing in Gorontalo, one of them is Paguyaman Bay. Tomini Paguyaman Bay is an estuary of the Paguyaman River whose watershed crosses three regencies, i.e., Gorontalo, Boalemo, and Pohuwato. Observation of the species making up the Nike fish schools is crucial in its contribution to fish biodiversity. The present study aims to determine the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species that have never been reported for their distribution in Gorontalo waters. A total of 1,773 samples of Nike fish were caught in the sea to the estuary of Paguyaman Bay on one period of their appearance on April 8–10, 2021. The species were further grouped based on the similarity of melanophore patterns and analyzed morphometrically. The molecular identification was performed on species with new melanophore patterns. The results indicated that the Nike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. The identification of *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay is a new record of the distribution of this species in the waters of Gorontalo. Based on the species composition, Nike fish schools in the waters of Paguyaman Bay showed a typical species dominance trend during the recruitment process back to freshwater, i.e., *S. longifilis* (52.00 %) on the first day, *Belobranchus segura* (63.27 %) on the second day, and *Stiphodon semoni* (83.43 %) on the third day.

Keywords: Belobranchus, Eleotris, Sicydiinae, Sicyopterus, Stiphodon.

Abbreviations: Basic Local Alignment Search Tools (BLAST), Cytochrome Oxidase I (COI), Deoxyribonucleic acid (DNA), Polymerase Chain Reaction (PCR)

Running title: Diversity of Nike fish schools in Paguyaman Bay

INTRODUCTION

Tomini Bay is the largest bay in Indonesia which has direct contact with 14 districts or cities in three provinces in Sulawesi, such as North Sulawesi, Central Sulawesi, and Gorontalo. One of the essential fishery commodities from Tomini Gorontalo Bay is Nike fish. They consist of amphidromous gobi schools in the pelagic to juvenile larval stages in the recruitment process from marine waters to freshwaters. Their embryos that hatch will be carried by rivers to the sea and develop as pelagic larvae before being recruited back into rivers and grow into adults and spawn (Thuesen et al. 2011; Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The diversity of species making up Nike fish schools (postlarva gobi) in Gorontalo waters is one of the studies that need to be continuously observed and conducted, considering the potential economic value of Nike fish as a food commodity in Gorontalo is quite high (Wolok et al. 2019). About 1,120 species from 30 genera in the Gobi group have been described to date. The gobies group plays a vital role in ichthyofauna diversity with a wide distribution area and a high number of species. This group of fish is dominantly found in eastern Indonesia, with a fairly high diversity of species (Tweedley 2013; Miesen et al. 2016; Hadiaty and Sauri 2017; Nurjirana et al. 2020).

There are, geographically, several rivers in Gorontalo which disembogue the sea waters of Tomini Bay. Some of the river estuaries are important locations for Nike fish catching in Gorontalo, including Gorontalo Bay and Paguyaman Bay (Salam et al. 2016). Nike fish in Gorontalo Bay has been studied by many researchers (Olii et al. 2017; Olii et al. 2019; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020a; Sahami et al. 2020) ~~due to its close location and significant productivity~~. Meanwhile, scientific data on Nike fisheries in the Paguyaman Bay estuary has not been widely observed. Paguyaman Bay is part of the Tomini Bay areas located on the border of Girisa Village, Paguyaman District, Boalemo Regency with Bilato Village, Boliyohuto District, Gorontalo Regency. This bay is the estuary of the Paguyaman River, whose watershed crosses three regencies, i.e., Gorontalo, Boalemo, and Pohuwato. The presence of the Paguyaman

Commented [D1]: Diversity of species which compose of the nike fish mobs and the new variant of Eleotris melanosoma in Tomini Paguyaman Bay, Gorontalo, Indonesia

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Commented [D3]: The observation of the species which compose of the Nike fish mobs is an crucial for fish biodiversity contribution.

Commented [D4]: The present study aims to determine the diversity of species composing the Nike fish mobs in Paguyaman Bay and reveals the types of species were unreported before which distributed in Gorontalo waters

Commented [D5]: A total of 1,773 samples of Nike fish were collected from the sea in the Paguyaman Bay estuary for a period of their first appearance on April 8–10, 2021. The species were grouped based on the melanophore patterns similarity and then analyzed by morphometrically. The molecular identification of species was performed refers to the new melanophore patterns.

Commented [D6]: The results showed the Nike fish mobs in Paguyaman Bay consist of seven species, four genera, and two families, i.e., Sicyopterus longifilis, S. parvei, S. cynocephalus, Stiphodon semoni, Belobranchus segura, B. belobranchus, and Eleotris melanosoma. The first finding of E. melanosoma as a species composing the Nike fish mobs in Paguyaman Bay is a new variant discovery of the distribution of this species in Gorontalo waters. Based on the species composition, Nike fish mobs in Paguyaman Bay waters show a typical species dominance trend during the mobilization process returning to freshwater, i.e., S. longifilis (52.00 %) on the first day, Belobranchus segura (63.27 %) on the second day, and Stiphodon semoni (83.43 %) on the third day.

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Commented [D8]: Tomini Bay is Indonesia's largest bay which is directly related with 14 districts in three provinces of Sulawesi, such as North Sulawesi, Central Sulawesi, and Gorontalo. Nike fish is one of the essential fishery commodities in Tomini Gorontalo bay communities. It consists of amphidromous gobi mobs in the pelagic to juvenile larval stages in the mobilization process from marine waters to freshwaters. The hatched embryos were carried by the rivers flow to the sea and develop as pelagic larvae before being recruited returning to rivers and grow until adults and spawn.

Commented [D9]: The diversity of species composing the Nike fish mobs (postlarva gobi) in Gorontalo waters is one of the important studies that should be conducted continuously, considering the high potential economic value as a food commodity in Gorontalo (Wolok et al. 2019). Currently, approximately 1,120 species from 30 genera in the Gobi group were described. The gobies group plays a vital role in ichthyofauna diversity with a wide area distribution beside a high number of species. These fish groups are dominantly found in eastern Indonesia waters, with a highly species diversity (Tweedley 2013; Miesen et al. 2016; Hadiaty and Sauri 2017; Nurjirana et al. 2020).

Commented [D10]: The geographically, there are several rivers in Gorontalo are flowing into the Tomini Bay. Some of these river estuaries are important locations for Nike fish catching in Gorontalo, including Gorontalo Bay and Paguyaman Bay (Salam et al. 2016). The studies on Nike fish in Gorontalo bay were reported by many researchers

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watershed as the second-largest watershed in Gorontalo and the direct contact with three administrative regencies make the location of the Paguyaman Bay estuary interesting to observe.

Demographic information on fish, such as life history at early stages, recruitment, migration patterns, and other biological characteristics, are crucial data for managing fish populations (Habibie et al. 2015). Besides, information on the identification and composition of species is also an essential part of management efforts, and thus, the management can be carried out effectively. The types of species making up the Nike fish schools in the waters of Gorontalo City Bay are reported consisting of nine species to date, i.e., *Sicyopterus pugnans, S. longifilis, S. lagocephalus, S. cynocephalus, S. parvei, Bunaka Gyrinoides, Belobranchus segura, B. Belobranchus,* and *Stiphodon semoni* (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). ~~The present study aims to find out the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species that have never been reported for their distribution in the waters of Gorontalo Bay.~~ The data are then expected to be significant as scientific information for the basis of Nike fish resource management in Paguyaman Bay and to support attempts to optimize Nike fishery potential in the Gorontalo waters.

MATERIALS AND METHODS

Sampling

The sampling of Nike fish in Paguyaman Bay (N 00º31’1.10” and E 122º39’0.73”) (Figure 1) was performed for three days in one appearance period on April 8–10, 2021. A total of 150 grams of Nike fish samples were taken from the catch of fishermen using nets every day. The samples were brought to the Hydrobioecology and Biometrics Laboratory, Faculty of Fisheries and Marine Sciences, Gorontalo State University for analysis. The samples were then identified for its species by referring to Sahami et al. (2019b) and Sahami et al. (2020) and counted for its number. The samples were then photographed for morphometric analysis. ~~If the number of samples were large, then the sample for morphometric analysis would only be limited to 50 individuals.~~ The species with new melanophores were also photographed, sketched, morphologically described, and molecularly analyzed.

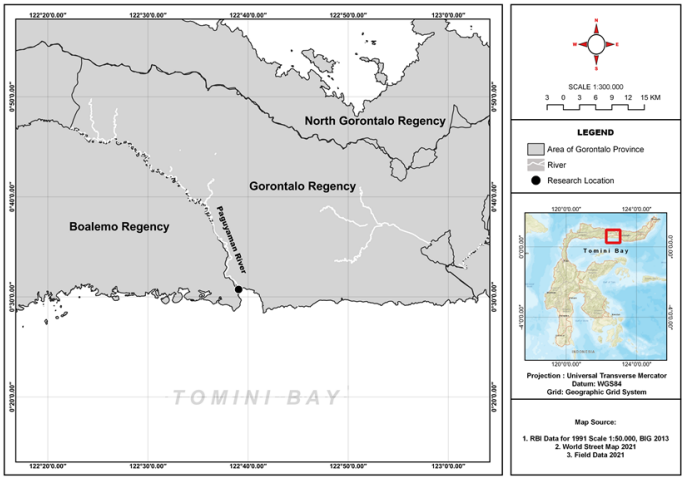

Figure 1. Research location map

Morphometric characters

Ten morphometric characters of Nike fish referring to Sahami et al. (2020) (Figure 2 and Table 1) were measured using the imageJ application with an accuracy of 0.001 cm.

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Commented [D13]: The demographic information of these fish such as life history at early stages, mobilizaion, migration patterns, and other biological characteristics are crucial information for managing fish populations (Habibie et al. 2015). In addition, information about the identification and composition of species are also an essential part of management efforts. Hence, the management could be carried out effectively. As currently reported, the types of species composing the Nike fish mobs in Gorontalo City Bay are composing to nine species i.e., Sicyopterus pugnans, S. longifilis, S. lagocephalus, S. cynocephalus, S. parvei, Bunaka Gyrinoides, Belobranchus segura, B. Belobranchus, and Stiphodon semoni (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020).

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Commented [D17]: The Nike fish samples were collected in Paguyaman Bay (N 00º31’1.10” and E 122º39’0.73”) (Figure 1) for three days at the first appearance period on April 8–10, 2021. A total of 150 grams of Nike fish samples were taken from the fishermen by using fishing nets. The samples were brought to the Hydrobioecology and Biometrics Laboratory, Faculty of Fisheries and Marine Sciences, Gorontalo State University for analysis. The number of samples were calculated and then identified referring to Sahami et al. (2019b) and Sahami et al. (2020) methods. The samples were documented by photographed for morphometric analysis, while the new melanophores were analyzed by DNA sequences

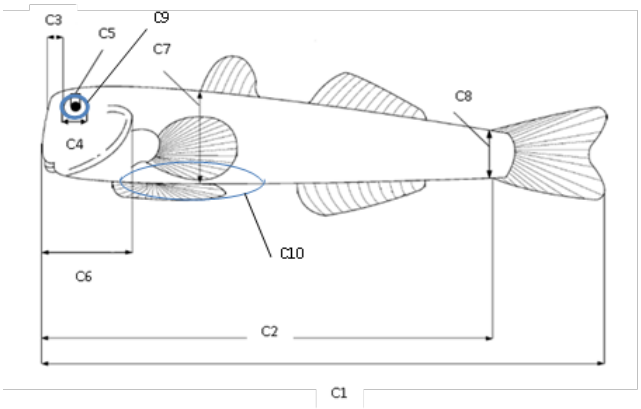

Figure 2. Morphometric characters of Nike fish (Sahami et al., 2020)

Table 1. Morphometric characters of Nike fish (Sahami et al. 2020)

No	Morphometric Characters	No	Morphometric Characters
C1	*Total Length* (TL)	C6	*Head Length (HL)*
C2	*Standard Length* (SL)	C7	*Body Depth (BD)*
C3	*Preorbital Length* (PL)	C8	*Peduncle Depth (PD)*
C4	*Eye Diameter (ED)*	C9	*Eye Area (EA)*
C5	*Eye Lens diameter (EL)*	C10	*Yolk Sac area (YS)*

Each measured morphometric character data was then standardized following the allometric formula according to Elliott et al. (1995) as follows:

$M_{adj} = M\ (L_s/L_0)^b$

M_{adj} is the standardized morphometric data, M is the measured morphometric data, L_0 is the total length of fish, L_s is the average total length, and parameter *b* is the slope of log linear curve M to log L_0 of all data.

DNA extraction, PCR amplification, and sequencing

The target gene for molecular analysis is the mitochondrial DNA Cytochrome Oxidase Subunit 1 (COI) gene. COI gene had better resolution at the intraspecific level than the other core genes (Bellagamba et al. 2015; Hubert et al. 2015; Rodrigues et al. 2017; Bingpeng et al. 2018; Roesma et al. 2018, Yulianto et al. 2020). In addition, mitochondrial COI genes are also widely and reliably used in identifying species in the gobi group (Jeon et al. 2012; Viswambharan et al. 2013; Laskar et al. 2016; Lejeune et al. 2016; Wang et al. 2017; Linh et al. 2018; Olii et al. 2019; Roesma et al. 2020; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020b; Sahami et al. 2020). Following the protocol kit, the DNA sample of fish tissue was isolated using Qiagen Tissue and Blood Extraction kits. The mitochondrial DNA COI gene was further amplified using the primer pair FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). Samples were amplified at pre-denaturation temperature of 94 ºC for five minutes, denaturation at 94 ºC for 30 seconds, annealing at 50 ºC for 30 seconds, extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 7 minutes. This Polymerase Chain Reaction (PCR) process lasted for 38 cycles.

Data analysis

The DNA samples that had been amplified and electrophoresed were then sequenced to obtain their nucleotide sequences using the Dideoxy Sanger Termination Method ~~through PT Genetika Science Indonesia~~. The nucleotide sequences resulting from DNA sequencing that had been processed and carried out contig. After that, the results were matched with the data available in the GenBank database (www.ncbi.nlm.nih.gov) through the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was arranged by aligning the DNA sequences of identified samples with some DNA of gobies (accession number KF489573, KU232392, KU692483, KU692484, and KU692490) and the species of Nike fish schools in Gorontalo Bay (accession number MN069306, MN069307, MN069308, MT706639, MT706640, MT706641, MT706720, MT706721, MT706722, MT706723, MT706724, MT706725, MT706726, and MT706791) available in the GenBank database using the Maximum Likelihood 1000 bootstrap method on MEGAX software (Kumar et al. 2018). The results of genetic identification were further confirmed morphologically, referring to Maeda and

Commented [D18]: The targeted gene for molecular analysis is the mitochondrial DNA Cytochrome Oxidase Subunit 1 (COI) gene. COI gene are the best resolution of the intraspecific level than other core genes

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Tachihara (2005). Furthermore, the Discriminant Function Analysis (DFA) (Landau and Everit 2004) was performed to find out the main distinctive characters among species by using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

New records of species that makes up the nike fish schools

The appearance of Nike fish in Paguyaman Bay does not have a regular rhythm like in Gorontalo Bay, where the periodization occurs almost every month. In 2021, Nike fish in Paguyaman Bay first appeared in April with a 3-day appearance period, namely April 8–10, 2021. A new melanophore pattern of species that make up the Nike fish school was found on the third day of its appearance. Mitochondrial COI gene sequencing results showed that the species with the new melanophore pattern was identified as *Eleotris melanosoma* (Figure 3) based on the NCBI database. The nucleotide arrangement of *E. melanosoma* had been entered in the NCBI database with accession number MZ401475.

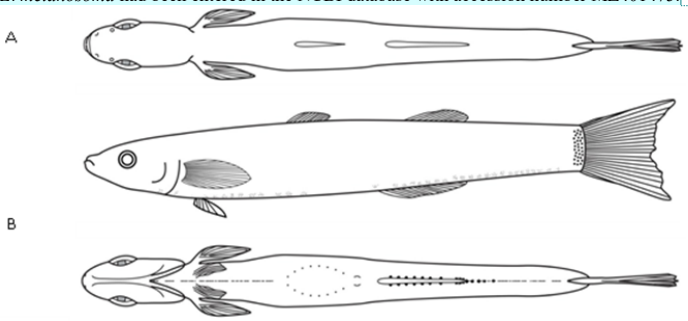

Figure 3. Pelagic larvae of *Eleotris melanosoma* in Paguyaman Bay. A. Dorsal view; B. Lateral view; C. Ventral view.

The caught species of *E. melanosoma* had a **standard length of 19.05–20.04 mm with an average standard length of 19.55 mm.** This species has a transparent body, an elongated and compressed body shape, has no scales, body fins are not perfect yet, pelvic fins are separated, and the caudal fin tends to form emarginate. There are no melanophore points distributed from head to body, and few melanophore points accumulate at the base of the caudal fin. This morphological feature is in line with the morphology of the pelagic larvae of *E. melanosoma* reported by Maeda and Tachihara (2005).

The results of the alignment of the DNA sequences of *E. melanosoma* with the DNA sequences of the species that make up the Nike fish school in Gorontalo Bay and several *Eleotris* species available in the NCBI Genbank are displayed through a phylogenetic tree (Figure 4). The analysis results show that *E. melanosoma* is very closely related to *Eleotris fusca* and is in the same monophyletic clade as *Belobranchus segura*, *B. belobranchus*, and *Bunaka gyrinoides*. The similarity of the *clades* in these five species is because they are members of the *Eleotridae* family, although it is clear that there are sub-clades based on the similarity of the genus in this clade. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species *Stiphodon semoni, Sicyopterus longifilis, S. lagocephalus, S. parvei,* and *S. cynocephalus.* The second monophyletic clade also clearly groups species in the genus *Sicyopterus* in the same sub-clade and separate from the genus *Stiphodon*.

Commented [D23]: New variant species compose the nike fish mobs

Commented [D24]: The appearance of Nike fish in Paguyaman Bay have not a regular rhythm like in Gorontalo Bay, where the periodization occurs almost every month. In 2021, Nike fish in Paguyaman Bay first appeared on April with a 3-day appearance period, namely April 8–10, 2021. A new melanophore pattern of species whose compose the Nike fish mobs was found on the third day of its appearance. Based on the NCBI database refer to the mitochondrial COI gene sequencing of new melanophore pattern species was identified as Eleotris melanosoma (Figure 3). The nucleotide sequences of E. melanosoma was listed in the NCBI database with accession number MZ401475.

Commented [D25]: Confuse!! Body length?

Commented [D26]: The average body length of E. melanosoma is 19.05–20.04 mm. These species with a transparent body, an elongated and compressed body shape, no scales, body fins are not perfect yet, pelvic fins are separated, and the caudal fin tends to form emarginate. There are no melanophore spots distributed from head to body, few melanophore spots accumulate at the base of the caudal fin. These morphological features corresponds to the morphology of the pelagic larvae of E. melanosoma as reported by Maeda and Tachihara (2005).

Commented [D27]: The similarity of the clades in these five species is because they are members of the Eleotridae family, although it is clear that there are sub-clades based on the similarity of the genus in this clade. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species Stiphodon semoni, Sicyopterus longifilis, S. lagocephalus, S. parvei, and S. cynocephalus. The second monophyletic clade also clearly groups species in the genus Sicyopterus in the same sub-clade and separate from the genus Stiphodon. It caused five species to be members of the Eleotridae family, although there are sub-clades based on the similarity of the genus in the clades. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species Stiphodon semoni, Sicyopterus longifilis, S. lagocephalus, S. parvei, and S. cynocephalus. The second monophyletic clade also grouping species in the genus Sicyopterus in the same sub-clade and separate from the genus Stiphodon

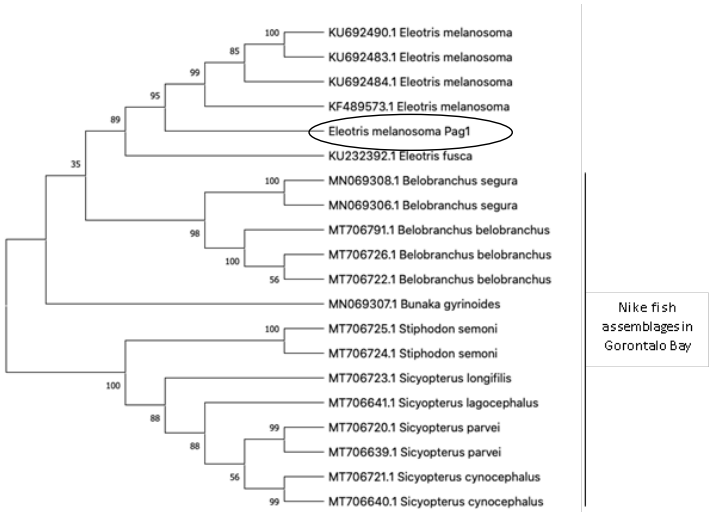

Figure 4. Phylogenetic tree of *Eleotris melanosoma* with several species of gobies and composers of Nike fish in Gorontalo Bay waters available in the NCBI database

Composition of species

A total of 1,773 specimens were found as composers making up Nike fish schools in Paguyaman Bay for three days of appearance in April 2021. Two families, i.e., Gobiidae and Eleotridae, were found to make up Nike fish schools. The Gobiidae family consists of four species, i.e., *Sicyopterus longifilis, S. parvei, S. cynocephalus,* and *Stiphodon semoni*. Meanwhile, the Eleotridae family consists of three species, i.e., *Belobranchus segura, B. belobranchus*, and *Eleotris melanosoma*. Among the seven species, three species from the family Gobiidae, i.e., *S. longifilis, S. parvei*, and *Stiphodon semoni* were found as composers making up the Nike fish schools during three days of their emergence. The type of species that was consistently found in considerable numbers was S. *longifilis.* Species of *B. segura* were only found on the second day, while *B. belobranchus* and *E. melanosoma* were only found on the third day of appearance of Nike fish. The most abundant species found were *S. semoni* (34.07 %), while the least were *S. parvei* and *B. segura* with 0.003 % each. Based on the species composition, the tendency of species dominance was different each day. On the first day, the school of Nike fish was dominated by *S. longifilis* (52.00 %), the second day was dominated by *Belobranchus segura* (63.27 %), and the third day was dominated by *Stiphodon semoni* with a composition value of 83.43 % (Figure 5).

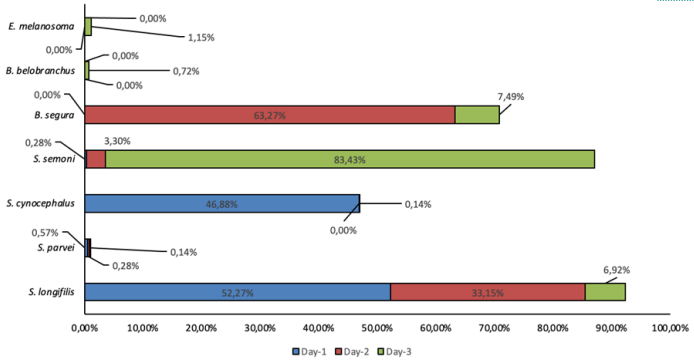

Figure 5. Nike fish schools composition in Paguyaman Bay waters

Commented [D28]: A total of 1,773 specimens were observed as composers of Nike fish mobs in Paguyaman Bay for three days of appearance in April 2021. Two families, i.e., Gobiidae and Eleotridae were recorded as the Nike fish mobs. The Gobiidae family consists of four species, i.e., Sicyopterus longifilis, S. parvei, S. cynocephalus, and Stiphodon semoni. Meanwhile, the Eleotridae family consists of three species, i.e., Belobranchus segura, B. belobranchus, and Eleotris melanosoma. Among seven species, three species members of the family Gobiidae, i.e., S. longifilis, S. parvei, and Stiphodon semoni were observed as composers of the Nike fish mobs during three days of their appearance. The species was consistently observed in considerable numbers i.e S. longifilis, whereas B. segura was observed only the second day, while B. belobranchus and E. melanosoma were only observed on the third day of appearance periods. S. semoni is the most abundant species observed (34.07 %), while S. parvei and B. segura are at least 0.003 %. Based on the species composition, species dominance was different each day. On the first, second and third day, the Nike fish mobs was dominated by S. longifilis, Belobranchus segura, and Stiphodon semoni with a composition value of 52.00 %, 63.27 %, and 83.43 %, respectively (Figure 5).

Commented [D29]: The nike fish mobs composotion in Paguyaman Bay waters

Size distribution and morphometric characters

The total length of Nike fish that had been caught, in general, ranged from 1,523 to 3,572 cm (Table 2). The species found with the smallest size was *Stiphodon semoni* (Gobiidae), while the largest was *S. parvei* (Gobiidae). The species with the broadest size range was *S. cynocephalus* (1,150 cm), while the species with the smallest size range was *B. belobranchus* (0,113 cm).

Table 2. Size Range of Each Species Making Up Nike Fish Schools in Paguyaman Bay Waters

No	Species	Mean of Size (cm)	Range of Size (cm)
1	*Sicyopterus longifilis*	2.666	2.262–3.044
2	*Sicyopterus parvei*	3.036	2.831–3.403
3	*Sicyopterus cynocephalus*	2.775	2.422–3.572
4	*Stiphodon semoni*	2.137	1.523–2.586
5	*Belobranchus segura*	2.311	2.005–2.638
6	*Belobranchus belobranchus*	2.347	2.290–2.403
7	*Eleotris melanosoma*	2.377	2.304–2.448

The morphometric characters can be used in taxonomy as early identification in fisheries science (Sara et al. 2016). The summary of data on the measurement results of morphometric characters that have been standardized following the allometric formula Elliott et al. (1995) is presented in Table 3. Furthermore, the results of the analysis on the discriminant function are shown in Figure 6. Two discriminant functions, respectively, can explain 67.3 % and 24.5 % of total variance of morphometric characters. Based on the results of the analysis, the main distinctive character of the Nike population in Paguyaman Bay was the C7 character (body depth). Figure 6 shows the tendency for the formation of groupings of Nike fish samples in Paguyaman Bay. *Sicyopterus longifilis, S. cynocephalus, S. Parvei*, and *S. semoni* indicated a tendency to form overlapping and close points since the four species are members of the Gobiidae family. Furthermore, *Belobranchus segura* and *Belobranchus belobranchus* have adjacent points because these two species are members of the same genus, the genus *Belobranchus*. The *Eleotris melanosoma*, although they have been distributed in separate areas, are quite close to the *B. belobranchus* species because these two species are members of the Eleotridae family.

Table 3. Morphometric Character Data of Nike Fish Schools in Paguyaman Bay Waters

Species	Unit of Characters (cm)								
	SL	PL	ED	EL	HL	BD	PD	EA	YS
S. longifilis	2.054 ± 0.05	0.111 ± 0.02	0.136 ± 0.02	0.060 ± 0.01	0.518 ± 0.04	0.381 ± 0.03	0.208 ± 0.02	0.013 ± 0.00	0.026 ± 0.00
S. parvei	2.086 ± 0.03	0.128 ± 0.03	0.133 ± 0.02	0.062 ± 0.01	0.535 ± 0.03	0.376 ± 0.01	0.205 ± 0.01	0.012 ± 0.00	0.031 ± 0.01
S. cynocephalus	2.043 ± 0.05	0.114 ± 0.02	0.133 ± 0.02	0.057 ± 0.01	0.521 ± 0.05	0.375 ± 0.03	0.203 ± 0.02	0.012 ± 0.00	0.029 ± 0.01
S. semoni	2.062 ± 0.05	0.104 ± 0.02	0.129 ± 0.02	0.056 ± 0.01	0.489 ± 0.04	0.343 ± 0.04	0.188 ± 0.03	0.012 ± 0.00	0.020 ± 0.01
B. segura	2.094 ± 0.04	0.126 ± 0.02	0.129 ± 0.02	0.061 ± 0.01	0.556 ± 0.05	0.392 ± 0.03	0.221 ± 0.02	0.012 ± 0.00	0.035 ± 0.01
B. belobranchus	2.051 ± 0.05	0.140 ± 0.03	0.128 ± 0.02	0.067 ± 0.01	0.579 ± 0.03	0.325 ± 0.01	0.213 ± 0.01	0.015 ± 0.00	0.021 ± 0.00
E. melanosoma	2.033 ± 0.02	0.123 ± 0.01	0.129 ± 0.00	0.058 ± 0.01	0.535 ± 0.04	0.280 ± 0.02	0.208 ± 0.01	0.012 ± 0.00	0.013 ± 0.00

Notes: SL= *Standard Length; PL = Preorbital Length; ED = Eye Diameter; EL = Eye Lens diameter; HL = Head Length; BD = Body Depth; PD = Peduncle Depth; EA = Eye Area; YS = Yolk Sac area*

Commented [D30]: The total length of Nike fish collected ranged from 1,523 to 3,572 cm (Table 2). The Stiphodon semoni (Gobiidae) and S. parvei (Gobiidae) species are the smallest (1,150 cm) and biggest size (0,113 cm), respectively.

Commented [D31]: The morphometric characters can be used in taxonomy as early identification in fisheries science (Sara et al. 2016). The summary of morphometric characters data were standardized following the allometric formula Elliott et al. (1995) is presented in Table 3. Furthermore, the analysis of discriminant function is shown in Figure 6. Two discriminant functions able to explain 67.3 % and 24.5 % of total variance of morphometric characters. Based on these works, the C7 character (body deep) is the main distinctive character of the Nike population in Paguyaman Bay. The tendency of Nike fish formation in Paguyaman Bay was shown in Figure 6. The species of Sicyopterus longifilis, S. cynocephalus, S. Parvei, and S. semoni tend to overlap and closed since four species are members of the Gobiidae family. Furthermore, Belobranchus segura and Belobranchus belobranchus are the most related spots because both species are the same genus members of Belobranchus. The Eleotris melanosoma, although was distributed in separate areas, however the B. belobranchus species are closest because both species are members of the Eleotridae family.

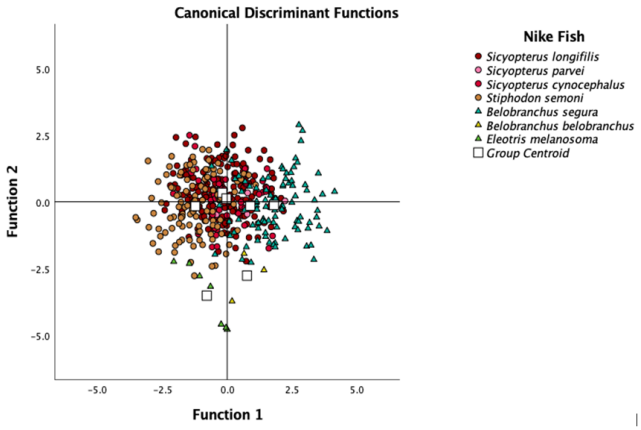

Figure 6. Diagram of the canonical discriminant function of Nike fish in Paguyaman Bay waters

Discussion

The types of species making up the Nike fish schools in Paguyaman Bay only consist of seven species, fewer than those making up the Nike fish schools in Gorontalo Bay, which has been reported to consist of nine species to date (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Two species of gobies, *S. pugnans* and *Bunaka gyrinoides*, were not found as the composers of Nike fish schools in Paguyaman Bay. However, the present study reported a new record of the distribution of *E. melanosoma*. ~~Identifying *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay based on molecular and morphological analysis is a new record of the distribution of this species in Gorontalo waters. A total of eight species of *E. melanosoma* were caught as the composers of the Nike fish schools in Paguyaman Bay when they began to approach the river estuary on the third day of their appearance.~~

The species *E. melanosoma* is one of three main species of *Eleotris* reported to be distributed in the Indo-Pacific waters. The other two species are *E. fusca* and *E. acanthopoma* (Maeda et al. 2011; Mennesson et al. 2019; Subchan et al. 2020). Additional *Eleotris* species may continue to be recorded as more research focuses on this research topic in more Indo-Pacific locations. Maeda and Tachihara (2005) reported that the pelagic and newly settled larvae of *E. fusca* had a larger body size than *E. melanosoma* and *E. acanthopoma*. *Eleotris* pelagic larvae migrate to river areas by utilizing the rising tides at night. *E. melanosoma* and *E. acanthopoma* settle at the upper end of the tidal affected area, while *E. fusca* migrates upstream of freshwater against river currents.

Based on the size, the species of *E. melanosoma* caught in Paguyaman Bay (average standard length 19.55 mm) should have been in the newly settled larval stage based on research Maeda and Tachihara (2005) in the Teima River estuary area, Okinawa Island with visible body pigmentation increasingly numerous and completely pigmented caudal fin with no distal border. However, the morphology of *E. melanosoma* species in Paguyaman Bay still characterizes the morphology of the pelagic larval stage, with the most striking feature that it does not have melanophore dots on its sides. This difference can occur due to differences in the aquatic habitat of the fishing area (Habibie et al. 2018). In addition, the fairly stable temperature throughout the year in the tropics area has no significant effect on the growth rate of fish (Habibie et al. 2015). The pelagic to juvenile larval stages of *E. melanosoma* species also recorded in the estuary area of the Teima River, Okinawa Island (Maeda and Tachihara 2005) and Sri Lankan waters estuary (Batuwita et al. 2017). Meanwhile, the adult species was found in the fresh waters of Japan (Maeda et al. 2011); rivers on the Buton and Kabaena Islands (Tweedley et al. 2013); Opak River, Yogyakarta (Djumanto et al. 2013); freshwater of Sri Lanka (Batuwita et al. 2017); freshwater of West Sulawesi (Nurjirana et al. 2020); and Sanenrejo and Wonoasri River Resorts (Subchan et al. 2020).

Among amphidromous fish, the Eleotridae and Gobiidae (Teleostei: Gobioidei) are the two most common families found in estuaries and freshwaters of the Indo-Pacific region. Eleotrid species most commonly inhabit the lower and middle parts of freshwater streams with the characteristic of sitting and waiting for prey (Mennesson et al. 2015; Mennesson and Keith 2020). *Eleotris* pelagic larvae morphological features are transparent and compressed bodies, conspicuous swim bladder, and emarginate caudal fin (Maeda and Tachihara 2005). While the adult species has a large blunt head, torpedo-shaped body, rounded caudal fin, prominent lower jaw, fin ventral discrete, has no lateral line on the sides of the body, and has an inconspicuous body-color, mostly light brown or dark brown or olive with some metallic sheen (Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species in the Gobiidae family are active swimmers. This species has pelvic fins that are fused and form a sucking disc that can be used to attach themselves to the substrate while actively swimming against currents upstream (Keith 2003; Taillebois et al. 2014). The

Commented [D32]: The species which compose the Nike fish mobs in Paguyaman Bay consist of seven species, less than in Gorontalo Bay are nine species as reported (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Two species of gobies are S. pugnans and Bunaka gyrinoides were not observed as the composers of Nike fish mobs in Paguyaman Bay. However, the present study reported a new variant species of E. melanosoma by morphological and molecular analysis.

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Commented [D34]: The species E. melanosoma is one of three main species of Eleotris reported distributed in the Indo-Pacific waters. Two of the other species are E. fusca and E. acanthopoma (Maeda et al. 2011; Mennesson et al. 2019; Subchan et al. 2020). The other Eleotris species are able to continue to be recorded when more research focuses on this topic in more locations in Indo-Pacific waters. As reported by Maeda and Tachihara (2005), the pelagic and newly settled larvae of E. fusca show a larger body size than E. melanosoma and E. acanthopoma. Eleotris pelagic larvae migrate to river areas by utilizing the rising tides at night. E. melanosoma and E. acanthopoma settle at the upper end of the tidal affected area, while E. fusca migrates upstream of freshwater against river water flow currents

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genus *Sicyopterus* uses its mouth as a secondary sucking disc that allows this genus to quickly access the headwaters above the waterfall (Keith 2003).

Like the Nike fish in Gorontalo Bay, the Nike fish in Paguyaman Bay is also an amphidromous species. As an amphidromous species, adults hatch in rivers, larvae then flow downstream (sea) and grow in coastal or offshore marine habitats, then recruitment occurs into rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively drift with the river currents shortly after hatching at dusk and reach the estuary area in the middle of the night (Maeda and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so the larvae are carried downstream with lots of yolks and the risk of starvation is minimized (McDowall 2009). One of the strategies that has been developed is spawning in river areas that are quite close to the estuary to shorten the larval drift time in freshwater environments (Lagarde et al. 2017).

The period of Nike fish appearance in groups in the bay area and moving into the estuary is pre-colonization. In this phase, gobies' postlarvae will swarm and choose to swim near the shoreline, where the flow is weaker and can even be reversed by tides or waves (Keith et al. 2008). Schooling is a gobies strategy to avoid predators and find food (Keith 2003). The diversity of species and sizes of species making up the fish schools is strongly influenced by the characteristics of the species, season, and hatching time. Taillebois et al. (2012) explained that the duration of pelagic larvae is not the only factor in determining species distribution. Still, it is most likely the result of interactions between larval behavior, environment, currents, and substrate preferences. Apart from these factors, fish larvae found in larger sizes are very likely to be the result of earlier hatching compared to smaller fish larvae (Mennesson et al. 2015). The rainy season will help the newly hatched larvae drift faster to the sea (Keith 2003).

The discriminant analysis determines the main distinctive character among populations (Landau and Everit 2004). The present study confirmed that the main distinctive character among Nike fish populations in Paguyaman Bay is body depth, in contrast to the main distinctive character among Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The distinction in the main distinctive characters between the two different locations could be found since the gobies species develop various morphological specificities as adaptations to their environment (Gani et al. 2019; Roesma et al. 2020). Thus, different backgrounds could affect the morphological characters of species in that environment.

Based on their species composition, the Nike fish schools in the Paguyaman Bay waters indicated a typical species dominance trend every day during the recruitment back to freshwaters. On the first day of their appearance in Paguyaman Bay, the Nike fish schools consisted of species in the Gobiidae family and was dominated by *S. longifilis* (52,00 %). Furthermore, the species composition was dominated by *Belobranchus segura* (63,27 %) on the second day and *Stiphodon semoni* (83,43%) on the third day when the schools began to enter the river estuary. Nevertheless, further study is needed to elucidate these results. In general, gobies are relatively easy to adapt to almost all types of aquatic habitats, although with varying degrees of abundance (Gani et al. 2019).

Finally, the present study has reported the diversity of species making up the Nike fish schools in Paguyaman Bay and a new record of *E. melanosoma* species as the first species of the genus *Eleotris* making up the Nike fish schools in Paguyaman Bay in specific and Gorontalo waters in general. Exploration of adult species and their distribution in the freshwaters of the Paguyaman River, which is thought to be the habitat of adult Nike in Paguyaman Bay, must be performed to ensure the sustainability of Nike fish resources in the future. Although the adult species of Nike fish are not economic value commodity, the postlarva stage of the species when it is about to be recruited back into the river is often targeted by fishermen for human consumption (Ellien et al. 2016; Roesma et al. 2020), including Nike fish in Gorontalo waters. Species identification needs to be done continuously to prevent the loss of certain fish species along with commodity fishing. This research indicates that many types of species that make up the Nike fish school may be found again distributed in Gorontalo waters if a more extensive study is carried out in the future, especially in river estuarine locations that have never been studied before.

ACKNOWLEDGEMENTS

The authors would like to thank the Institute of Research and Community Service (LPPM) of the Gorontalo State University, which funded this research through the Penelitian Dasar scheme for the 2021 Fiscal Year. The thanks are also conveyed to Thomas Tammu and Adistya for technical assistance during the study and all parties who contributed to the implementation of the research.

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Commented [D36]: Among amphidromous fish, the Eleotridae and Gobiidae (Teleostei: Gobioidei) are the two most common families found in estuaries and freshwaters of the Indo-Pacific region. The Eleotrid species most commonly inhabit the lower and middle parts of freshwater flows characterized by sitting and waiting for prey (Mennesson et al. 2015; Mennesson and Keith 2020). Eleotris pelagic larvae morphological features are transparent and compressed bodies, conspicuous swim bladder, and emarginate caudal fin (Maeda and Tachihara 2005). While the adult species has a large blunt head, torpedo-shaped body, rounded caudal fin, prominent lower jaw, fin ventral discrete, has no lateral line on the sides of the body, and an inconspicuous body-color, mostly light brown or dark brown or olive with some dazzled metallic (Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species of the Gobiidae family are active swimmers. This species with pelvic fins was fused and formed a sucking disc used to attach to the substrate while actively swimming against currents upstream (Keith 2003; Taillebois et al. 2014). The genus Sicyopterus uses its mouth as a secondary sucking disc that allows this genus to quickly access the upstream of the waterfall (Keith 2003).

Commented [D37]: The Nike fish in Gorontalo Bay is an amphidromous species as well as in Paguyaman Bay. As an amphidromous species, the adults are hatch in rivers, then larvae flow downstream (sea) and grow in coastal or offshore marine habitats, then mobilization into rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively drift follow the river water currents after hatching in the afternoon and they are reach the estuary in the middle night (Maeda and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so the larvae are carried downstream with lots of yolks and the risk of starvation was minimized (McDowall 2009). One of the strategies developed of the fish is spawning in river areas near the estuary to shorten the larval drift time in freshwater environments (Lagarde et al. 2017).

Commented [D38]: The appearance period of Nike fish making mobs in the bay and moving into the estuary is the pre-colonization phase. In this phase, gobies' post larvae will mob and prefer to swim near the shoreline, because the water flowing is slower and could even be reversed by tides or waves (Keith et al. 2008). Making mobs is a gobies strategy to avoid predators beside finding food (Keith 2003). The diversity of species and sizes of the species which compose the fish mobs is strongly influenced by the character ... [1]

Commented [D39]: According to (Landau and Everit 2004), the discriminant analysis determines the main distinctive character among populations . The present study confirmed the main distinguishing trait among Nike fish populations in Paguyaman Bay is body depth. However, in contrast to the main distinguishing trait among Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The difference of the main distinguishing traits between two locations since the gobies species are able to develop vari ... [2]

Commented [D40]: Based on their species composition, the Nike fish mobs in Paguyaman Bay waters indicated a trend of typical species dominance every day during the mobilization back to freshwaters. On the first day of their appearance in Paguyaman Bay, the Nike fish mobs composed by species in the Gobiidae family and dominated by S. longifilis (52,00 %), followed the species was dominated by Belobranchus segura (63,27 %) on the second day, the last Stiphodon semoni (83,43%) on the third day when the fish ... [3]

Commented [D41]: Finally, the present study was reported the diversity of species compose the Nike fish mobs in Paguyaman Bay and a new variant of E. melanosoma species as the first species of the genus Eleotris compose the Nike fish mobs especially in Paguyaman Bay and Gorontalo waters in general. Exploration of adult species and their distribution in the freshwaters of Paguyaman River, which assumpted recognition as the habitat of adult Nike fish should be performed to ensure the sustainability of Nike fish ... [4]

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PROSES SUBMISSION
BIODIVERSITAS JOURNAL OF BIOLOGICAL DIVERSITY

(*REVISED MANUSCRIPT*)

Diversity of species making up nike fish schools and a new record of *Eleotris melanosoma* in Tomini Paguyaman Bay, Gorontalo, Indonesia

Abstract. Several estuaries in the waters of Tomini Bay are reported as the primary location for Nike fishing in Gorontalo, one of which is Paguyaman Bay. However, scientific information on Nike fish in the Tomini Bay waters is currently limited in Gorontalo Bay. The present study aims to determine the diversity of species making up the Nike fish schools in Paguyaman Bay and show the types of constituent species whose distribution in Gorontalo waters has never been reported. A total of 1,773 samples of Nike fish were collected from the sea to the estuary of Paguyaman Bay in one period of their emergence on April 8–10, 2021. The species were grouped based on the melanophore patterns similarity and then analyzed morphometrically. The molecular identification of COI mitochondrial DNA was performed on species with different morphological appearances from those found in Gorontalo Bay. The Discriminant Function Analysis (DFA) indicated that the main distinguishing character of morphometrics is body depth. The morphological results suggested that the Nike fish schools in Paguyaman Bay consisted of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. The first finding of *E. melanosoma* as a species making up the Nike fish schools in Paguyaman Bay was a new variant of the distribution of this species in Gorontalo waters and confirmed using morphology and molecular analysis. Further, based on the species composition, Nike fish schools in the waters of Paguyaman Bay show a typical species dominance trend during the recruitment process returning to freshwater, i.e., *S. longifilis* (52.00 %) on the first day, *Belobranchus segura* (63.27 %) on the second day, and *Stiphodon semoni* (83.43 %) on the third day.

Keywords: *Belobranchus*, *Eleotris*, Sicydiinae, *Sicyopterus*, *Stiphodon*.

Abbreviations: Basic Local Alignment Search Tools (BLAST), Cytochrome Oxidase I (COI), Deoxyribonucleic acid (DNA), Polymerase Chain Reaction (PCR)

Running title: Diversity of Nike fish schools in Paguyaman Bay

INTRODUCTION

Tomini Bay is Indonesia's largest bay intersecting related with 14 regencies in three provinces of Sulawesi, namely North Sulawesi, Central Sulawesi, and Gorontalo. Nike fish is among the essential fishery commodities in Tomini Gorontalo Bay communities. It consists of amphidromous gobi schools in the pelagic to juvenile larval stages in the recruitment process from marine waters to freshwaters. Their hatched embryos are carried by the river flow to the sea and develop as pelagic larvae before being recruited, returning to rivers and growing as adults and spawn (Thuesen et al. 2011; Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The diversity of species making up Nike fish schools (postlarva gobi) in Gorontalo waters is one of the important studies that should be conducted continuously, considering the high economic value potential as a food commodity in Gorontalo with an R/C ratio of 2.68 (economically feasible) (Wolok et al. 2019). As of today, approximately 1,120 species from 30 genera in the gobi group are described. The gobi group plays a vital role in ichthyofauna diversity with a wide area distribution beside a high number of species. These fish groups are dominantly found in the eastern part of Indonesia, with high species diversity (Tweedley 2013; Miesen et al. 2016; Hadiaty and Sauri 2017; Nurjirana et al. 2020).

Geographically, there are several rivers in Gorontalo which empty into Tomini Bay. Some of these river estuaries are important locations for Nike fish catching in Gorontalo, including Gorontalo Bay and Paguyaman Bay (Salam et al. 2016). Numerous studies have reported Nike fish in Gorontalo Bay (Olii et al. 2017; Olii et al. 2019; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020a; Sahami et al. 2020). Meanwhile, scientific data on Nike fisheries in the Paguyaman Bay estuary are not widely observed. Paguyaman Bay is part of the Tomini Bay areas located on the border of Girisa Village, Paguyaman District, Boalemo Regency with Bilato Village, Boliyohuto District, Gorontalo Regency. The bay is

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the estuary of the Paguyaman River, whose watershed flows across three regencies, i.e., Gorontalo, Boalemo, and Pohuwato. The presence of the Paguyaman watershed as the second-largest watershed in Gorontalo and directly intersecting with three administrative regencies draws the interest to observe the Paguyaman Bay estuary.

The demographic information, such as life history at early stages, recruitment, migration patterns, and other biological characteristics, is crucial for managing fish populations (Habibie et al. 2015). Information about the identification and composition of species is also essential in the management process. Hence, the management can be carried out effectively. As currently reported, the types of species making up the Nike fish schools in the waters of Gorontalo Bay consist of nine species i.e., *Sicyopterus pugnans, S. longifilis, S. lagocephalus, S. cynocephalus, S. parvei, Bunaka Gyrinoides, Belobranchus segura, B. Belobranchus,* and *Stiphodon semoni* (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Although the goby is widely distributed in tropical Indo-Pacific waters (Keith et al. 2015; Lord et al. 2019), the diversity of species in each water location needs to be examined further. Therefore, the present study aims to find out the diversity of species making up the Nike fish schools in Paguyaman Bay and reveal the types of constituent species whose distribution in the waters of Gorontalo Bay has never been reported. The present work is also expected to provide significant scientific information for Nike fish resource management in Paguyaman Bay and to support and optimize Nike fisheries in the Gorontalo waters.

MATERIALS AND METHODS

Sampling

The samples of Nike fish were collected in Paguyaman Bay (N 00º31'1.10" and E 122º39'0.73") (Figure 1) for three days in one period of emergence on April 8–10, 2021. A total of 150 grams of Nike fish samples were taken from the fishermen by using fishing nets. The samples were then brought to the Hydrobioecology and Biometrics Laboratory, Faculty of Fisheries and Marine Sciences, Gorontalo State University, for analysis. The number of samples were calculated and then identified, referring to Sahami et al. (2019b) and Sahami et al. (2020) methods. The samples were photographed for morphometric analysis. After this, five individuals of the new variant discovery were taken and placed in a sample bottle with 95 % ethanol solution for genetic analysis.

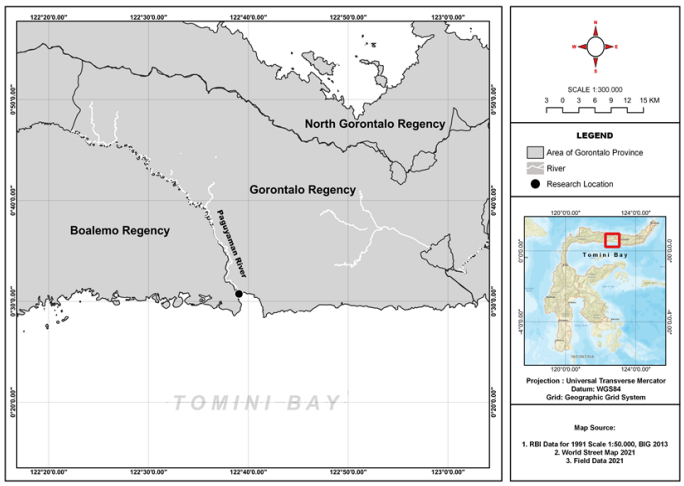

Figure 1. Research site map

Morphometric characters

Ten morphometric characters of Nike fish referring to Sahami et al. (2020) (Figure 2 and Table 1) were measured using the imageJ application with an accuracy of 0.001 cm.

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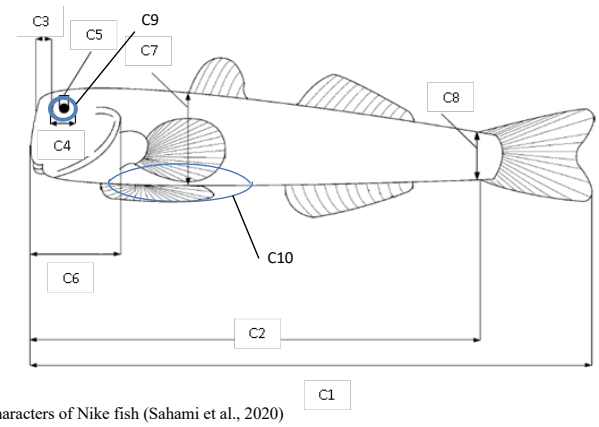

Figure 2. Morphometric characters of Nike fish (Sahami et al., 2020)

Table 1. Morphometric characters of Nike fish (Sahami et al. 2020)

No	Morphometric Characters	No	Morphometric Characters
C1	*Total Length* (TL)	C6	*Head Length (HL)*
C2	*Standard Length* (SL)	C7	*Body Depth (BD)*
C3	*Preorbital Length* (PL)	C8	*Peduncle Depth (PD)*
C4	*Eye Diameter (ED)*	C9	*Eye Area (EA)*
C5	*Eye Lens diameter (EL)*	C10	*Yolk Sac area (YS)*

Each measured morphometric character datum was then standardized following the allometric formula according to Elliott et al. (1995) as follows:

$M_{adj} = M\ (L_s/L_0)^b$

M_{adj} is the standardized morphometric data, M is the measured morphometric data, L_0 is the total length of fish, L_s is the average total length, parameter *b* is the slope of log linear curve M to log L_0 of all data.

DNA extraction, PCR amplification, and sequencing

The target gene for molecular analysis was the mitochondrial DNA Cytochrome Oxidase Subunit 1 (COI) gene. COI gene is the best resolution of the intraspecific level than other core genes (Bellagamba et al. 2015; Hubert et al. 2015; Rodrigues et al. 2017; Bingpeng et al. 2018; Roesma et al. 2018, Yulianto et al. 2020). In addition, mitochondrial COI genes are also widely and reliably utilized to identifying species in the gobi group (Jeon et al. 2012; Viswambharan et al. 2013; Laskar et al. 2016; Lejeune et al. 2016; Wang et al. 2017; Linh et al. 2018; Olii et al. 2019; Roesma et al. 2020; Sahami et al. 2019a; Sahami et al. 2019b; Pasisingi et al. 2020b; Sahami et al. 2020). Following the protocol kit, the 20 grams of fish meat tissues was isolated using Qiagen Tissue and Blood Extraction kits for genetic analysis. The mitochondrial DNA COI gene was further amplified using the primer pair FishF1 5'-TCAACCAACCACAAAGACATTGGCAC-3' and FishR1 5'-TAGACTTCTGGGTGGCCAAAGAATCA-3' (Ward et al. 2005). Samples were amplified at pre-denaturation temperature of 94 ºC for five minutes, denaturation at 94 ºC for 30 seconds, annealing at 50 ºC for 30 seconds, extension at 72 ºC for 30 seconds, and final extension at 72 ºC for 7 minutes. This Polymerase Chain Reaction (PCR) process lasted for 38 cycles.

Data analysis

The DNA samples were amplified and electrophoresed, and then the gene was sequenced using the Dideoxy Sanger Termination Method. Contig was done for the nucleotide sequences. After that, the results were matched with the data available in the GenBank database (www.ncbi.nlm.nih.gov) through the Basic Local Alignment Search Tool (BLAST). The phylogenetic tree was arranged by aligning the DNA sequences of identified samples with some DNA of gobies (accession number KF489573, KU232392, KU692483, KU692484, and KU692490) and the species of Nike fish schools in Gorontalo Bay (accession number MN069306, MN069307, MN069308, MT706639, MT706640, MT706641, MT706720, MT706721, MT706722, MT706723, MT706724, MT706725, MT706726, and MT706791) available in the GenBank database using the Maximum Likelihood 1,000 bootstrap method on MEGAX software (Kumar et al. 2018). The results of genetic identification were further confirmed morphologically, referring to Maeda and Tachihara (2005).

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Furthermore, the Discriminant Function Analysis (DFA) (Landau and Everit 2004) was performed to determine the main distinctive characters among species using IBM SPSS Statistics 25.

RESULTS AND DISCUSSION

New variant of species that makes up the Nike fish schools

The emergence of Nike fish in Paguyaman Bay does not have a regular pattern as in Gorontalo Bay, which the periodization occurs almost every month. In 2021, Nike fish in Paguyaman Bay first appeared in April with a 3-day emergence period (April 8–10, 2021). Based on the NCBI database referring to the mitochondrial COI gene sequencing, a new melanophore pattern species was identified as *Eleotris melanosoma* (Figure 3). The nucleotide sequence of *E. melanosoma* was listed in the NCBI database with accession number MZ401475.

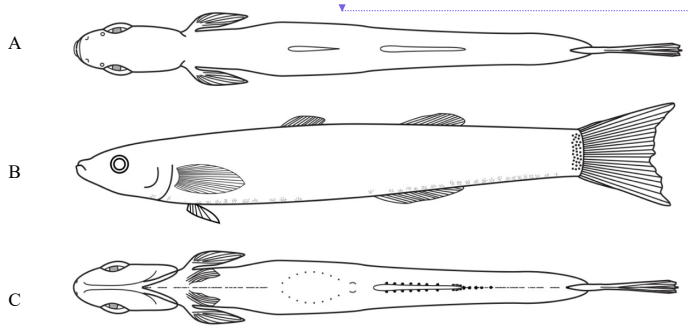

Figure 3. Pelagic larvae of *Eleotris melanosoma* in Paguyaman Bay. A. Dorsal view; B. Lateral view; C. Ventral view.

The caught species of *E. melanosoma* have a standard length of 19.05–20.04 mm with an average standard length of 19.55 mm. These species have a transparent body, an elongated and compressed body shape, and no scales. In addition, the body fins are not perfect yet, pelvic fins are separated, and the caudal fin tends to form emarginate. There are no melanophore spots distributed from head to body, and few melanophore spots accumulated at the base of the caudal fin. These morphological features correspond to the morphology of the pelagic larvae of *E. melanosoma* as reported by Maeda and Tachihara (2005).

The results of the alignment of the DNA sequences of *E. melanosoma* with the DNA sequences of the species that make up the Nike fish school in Gorontalo Bay and several *Eleotris* species available in the NCBI Genbank are displayed through a phylogenetic tree (Figure 4). The analysis results show that *E. melanosoma* is closely related to *Eleotris fusca* and is in the same monophyletic clade as *Belobranchus segura*, *B. belobranchus*, and *Bunaka gyrinoides*. The similarity of the *clades* in these five species is because they are members of the *Eleotridae* family, although it is clear that there are sub-clades based on the similarity of the genus in this clade. Furthermore, the second monophyletic clade is the family clade Gobiidae which includes the species *Stiphodon semoni, Sicyopterus longifilis, S. lagocephalus, S. parvei,* and *S. cynocephalus.* The second monophyletic clade also groups the species in the genus *Sicyopterus* in the same sub-clade and separated from the genus *Stiphodon*.

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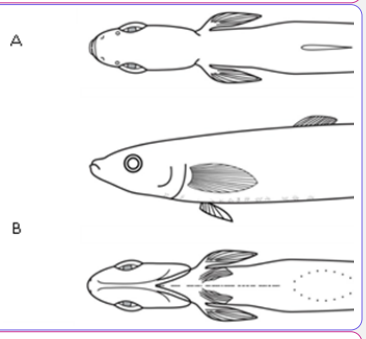

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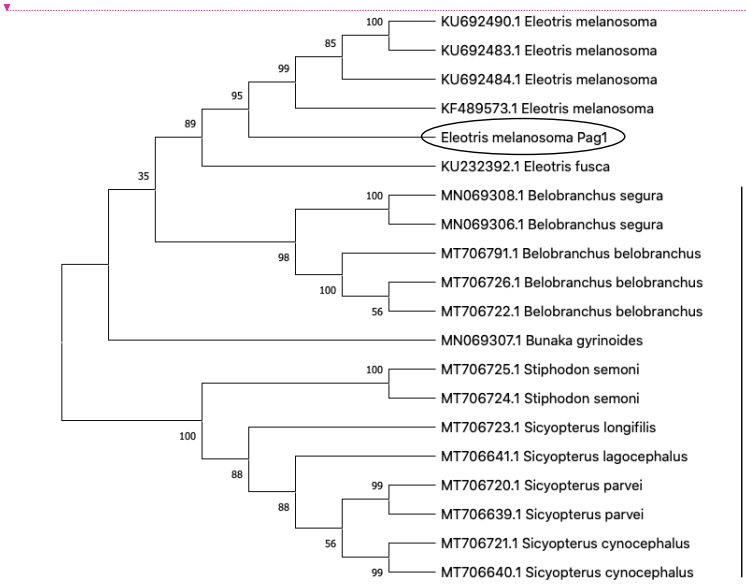

Figure 4. Phylogenetic tree of *Eleotris melanosoma* with several species of gobies and composers of Nike fish in Gorontalo Bay waters available in the NCBI database

Composition of species

A total of 1,773 specimens were observed as constituents of Nike fish schools in Paguyaman Bay for three days of emergence in April 2021. Two families, i.e., Gobiidae and Eleotridae, were recorded to make up Nike fish schools. The Gobiidae family consists of four species, i.e., *Sicyopterus longifilis, S. parvei, S. cynocephalus,* and *Stiphodon semoni*. Meanwhile, the Eleotridae family consists of three species, namely *Belobranchus segura, B. belobranchus*, and *Eleotris melanosoma*. Among seven species, three species in family Gobiidae, i.e., *S. longifilis, S. parvei*, and *Stiphodon semoni* were observed as species that made up Nike fish schools during their emergence. The species was consistently observed in considerable numbers i.e. S. *longifilis*, whereas *B. segura* was observed only on the second day. On the third day of emergence, *B. belobranchus* and *E. melanosoma* were observed, *S. semoni* is the most abundant species observed (34.07 %), meanwhile *S. parvei* and *B. segura* are at least 0.003 %. Based on the species composition, species dominance was different each day. On the first, second, and thir day, the school of Nike fish were dominated by *S. longifilis*, *Belobranchus segura*, and *Stiphodon semoni* with a composition value of (52.00 %), 63.27 %, and 83.43 %, respectively (Figure 5).

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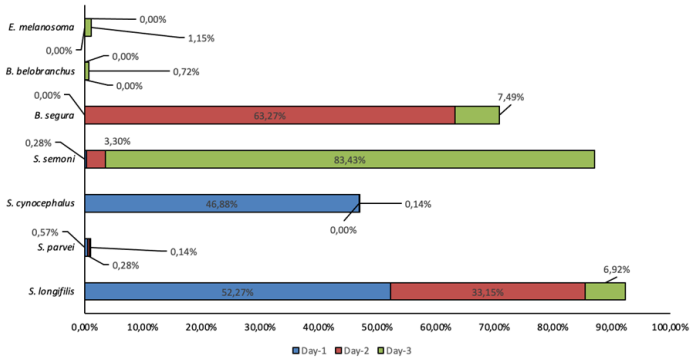

Figure 5. Nike fish schools composition in Paguyaman Bay waters

Size distribution and morphometric characters

The total length of the collected Nike fish ranged from 1,523 to 3,572 cm (Table 2). The *Stiphodon semoni* (Gobiidae) and *S. parvei* (Gobiidae) species have the smallest and biggest size. The species with the widest size range is *S. cynocephalus* (2.422–3.572 cm), and the species with the smallest size range is *B. belobranchus* (2.290–2.403 cm).

Table 2. Size range of each species making up Nike fish schools in Paguyaman Bay waters

No	Species	Mean of Size (cm)	Range of Size (cm)
1	*Sicyopterus longifilis*	2.666	2.262–3.044
2	*Sicyopterus parvei*	3.036	2.831–3.403
3	*Sicyopterus cynocephalus*	2.775	2.422–3.572
4	*Stiphodon semoni*	2.137	1.523–2.586
5	*Belobranchus segura*	2.311	2.005–2.638
6	*Belobranchus belobranchus*	2.347	2.290–2.403
7	*Eleotris melanosoma*	2.377	2.304–2.448

The morphometric characters can be used in taxonomy as early identification in fisheries science (Sara et al. 2016). The summary morphometric characters data standardized following the allometric formula Elliott et al. (1995) is presented in Table 3. Moreover, the analysis of discriminant function is presented in Figure 6. Two discriminant functions are able to explain 67.3 % and 24.5 % of the total variance of morphometric characters. Based on this work, the C7 character (body depth) is the main distinctive character of the Nike population in Paguyaman Bay. The tendency of Nike fish formation in Paguyaman Bay is clearly shown by a group of centroid in Figure 6. The species of *Sicyopterus longifilis, S. cynocephalus, S. Parvei*, and *S. semoni* tend to overlap and adjacent since four species are members of the Gobiidae family. Furthermore, *Belobranchus segura* and *Belobranchus belobranchus* are the most related spots because both species are from the same genus of *Belobranchus*. The *Eleotris melanosoma*, although was distributed in separate areas, is close to *B. belobranchus* because both species are members of the Eleotridae family. In general, species identification through canonical discriminant diagrams (Figure 6) is in line with the phylogenetic tree shown in Figure 4.

Table 3. Morphometric character data of Nike fish schools in Paguyaman Bay waters

Species	Unit of Characters (Mean (cm) ± SD)								
	SL	PL	ED	EL	HL	BD	PD	EA	YS
S. longifilis	2.054 ± 0.05	0.111 ± 0.02	0.136 ± 0.02	0.060 ± 0.01	0.518 ± 0.04	0.381 ± 0.03	0.208 ± 0.02	0.013 ± 0.00	0.026 ± 0.00
S. parvei	2.086 ± 0.03	0.128 ± 0.03	0.133 ± 0.02	0.062 ± 0.01	0.535 ± 0.03	0.376 ± 0.01	0.205 ± 0.01	0.012 ± 0.00	0.031 ± 0.01
S. cynocephalus	2.043 ± 0.05	0.114 ± 0.02	0.133 ± 0.02	0.057 ± 0.01	0.521 ± 0.05	0.375 ± 0.03	0.203 ± 0.02	0.012 ± 0.00	0.029 ± 0.01
S. semoni	2.062 ± 0.05	0.104 ± 0.02	0.129 ± 0.02	0.056 ± 0.01	0.489 ± 0.04	0.343 ± 0.04	0.188 ± 0.03	0.012 ± 0.00	0.020 ± 0.01
B. segura	2.094 ± 0.04	0.126 ± 0.02	0.129 ± 0.02	0.061 ± 0.01	0.556 ± 0.05	0.392 ± 0.03	0.221 ± 0.02	0.012 ± 0.00	0.035 ± 0.01
B. belobranchus	2.051 ± 0.05	0.140 ± 0.03	0.128 ± 0.02	0.067 ± 0.01	0.579 ± 0.03	0.325 ± 0.01	0.213 ± 0.01	0.015 ± 0.00	0.021 ± 0.00
E. melanosoma	2.033 ± 0.02	0.123 ± 0.01	0.129 ± 0.00	0.058 ± 0.01	0.535 ± 0.04	0.280 ± 0.02	0.208 ± 0.01	0.012 ± 0.00	0.013 ± 0.00

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Notes: SL= *Standard Length; PL = Preorbital Length; ED = Eye Diameter; EL = Eye Lens diameter; HL = Head Length; BD = Body Depth; PD = Peduncle Depth; EA = Eye Area; YS = Yolk Sac area*

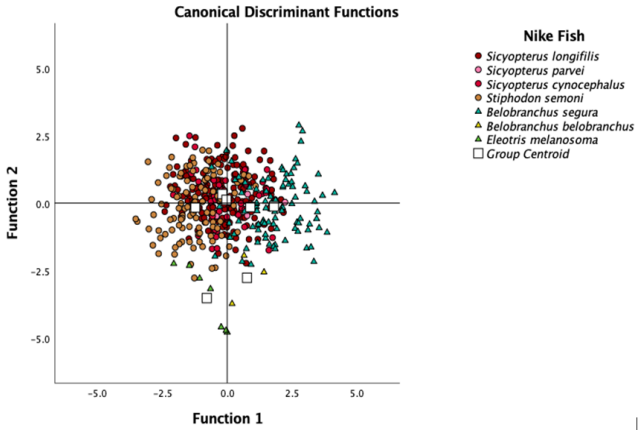

Figure 6. Diagram of the canonical discriminant function of Nike fish in Paguyaman Bay waters

Discussion

The species that make up the Nike fish schools in Paguyaman Bay consist of seven species, less than nine species reported in Gorontalo Bay (Olii et al. 2019; Sahami et al. 2019b; Sahami et al. 2020). Two species of gobies are, *S. pugnans* and *Bunaka gyrinoides*, are not observed as the constituents of Nike fish schools in Paguyaman Bay. However, the present study reports a new record of the distribution of *E. melanosoma*. Identifying *E. melanosoma* as one of the species making up the Nike fish schools in Paguyaman Bay based on molecular and morphological analysis is a new record of the distribution of this species in Gorontalo waters. A total of eight species of *E. melanosoma* were caught as the constituents of the Nike fish schools in Paguyaman Bay when they began to approach the river estuary on the third day of their emergence.

The *E. melanosoma* species is one of the three main species of *Eleotris* reported to be distributed in the Indo-Pacific waters. Two of the other species are *E. fusca* and *E. acanthopoma* (Maeda et al. 2011; Mennesson et al. 2019; Subchan et al. 2020). The other *Eleotris* species can continue to be recorded when more studies focus on this topic in more locations in Indo-Pacific waters. As reported by Maeda and Tachihara (2005), the pelagic and newly settled larvae of *E. fusca* show a larger body size than *E. melanosoma* and *E. acanthopoma*. *Eleotris* pelagic larvae migrate to river areas by utilizing the rising tides at night. *E. melanosoma* and *E. acanthopoma* settle at the upper end of the tidal affected area, meanwhile *E. fusca* migrates to freshwater upstream against river water flow currents.

In terms of the size, the species of *E. melanosoma* caught in Paguyaman Bay (average standard length 19.55 mm) should in the newly settled larval stage based on research Maeda and Tachihara (2005) in the Teima River estuary area, Okinawa Island, with increasingly numerous visible body pigmentation and completely pigmented caudal fin with no distal border. However, the morphology of *E. melanosoma* species in the site area still characterizes the morphology of the pelagic larval stage, with the most conspicuous feature and no melanophore spots on its sides. Such a difference in feature is due to the difference in the aquatic habitat area (Habibie et al. 2018). Besides, the tropical area's stable temperature throughout the year has no significant effect on the fish rate growth (Habibie et al. 2015). The pelagic to juvenile larval stages of *E. melanosoma* species were also recorded in the estuary area of the Teima River, Okinawa Island (Maeda and Tachihara 2005) and Sri Lankan waters estuary (Batuwita et al. 2017). Meanwhile, the adult species were found in the fresh waters of Japan (Maeda et al. 2011); rivers on the Buton and Kabaena Islands (Tweedley et al. 2013); Opak River, Yogyakarta (Djumanto et al. 2013); freshwater of Sri Lanka (Batuwita et al. 2017); freshwater of West Sulawesi (Nurjirana et al. 2020); and Sanenrejo and Wonoasri River Resorts (Subchan et al. 2020).

Among amphidromous fish, the Eleotridae and Gobiidae (Teleostei: Gobioidei) are the most common families discovered in estuaries and freshwaters of the Indo-Pacific area. The Eleotrid species mostly inhabits the lower and middle parts of freshwater flows characterized by waiting for prey (Mennesson et al. 2015; Mennesson and Keith 2020). *Eleotris* pelagic larvae morphological features are transparent and compressed bodies, conspicuous swim bladder, and emarginate caudal fin (Maeda and Tachihara 2005). On the other hand, the adult species has a large blunt head, torpedo-shaped body, rounded caudal fin, prominent lower jaw, discrete fin ventral, has no lateral line on the sides of the body, and

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inconspicuous body color, mostly light brown, dark brown, or olive with some dazzled metallic (Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species of the Gobiidae family are active swimmers. They have pelvic fins that are fused and form a sucking disc to attach themselves to the substrate while actively swimming against currents towards the upstream (Keith 2003; Taillebois et al. 2014). The genus *Sicyopterus* uses its mouth as a secondary sucking disc that allows this genus to quickly access the upstream of the waterfall (Keith 2003).

The Nike fish both in Paguyaman Bay and Gorontalo Bay is an amphidromous species. As an amphidromous species, the adults are hatch in rivers, then larvae flow downstream (sea) and grow in coastal or offshore marine habitats, then recruitment occurs in rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively follow the river water currents after hatching in the afternoon and reach the estuary in the middle of the night (Maeda and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so that the larvae are carried downstream with lots of yolks, and the risk of starvation is minimized (McDowall 2009). One of the strategies employed is spawning in river areas near the estuary to shorten the larval drift time in freshwater environments (Lagarde et al. 2017).

The emergence period of Nike fish schools in the bay and moving into the estuary is pre-colonization phase. In this phase, gobies' post-larvae will swarm and prefer to swim near the shoreline because the water flowing is slower and can even be reversed by tides or waves (Keith et al. 2008). Making schools is a gobies strategy to avoid predators besides finding food (Keith 2003). The species' diversity and sizes making up the fish schools is strongly influenced by the characteristics of the species, season, and hatching time. According to Taillebois et al. (2012), the duration of the pelagic larvae is not the only factor determining species distribution; It is most likely interactions between larval behavior, environment, currents, and substrate preferences. Apart from these factors mentioned previously, the larger fish larvae possibly resultes from earlier hatching compared to smaller fish larvae (Mennesson et al. 2015). In addition, the rainy season will support the newly hatched larvae drifting into the sea faster (Keith 2003).

According to Landau and Everit (2004), the discriminant analysis determines the main distinctive character among populations. The present study confirmed that the main distinguishing characteristic among Nike fish populations in Paguyaman Bay is body depth. It is contrary to the main distinctive character of Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The difference in the main distinguishing characters between two locations since the gobies species are able to develop various morphological specificities as adaptations strategy for their environment (Gani et al. 2019; Roesma et al. 2020). Thus, the different backgrounds are able to affect the morphological characters of species in their environment. Populations in different environments are likely to have different population structures. Differences in population structure will influence population size, which can be observed through differences in morphometric and meristic characters (Aisyah and Syarif 2018).

Based on their species composition, the Nike fish schools in Paguyaman Bay waters indicate a trend of typical species dominance every day during the recruitment back to freshwaters. On the first day of their emergence in Paguyaman Bay, the Nike fish schools were composed of species in the Gobiidae family and dominated by *S. longifilis* (52,00 %). Furthermore, the species were dominated by *Belobranchus segura* (63,27 %) on the second day and *Stiphodon semoni* (83,43%) on the third day when the fish schools started to enter the river estuary. Nevertheless, further studies are required to explain these phenomena. In general, the gobies are adapt mostly to aquatic habitats, although with various abundance degrees (Gani et al. 2019).

In conclusion, the Nike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. Species with the new melanophore pattern is *E. melanosoma*, confirmed through morphological and molecular analysis. A new variant of *E. melanosoma* species as the first species of the genus *Eleotris* composes the Nike fish schools in Paguyaman Bay and Gorontalo waters in general. Exploration of adult species and their distribution in the freshwaters of the Paguyaman River assumed to be the habitat of adult Nike fish should be performed to ensure the sustainability of Nike fish resources in the future. Although the adult species of Nike fish is an unvalued economic commodity, unfortunately the post larva stage when it is recruiting back into the river is often targeted by fishermen for consumption (Ellien et al. 2016; Roesma et al. 2020), including in Gorontalo waters. Identification of these species must be carried out continuously to prevent the loss of these fish species while catching along with commodity fishing. This research indicates many types of species constituents in Gorontalo waters may be collected when an extensive study is conducted later in the future, specifically in river estuarine locations that are not explored.

ACKNOWLEDGEMENTS

The authors express our deep gratitude to the Institute of Research and Community Service (LPPM) of the Gorontalo State University that has funded this research through the scheme of Penelitian Dasar (Basic Research) for the 2021 Fiscal Year. We also would like to thank Thomas Tammu, Adistya, and all parties for their technical assistance and contribution during the study.

Deleted: has an …nconspicuous body -…olor, mostly light brown, or dark brown, or olive with some dazzled metallic sheen …Murdy and Hoese 2002; Batuwita et al. 2017; Mennesson and Keith 2020). In contrast, the species in …f the Gobiidae family are active swimmers. This species…hey has …ave pelvic fins that are fused and form a sucking disc that can be used …o attach themselves to the substrate while actively swimming against currents towards the upstream (Keith 2003; Taillebois et al. 2014). The genus *Sicyopterus* uses its mouth as a secondary sucking disc that allows this genus to quickly access the headwaters above… ... [13]

Deleted: Like tThe Nike fish both in Paguyaman Bay and Gorontalo Bay is an amphidromous speciesGorontalo Bay, the Nike fish in Paguyaman Bay is also an amphidromous species… As an amphidromous species, the adults are hatch in rivers, then larvae then flow downstream (sea) and grow in coastal or offshore marine habitats, then recruitment occurs into…rivers (Yamasaki et al. 2011; Taillebois et al. 2012; Mennesson et al. 2019; Iida et al. 2017; Mennesson and Keith 2020; Sahami and Habibie 2020). The amphidromous larvae passively drift with the…ollow the river water currents shortly …fter hatching at …n the afternoon dusk …nd reach reach the estuary area …n the middle of the of the …ight (Maeda and and Tachihara 2010). Some amphidromous gobies, especially subfamily Sicydiinae, often hatch less than 48 hours after fertilization, so that the larvae are carried downstream with l ... [14]

Deleted: appearance in groupsschools in the bay area …nd moving moving into the estuary is pre-colonization phase. In this phase, gobies' post-larvae will swarm and choose …refer to swim near the shoreline,…where …ecause the water flowing is weaker …lower and can can even be reversed by tides or waves (Keith et al. 2008). Making Schooling …chools is a gobies strategy to avoid predators and besides finding food (Keith 2003). The species' diversity of species and sizes of species …aking up the fish schools is strongly influenced by the characteristics of the species, season, and hatching time. According to Taillebois et al. (2012), explained that … ... [15]

Deleted: The the discriminant analysis determines the main distinctive character among populations (Landau and Everit 2004)… The present study confirmed that the main distinctive characterdistinguishing characteristic among Nike fish populations in in Paguyaman Bay is body depth. It is, in…contrast …ontrary to the main distinctive character distinctive character among…f Nike populations in Gorontalo Bay, i.e., head length (Sahami et al. 2020). The distinction …ifference in in …he main distinguishing charactersdistinctive characters…between the …wo different locations could be found …ince the gobies species are able t ... [16]

Deleted: the Paguyaman Bay waters indicated…a trend of typical species dominance trend …very day during the recruitment back to freshwaters. On the first day of their appearance …mergence in Paguyaman Bay, the Nike fish schools were consisted of…omposed of species in the Gobiidae family and was …ominated by *S. longifilis* (52,00 %). Furthermore, the species composition was…ere dominated by *Belobranchus segura* (63,27 %) on the second day and *Stiphodon semoni* (83,43%) on the third day when the fish schools began …tarted to enter the river estuary. Nevertheless, further studiesy…is needed…re required to elucidate …xplain these ... [17]

Deleted: Finally, the present study …ike fish schools in Paguyaman Bay consist of seven species, four genera, and two families, i.e., *Sicyopterus longifilis*, *S. parvei*, *S. cynocephalus*, *Stiphodon semoni*, *Belobranchus segura*, *B. belobranchus*, and *Eleotris melanosoma*. Species with the new melanophore pattern is *E. melanosoma*, confirmed through morphological and molecular analysis. A new variant of *E. melanosoma* species as the first species ofhas reported the diversity of species making up the Nike fish schools in Paguyaman Bay and a new record of *E. melanosoma* species as the first species of…the genus *Eleotris* making ... [18]

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Uncorrected Proof

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We have reached a decision regarding your submission to Biodiversitas Journal of Biological Diversity, "Diversity of species in making up Nike fish schools and a new record of Eleotris melanosoma in Tomini Paguyaman Bay, Gorontalo, Indonesia".

Our decision is to: Accept Submission

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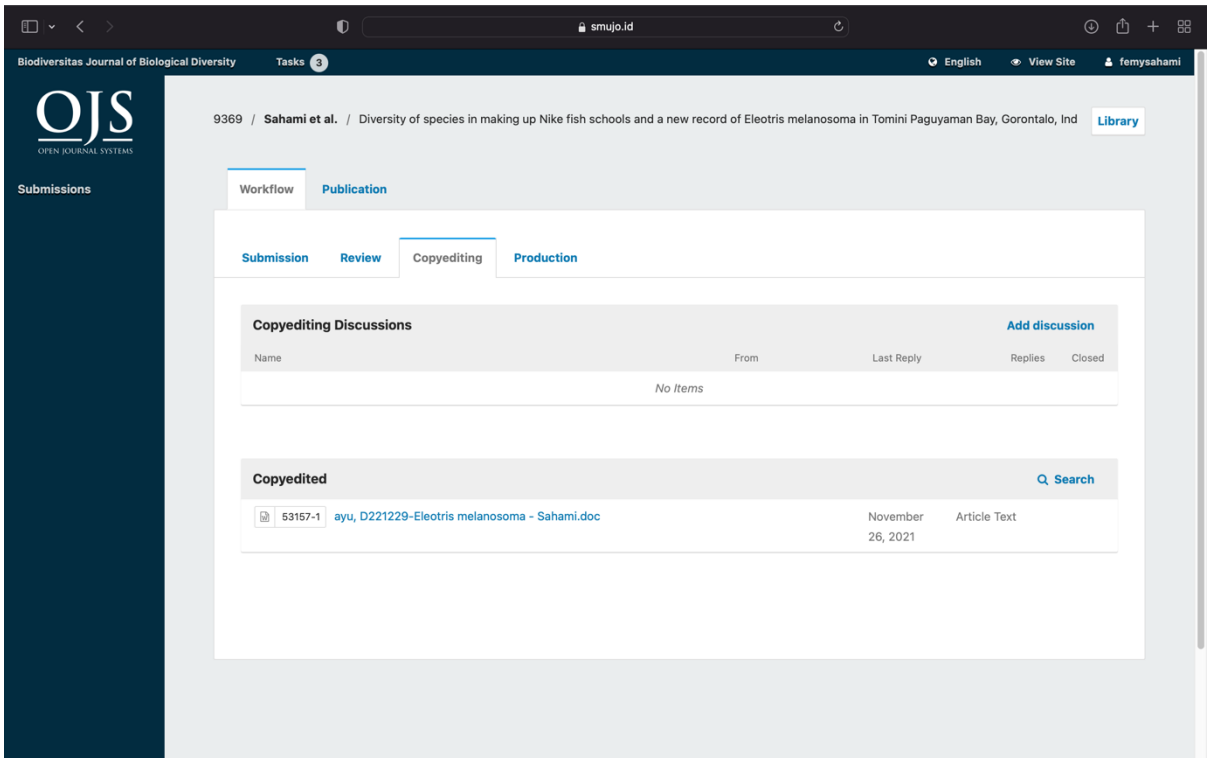

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The editing of your submission, "Diversity of species in making up Nike fish schools and a new record of Eleotris melanosoma in Tomini Paguyaman Bay, Gorontalo, Indonesia," is complete. We are now sending it to production.

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