BIODIVERSITAS
Volume 25, Number 1, January 2024
Pages: 223-231

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d250125

Morphological characteristic and truss morphometric analysis of amphidromous goby (‘nike') (Teleostei: Gobiiformes) in Bone River, Gorontalo, Indonesia

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Manuscript received: 20 November 2023. Revision accepted: 23 January 2024.

Abstract. *Pasisingi N, Suci D, Panigoro C, Kadim MK. 2024. Morphological characteristic and truss morphometric analysis of amphidromous goby (‘nike') (Teleostei: Gobiiformes) in Bone River, Gorontalo, Indonesia. Biodiversitas 25: 223-231.* One of the preferred fish resources in the Gorontalo region is amphidromous goby species, commonly known by local community as nike fish. In rivers, at the adult stage, these fish are morphologically and potentially morphometrically not necessarily distinguishable among species and between sexes. This research aimed to identify the morphological and morphometric truss characters that distinguish the sexual characteristics of goby fishes in the Bone River, Gorontalo. Fish samples were purposefully collected from eight sites along the river, spanning from upstream to downstream, using a backpack electrofishing device. Sampling occurred in March and November 2022, with morphological analysis and measurements conducted on 336 individuals. The morphometric method measured data from 13 points on the sample body, forming 28 combinations of truss distance characters (A1-A3; B1-B12; C1-C10; D1-D3) connecting these points. Morphologically, the samples were identified as three species, namely *Sicyopterus longifilis*, *Belobranchus belobranchus*, and *Awaous grammepomus*. This study revealed distinctions in morphological characters between male and female individuals. In *S. longifilis*, males exhibited a relatively slimmer body shape compared to the rounded form of females. Male *B. belobranchus* displayed more striking coloration than females, while male *A. grammepomus* featured a lighter brown body color than the paler females. All the morphometric truss characters in this study can be reliably used as a sexual distinguishing characteristic in *S. longifilis*. As for species *B. belobranchus*, B7 truss cannot be considered a differentiator. In *A. grammepomus,* thirteen truss characters (B3, B4, B6, B7, B8, B10, B12, C3, C6, C7, C9, C10, D1) cannot be used to differentiate males and females.

Keywords: *Awaous grammepomus*, *Belobranchus belobranchus*, Gorontalo, nike, *Sicyopterus longifilis*

Abbreviations: SL: Standard Length

INTRODUCTION

The presence of aquatic amphidromous biota is distinctive in living organisms as it encompasses a considerable spectrum of water salinity. The spawning process takes place in freshwater by matured adult fish, and shortly after hatching, the larvae are transported by currents to brackish or sea waters. Subsequently, they return to freshwater after a certain period. The goby is frequently documented as a species with amphidromous behavior such as *Redigobius bikolanus* (Tran et al. 2018), *Gymnogobius petschiliensis* (Oto 2020; Oto 2021), *Rhinogobius nagoyae* (Iida et al. 2021), and *Sicyopterus lagocephalus* (Teichert et al. 2021). Other amphidromus goby species have also recently been reported to be recorded in Indonesian waters, such as *Sicyopus auxilimentus* on Sangihe Island (Hasan et al. 2021), *Sicyopus discordipinnis* in Central Sulawesi (Nurjirana et al. 2022a), *Belobranchus segura* in the south of Java (Hasan et al. 2023a) and Sumatera (Hasan et al. 2023b), *Stiphodon surrufus* (Ndobe et al. 2022) and *Stiphodon annieae* (Gani et al. 2021) in Sulawesi. Some groups of goby larvae and juveniles found in the estuary and sea areas of Gorontalo waters by the local people are named “Nike” fish. In West Sulawesi, the stadia of this goby is named by local people as “Penja” (Nurjirana et al. 2022b).

It is notable that studies on Nike fish in Gorontalo have only begun to be published in the last decade (Olii et al. 2017; Pasisingi and Abdullah 2018; Olii et al. 2019; Sahami et al. 2019; Pasisingi et al. 2020a; Pasisingi et al. 2020b; Sahami et al. 2020; Sahami and Habibie 2020; Pasisingi et al. 2021; Olii and Pasisingi 2022a; Olii and Pasisingi 2022b; Pasisingi and Olii 2023). This suggests an emerging interest and focus on understanding and documenting Nike fish's characteristics, behavior, and ecological significance in the Gorontalo region during this relatively recent period. Recent research and the publication of findings have contributed to expanding knowledge about the Nike fish population and its role in the local ecosystem. Nike fish in various references have been reported as a goby species in the pre-adult stage (Olii et al. 2017; Olii et al. 2019; Sahami et al. 2019; Sahami et al. 2020), but it has not been confirmed whether all the goby fish found in the Bone River are Nike fish. On the other hand, Nike's resources continue to be exploited to meet market demand as consumption fish (Pasisingi and

Olii 2023), as is also experienced by Penja fish in West Sulawesi (Nurjirana et al. 2022b), raising concerns about the threat of extinction. Hence, conducting a comprehensive study that traces the species comprising adult Nike fish is imperative. This research is crucial for developing effective management strategies to preserve the sustainability of amphidromous fish resources in nature, particularly in the waters of Gorontalo. Morphological and morphometric analyses represent commonly employed approaches to ascertain the certainty and composition of fish species in aquatic environments as applied to the goby species *Ponticola bathybius* (Tajbakhsh et al. 2018) and *Neogobius melanostomus* (Yurtseva and Uspenskiy 2022).

Morphological and morphometric aspects have a high potential to be applied as techniques for distinguishing and identifying fish species. Morphological methods have made rapid progress in collecting precise data, so this method is important for visually revealing prominent differences in form, in this case, the color, size, and shape of fish as research objects as applied by Hasan et al. (2023c) to *Lobocheilos falcifer* species. Morphometric characterization using truss network systems is another approach which also has been widely used for fish identification. Truss network measurements involve determining distances between specific landmarks and creating a structured network of interconnected quadrilaterals or cells across the body. Assessing morphometric variations among species stocks is crucial for understanding population structures and identifying distinct stocks (Rawat et al. 2017). Utilizing advanced techniques in morphometric analysis provides more effective and potent tools for discerning variations among fish populations. These methods enhance the ability to detect differences among groups and differentiate between species with similar shapes (Mojekwu and Anumudu 2015). Research on sexual dimorphism using morphological and truss morphometric approaches for goby species in Bone River has never been carried out. Given these circumstances, this research aimed to identify the morphological and morphometric truss characters that distinguish the sexual characteristics of goby fishes in the Bone River, Gorontalo.

MATERIALS AND METHODS

Study area

The research was conducted at eight observation sites along the Bone River in Gorontalo Province, Indonesia. These sites were purposively selected to cover areas from upstream to downstream, considering accessibility. Figure 1 depicts the map of the sampling locations. In detail, the description of each station is provided in Table 1.

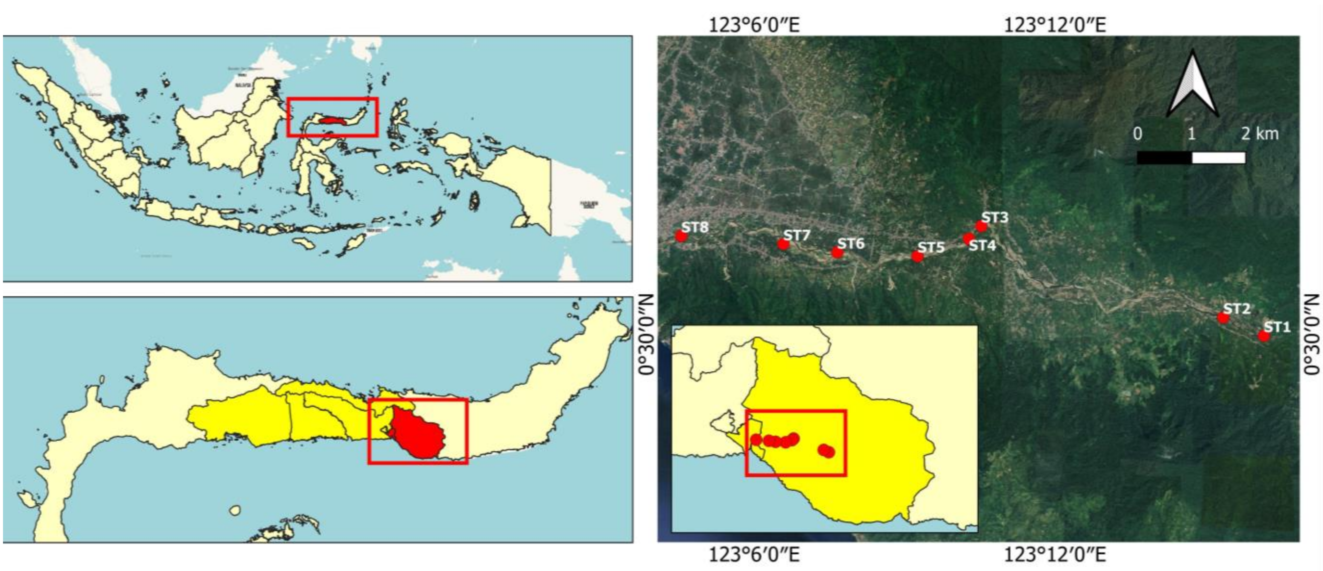

Figure 1. Research location and fish sample sites of Bone River, Gorontalo Province, Indonesia (ST: Sampling Station)

Table 1. Coordinates and surrounding land use of the sampling sites along the Bone River, Gorontalo, Indonesia

Sampling sites	Coordinate	Substrate	Land use
Station 1. Poduwoma Village	00° 29.973'N 123° 16.167'S	Rock and sandy	Settlements, sand mining, rafting tourism
Station 2. Tulabongo Village	00° 30.338'N 123° 15.355'S	Rocky	Agriculture and settlement
Station 3. Lombongo Village	00° 32.163'N 123° 10.535'S	Sandy and rocky	Dams, agriculture, settlements
Station 4. Alale Village	00° 31.909'N 123° 10.285'S	Sandy and rocky	Settlement
Station 5. Duano Village	00° 31.563'N 123° 09.262'S	Sandy and rocky	Agriculture, sand mining
Station 6. Bubeya Village	00° 31.640'N 123° 07.662'S	Sandy and rocky	Settlements, agriculture and livestock
Station 7. Tanggilingo Village	00° 31.806'N 123° 06.587'S	Sandy and rocky	Settlement, agriculture, sand mining
Station 8. Talumolo Village	00° 31.966'N 123° 04.552'S	Sandy and rocky	Settlements, agriculture, former sand mining

Procedures

Sampling technique

Sampling was conducted in both March and November 2022. The minimum number of samples per species is 50 individuals, depending on their availability in the waters during the sampling period. Fish were collected at each site using a backpack electrofishing device (5 Amperes) equipped with a stemmed fishing-net to catch fish paralyzed by electric shock. The samples were put in a ziplock plastic and stored in a coolbox containing ice cubes for laboratory analysis. Once arriving at the lab, the samples were carefully stored in a refrigerator. This electric stunning method was chosen since it has a very low impact on fish welfare compared to other methods (Robb and Kestin 2002). This precautionary also preventing alterations in color and stomach contents and maintain the morphological condition of the samples, facilitating their identification and documentation. The collected specimens were fixed with the internal injection and immersion in 85% formalin solution. Samples were coded and stored in specimen collection at the Hydrobioecology Laboratory, Faculty of Fisheries and Marine Sciences, Universitas Negeri Gorontalo.

Fish morphology analysis

The morphological identification of the sampled fishes was conducted according to the identification guide by Kottelat et al. (1993), Carpenter and Niem (2001), Keith et al. (2015a), Keith et al. (2015b), and Froese and Pauly (2019). To facilitate this process, the fins of the sample fish were carefully stretched using pins on a styrofoam-lined tray. Subsequently, detailed observations were made regarding various morphological characteristics, including the shape of the body, mouth, and pelvic fins, as well as the position of the mouth, scales, and special features (if any) of the fish.

Morphometric truss analysis and sample sexuality

Following the morphological identification, morphometric measurements were conducted on each fish, focusing on measuring the fish's standard length. The morphometric truss method employed in this study was based on measurements calculated from 13 specific points that formed regular and connected patterns across the body shape. Each sample was marked at these 13 points, serving as morphometric truss points, and creating 28 distance character combinations by connecting all these points. The marking process involved inserting a needle into the preparation until it penetrated the styrofoam sample base at each designated point. Subsequently, measurements were taken for the distance of each character combination using a caliper with an accuracy of 0.01 mm. This meticulous approach allows for precise and standardized morphometric data collection. The 13 truss morphometric points in this study include 1) outer tip of the snout, 2) head-dorsal body border point, 3) anterior base of dorsal fin, 4) posterior base of dorsal fin, 5) anterior base of second dorsal fin, 6) posterior base of second dorsal fin, 7) anterior base of dorsal caudal fin, 8) midpoint of the anterior base of the caudal fin, 9) anterior base of ventral caudal fin, 10) posterior base of anal fin, 11) anterior base of anal fin, 12) anterior base of the ventral fin, 13) base of the lower jaw. Subsequent to the morphometric measurements, a dissection of the abdominal cavity was performed on each fish sample to determine gender. This determination was made by observing the gonads. A sketch illustrating the morphometric truss measurements in gobies can be found in Figure 2, providing a visual representation of the measurement points. Additionally, a detailed description of the combination of morphometric truss distances used in the research is provided in Table 3, offering a comprehensive reference for the specific measurements taken at each designated point.

Data analysis

Morphological character analysis involved recording specific features such as body, mouth, and pelvic fin shapes, mouth position, the presence or absence of scales, and any special visible characteristics for all samples. Subsequently, representative samples of each species and sex were documented using a digital camera Fujifilm X-A5 24.2 million pixels. The results of the morphological observations were described descriptively and supported by relevant references. For the morphometric truss measurements, data was tabulated using the microsoft excel and standardized. Each truss morphometric measurement of each species and sexes was standardized for fish size prior to multivariate analysis, to eliminate any size effect in the data set as more effective transformation method recommended by Fernando and Amarasinghe (2011). The truss standardization method according to Senar et al. (1994) and Doherty and McCarthy (2004) is as follows:

$$LTs_i = \log_{10} LT_i \left[\frac{\log_{10} TL_m}{\log_{10} TL_i}\right]^b \quad \text{……..…….. (equation 1)}$$

Where: TL: the total length; LT_i: the truss length characters of i^{th} fish, TL_m: overall mean total length of samples; b: the slope from linear regression transformation of the logarithms of LT and TL. Principal Component Analysis using Minitab Ver. 14 was applied to differenciate group of each truss morphometric character between the sexes of each goby species.

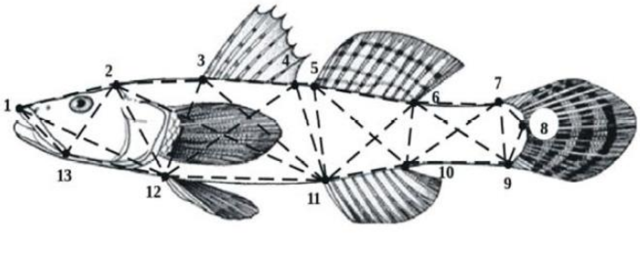

Figure 2. Sketch of morphometric truss measurement points and distances on fish sample (Islam et al. 2016) applied to goby samples from Bone River, Gorontalo, Indonesia

RESULTS AND DISCUSSION

Morphology identification

Morphologically, the results of observations of 336 individual goby samples taken purposively were identified as consisting of three species, namely *Sicyopterus longifilis*, *Belobranchus belobranchus*, and *Awaous grammepomus* (Table 2). The standard length range of samples for morphological analysis is 50.24±13.78 mm (male) and 53.65±14.53 mm (female) mm for *S. longifilis*; 67.94±26.99 mm (male) and 60.88±28.17 mm (female) mm for *B. belobranchus*, and 54.84±13.04 mm (male) and 53.04±9.13 mm (female) mm for *A. grammepomus*. Apart from several morphological characters that correspond to several key morphological identification literature, the most easily recognized difference between these three species is the pattern along the body.

Three species of adult goby fish in the waters of the Bone River show different morphological phenotypes. Their distinctive characters are presented in Figure 3.

Morphometric truss characters

Comparison of standardized morphometric truss character data using equation 1 for males and females of three goby species as research objects in this research are presented in Table 4.

In current research, multivariate truss morphometric analysis between sexes of three goby species is presented in Figure 4. In PCA ordination of the standardized morphometric characteristics, the first two components each of the first two components is quite good at explaining the overall variance of the data set (greater than 50%). The cumulative value of the eigenanalysis of the covariance matrix is 75.6, 78.3, and 62.5% for *S. longifilis*, *B. belobranchus*, and *A. grammepomus* respectively. Therefore, the PCA ordination of the first two components (PC1 and PC2) sufficiently described an underlying pattern of the multivariate data set. The clusters of male and female *S. longifilis* (Figure 4.A) and *B. belobranchus* (Figure 4.B) ordinated by PCA do not overlap. Meanwhile, a small portion of *A. grammepomus* (Figure 4.C) dataset shows an overlap in data between the sexes.

Table 2. Three species of gobies caught from Bone River, Gorontalo, Indonesia

Species	Number of samples		Total
	March	November	
Sicyopterus longifilis	133	93	226
Belobranchus belobranchus	33	24	57
Awaous grammepomus	25	28	53
Total			336

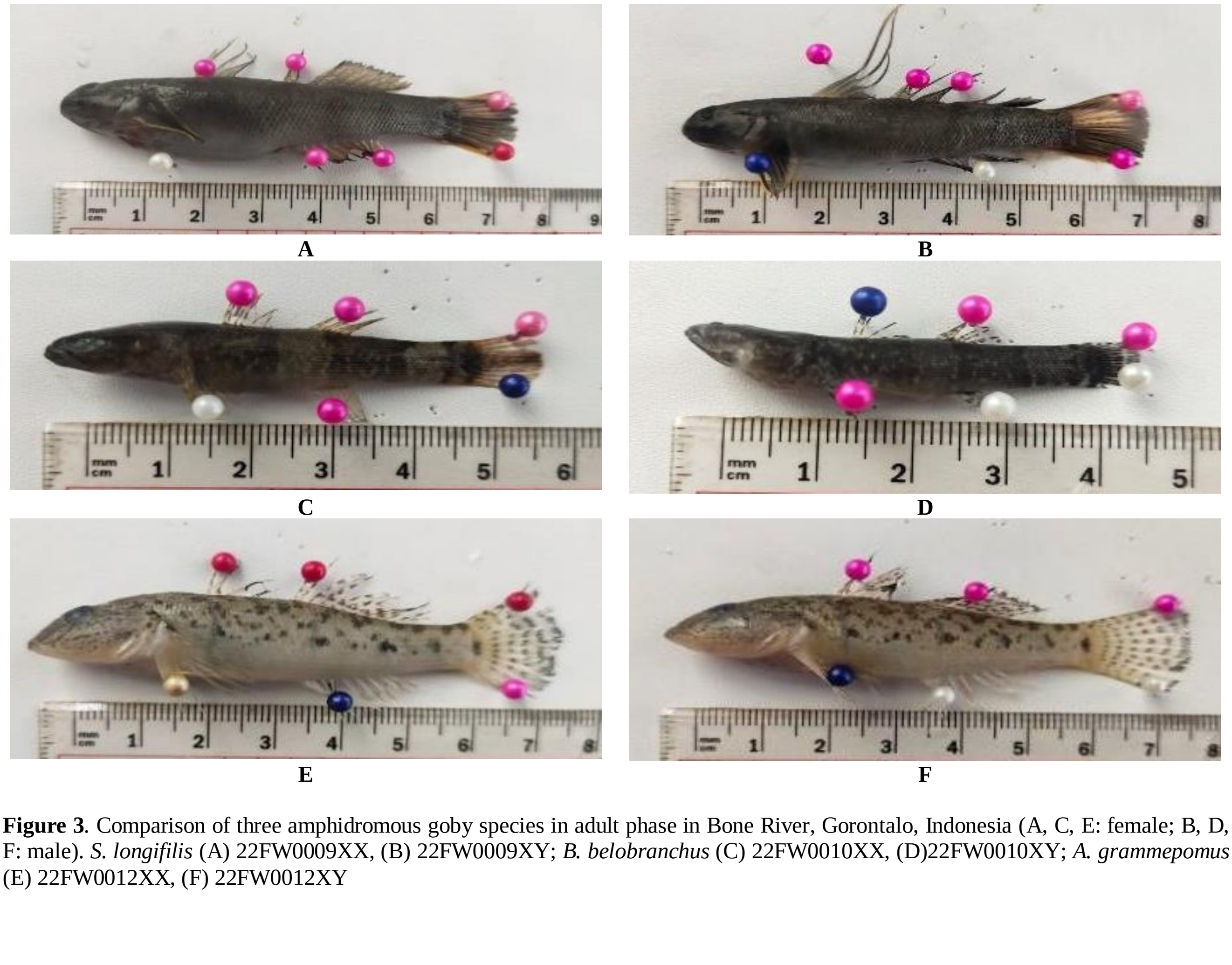

Figure 3. Comparison of three amphidromous goby species in adult phase in Bone River, Gorontalo, Indonesia (A, C, E: female; B, D, F: male). *S. longifilis* (A) 22FW0009XX, (B) 22FW0009XY; *B. belobranchus* (C) 22FW0010XX, (D)22FW0010XY; *A. grammepomus* (E) 22FW0012XX, (F) 22FW0012XY

Table 3. Code and description of the truss morphometric characteristic standards in research on adult goby fish in the Bone River, Gorontalo, Indonesia

Body part	Truss code	Description
Head	1-2 (A1)	Distance between outer tip of the snout and head-dorsal body border point
	1-13 (A2)	Distance between outer tip of the snout and base of the lower jaw
	2-13 (A3)	Distance between head-dorsal body border point and base of the lower jaw
Anterior	2-3 (B1)	Distance between head-dorsal body border point and anterior base of dorsal fin
	3-4 (B2)	Distance between anterior base of dorsal fin and posterior base of dorsal fin
	4-5 (B3)	Distance between posterior base of the dorsal fin and the anterior base of the second dorsal fin
	11-12 (B4)	Distance between anterior base of the anal fin and the anterior base of the ventral fin
	12-13 (B5)	Distance between anterior base of the ventral fin and the base of the lower jaw
	1-12 (B6)	Distance between outer tip of the snout and the anterior base of the ventral fin
	2-11 (B7)	Distance between head-dorsal body border point and the anterior base of the anal fin
	2-12 (B8)	Distance between head-dorsal body border point and the anterior base of the ventral fin
	3-11 (B9)	Distance between anterior starting point of the dorsal fin and anterior base of the anal fin
	3-12 (B10)	Distance between anterior starting point of the dorsal fin and anterior base of the ventral fin
	4-11 (B11)	Distance between posterior starting point of dorsal fin and anterior base of the anal fin
	4-12 (B12)	Distance between posterior starting point of dorsal fin and anterior base of the ventral fin
Posterior	5-6 (C1)	Distance between anterior base of the second dorsal fin and posterior base of the second dorsal fin
	6-7 (C2)	Distance between posterior base of the second dorsal fin and anterior base of the dorsal caudal fin
	9-10 (C3)	Distance between anterior base of ventral fin and posterior base of anal fin
	10-11 (C4)	Distance between posterior base of anal fin and anterior base of anal fin
	5-10 (C5)	Distance between anterior base of the second dorsal fin and posterior base of anal fin
	5-11 (C6)	Distance between anterior base of the second dorsal fin and anterior base of anal fin
	6-9 (C7)	Distance between posterior base of the second dorsal fin and anterior base of ventral fin
	6-10 (C8)	Distance between posterior base of the second dorsal fin and posterior base of anal fin
	6-11 (C9)	Distance between posterior base of the second dorsal fin and anterior base of anal fin
	7-10 (C10)	Distance between anterior base of dorsal caudal fin and posterior base of anal fin
Caudal	7-8 (D1)	Distance between anterior base of dorsal caudal fin and midpoint of anterior base of the caudal fin
	8-9 (D2)	Distance between midpoint of anterior base of the caudal fin and anterior base of ventral caudal fin
	7-9 (D3)	Distance between anterior base of dorsal caudal fin and anterior base of ventral caudal fin

Table 4. Average values of twenty eight standardized morphometric truss characters of three goby species from Bone River, Gorontalo, Indonesia

Standardized truss code	*S. longifilis*		Standardized truss code	*B. belobranhcus*		Standardized truss code	*A. grammepomus*	
	Male (M) n=108	Female (F) n=118		Male (M) n=31	Female (F) n=26		Male (M) n=30	Female (F) n=23
LT-A1	0.940±0.135	0.959±0.131	LT-A1	1.204±0.088	1.106±0.124	LT-A1	1.032±0.165	1.063±0.094
LT-A2	0.791±0.135	0.829±0.128	LT-A2	0.991±0.110	0.946±0.946	LT-A2	0.910±0.147	0.873±0.105
LT-A3	0.793±0.161	0.827±0.151	LT-A3	0.835±0.159	0.795±0.115	LT-A3	0.809±0.155	0.808±0.157
LT-B1	0.537±0.211	0.555±0.194	LT-B1	0.756±0.119	0.752±0.160	LT-B1	0.642±0.309	0.634±0.146
LT-B2	1.026±0.148	1.071±0.136	LT-B2	0.931±0.152	0.886±0.178	LT-B2	1.051±0.165	1.066±0.081
LT-B3	0.943±0.153	0.953±0.133	LT-B3	1.175±0.191	1.105±0.171	LT-B3*	0.955±0.200	0.958±0.103
LT-B4	0.502±0.158	0.518±0.177	LT-B4	0.593±0.190	0.631±0.166	LT-B4*	0.483±0.159	0.506±0.142
LT-B5	0.506±0.164	0.533±0.167	LT-B5	0.656±0.157	0.613±0.202	LT-B5	0.567±0.211	0.564±0.171
LT-B6	0.963±0.149	1.002±0.099	LT-B6	1.145±0.134	1.080±0.223	LT-B6*	1.022±0.110	1.032±0.163
LT-B7	0.987±0.170	1.007±0.166	LT-B7*	0.877±0.169	0.890±0.177	LT-B7*	1.018±0.187	1.037±0.144
LT-B8	1.197±0.156	1.221±0.179	LT-B8	1.330±0.092	1.241±0.235	LT-B8*	1.197±0.153	1.176±0.150
LT-B9	0.770±0.182	0.804±0.144	LT-B9	1.015±0.148	0.997±0.179	LT-B9	0.926±0.200	0.885±0.169
LT-B10	0.956±0.165	0.976±0.181	LT-B10	1.158±0.217	1.120±0.246	LT-B10*	1.061±0.237	0.989±0.157
LT-B11	0.725±0.219	0.761±0.252	LT-B11	0.902±0.246	0.875±0.248	LT-B11	0.828±0.245	0.882±0.293
LT-B12	1.172±0.223	1.217±0.177	LT-B12	1.339±0.220	1.244±0.270	LT-B12*	1.229±0.216	1.196±0.173
LT-C1	0.875±0.154	0.899±0.143	LT-C1	1.030±0.153	1.003±0.154	LT-C1	0.949±0.141	0.985±0.139
LT-C2	0.922±0.156	0.953±0.205	LT-C2	1.101±0.115	1.034±0.114	LT-C2	1.068±0.141	1.054±0.129
LT-C3	1.074±0.184	1.099±0.151	LT-C3	1.207±0.133	1.170±0.161	LT-C3*	1.132±0.140	1.161±0.050
LT-C4	1.010±0.118	1.020±0.116	LT-C4	1.143±0.104	1.136±0.127	LT-C4	1.098±0.130	1.076±0.093
LT-C5	0.980±0.140	1.027±0.151	LT-C5	1.082±0.206	1.065±0.145	LT-C5	1.069±0.142	1.096±0.135
LT-C6	1.119±0.138	1.126±0.166	LT-C6	1.221±0.160	1.204±0.196	LT-C6*	1.162±0.147	1.161±0.076
LT-C7	1.024±0.185	1.061±0.192	LT-C7	1.128±0.121	1.100±0.141	LT-C7*	1.067±0.197	1.116±0.126
LT-C8	0.923±0.144	0.936±0.139	LT-C8	0.990±0.119	0.971±0.144	LT-C8	0.969±0.122	0.980±0.121
LT-C9	0.918±0.156	0.981±0.128	LT-C9	1.104±0.151	1.027±0.163	LT-C9*	0.984±0.123	0.947±0.119
LT-C10	0.865±0.162	0.852±0.176	LT-C10	0.901±0.115	0.905±0.199	LT-C10*	0.898±0.178	0.844±0.155
LT-D1	0.971±0.229	1.019±0.228	LT-D1	1.068±0.119	1.096±0.197	LT-D1*	0.955±0.285	1.005±0.215
LT-D2	0.727±0.172	0.760±0.158	LT-D2	0.846±0.140	0.843±0.176	LT-D2	0.726±0.129	0.782±0.157
LT-D3	0.953±0.137	0.986±0.124	LT-D3	1.136±0.147	1.084±0.169	LT-D3	1.059±0.098	0.985±0.138

Note: LT-(A1-D3)-M: Standardized male truss morphometric (A1-D3); LT-(A1-D3)-F: Standardized female truss morphometric (A1-D3); *cannot be used to differentiate between males and females

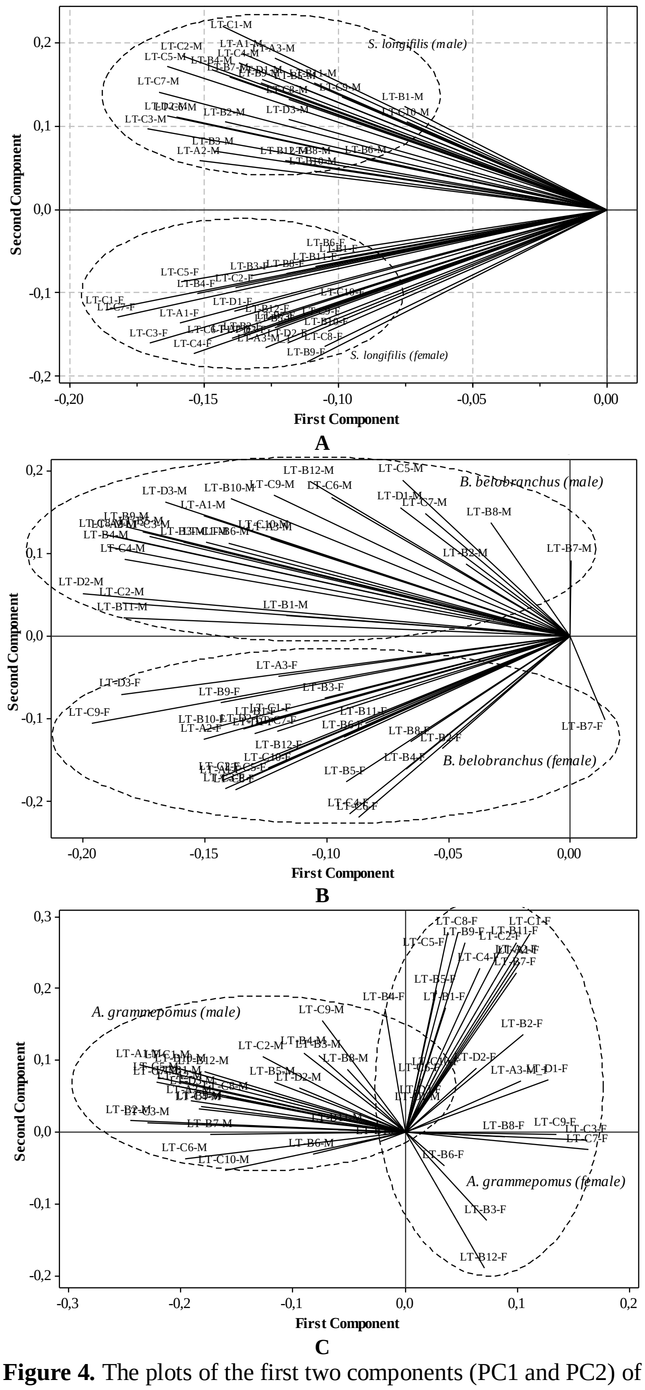

Figure 4. The plots of the first two components (PC1 and PC2) of standardized morphometric truss of male and female for three goby species sampled from Bone River, Gorontalo, Indonesia: A. *S. longifilis,* B. *B. belobranchus,* C. *A. grammepomus*

In *S. longifilis*, all morphometric truss characters in both males and females were negatively correlated with PC1. All male characters are positively correlated with PC2, whereas all female characters have negative correlation to PC2. The respective ordinates of the male and female truss data sets of the *B. belobranchus* species do not overlap. All the truss characters of this species were negatively correlated with PC1 except for LT-B7-M and LT-B7-F truss. As for PC2, all male truss morphometric characters have a positive correlation with PC2, while all female trusses have a negative correlation with PC2. In contrast to the two goby species in this study, several morphometric truss characters of the male and female *A. grammepomus* species do not show a uniform correlations trend to both PC1 and PC2 and appear to overlap in the PCA quadrant. In detail, almost all male truss characters were positively correlated with PC1 except truss LT-D1-M. Meanwhile, in females, all morphometric truss characters were negatively correlated with PC1 unless LT-B4-F and LT-B10-F. As for the correlation with PC2 for both sex's morphological trusses, almost all are positive except for the characters LT-B6-M, LT-B7-M, LT-C6-M, LT-C10-M, LT-B3-F, LT -B6-F, LT-B8-F, LT-B10-F, LT-B12-F, LT-C3-F, LT-C7-F, and LT-C9-F.

Discussion

Sicyopterus longifilis and *B. belobranchus* have been identified as the two species constituting Nike fish according to Sahami et al. (2019). While *A. grammepomus* has not been previously reported as part of Nike fish, the assumption is made in this research based on the indication in Ali et al. (2015) that *A. grammepomus* is an amphidromous species. Thus, it is considered that these three species collectively make up Nike fish in Gorontalo waters. The identification results reveal that the most prevalent goby fish type captured is *S. longifilis*. Additionally, Olii et al. (2019) noted that *S. longifilis* is the predominant goby species in the Bone River Gorontalo. The observations conducted on the three goby species in this study reveal distinctive phenotypic appearances, making it easy to recognize and differentiate each species. Although these three species were most recently assessed for The IUCN Red List of Threatened Species and listed as Least Concern (https://www.iucnredlist.org), morphological and morphometric studies of these fishes are still rare, particularly for *A. grammepomus.*

Sicyopterus longifilis species exhibits distinctive morphological features, including an elongated body with a rounded and prominent snout, an inferior mouth where the upper jaw protrudes from the lower jaw. The first dorsal fin consists of six hard fins, with an elongated shape, and the third fin is the longest. The second dorsal fin has one hard fin and ten soft fins. The tail fin is rounded with 17 fin segments, and the pectoral fins have 20 rays. Notably, the pelvic fins are fused to form a robust cup-like disc. The fin formula for *S. longifilis* is D VI, I 10; A I 10; P 19. It has ctenoid scale type with 66 longitudinal scales, 17 transverse scales, and 25 predorsal scales. The second to fourth hard rays of the dorsal fin form long filaments. The pectoral and caudal fins are fringed with pale yellow. The body color of *S. longifilis* is gray, featuring vertical stripes that sometimes extend towards the stomach. The pectoral fins are black with white bands at the edges, and there is a hint of orange with red and blue stripes at the tip of the dorsal and ventral caudal fins. As a comparison, Muthiadin et al. (2020) reported that the body shape of *S. longifilis* is fusiform and elongated with whitish brown coloration and seven-eight black bands on the body. Although they did not report the results of tracking the morphology of males and females adults separately, the ventral margin of the eye of

S. longifilis is marked with triangular black to the posterior end of the mouth. Mouth ventrally oriented with lateral cleft of the lip and without a median cleft. Dentary with four or seven canine (cone)-like symphyseal teeth on each side. Premaxillary teeth are tricuspid. The first dorsal fin is white with elongated spines and some black spots on the base. One spine in the second dorsal fin and ten soft rays. The second to fourth rays of the dorsal fin are filamentous. Caudal fins are about 1-1.2 mm, have pale to grayish coloration, and are rounded. The anal fin is dark gray with one spine, ten soft rays, and cycloid scales on the base. Pectoral fins are blackish with white bands at the end. Pelvic fins about 0.6 mm in length are translucent and form a strong disk. In this study result, all the morphometric truss characters in this study can be reliably used as a sexual distinguishing characteristic in *S. longifilis*. The standard length of the adult *Sicyopterus* group reported by Lestari et al. (2021) reached 10.4 ± 0.31 mm. This size is much shorter than the results of this research of 50.24-53.65 mm. Keith et al. (2015c) also reported that the standard length of male *S. longifilis* from Tubah River, western Ceram, Indonesia was smaller (36.7-74.5 mm), while the range of 48.4-62.5 mm came from Reifafeif River, Papua Province, Indonesia.

Belobranchus belobranchus has an elongated, torpedo-shaped body. The mouth shape is terminal and has 70 rows of scales on its body. The first stilt fin has one hard fin and eight soft fins. The anal fin has one hard fin and seven soft fins. This species has many narrow dark horizontal stripes on its body. The second dorsal fin has eight hard fins. The caudal fin has spots, white stripes and alternating shades of light brown and dark brown. The scales are ctenoid type with the head having no scales. Keith et al. (2012) also reported that *B. belobranchus* has many narrow dark horizontal stripes on its sides with one per row of scales. There are generally light brown stripes alternating with dark brown central stripes, and the tail fin is always spotted, alternating white and brown stripes. This is in line with research by Bataragoa and Kambey (2021) who reported that this species has a fin formula of D VI, I 7; A I 7; Longitudinal Scales 70. The head has no scales, the gill spine ends with spines, the body is dark brown and there are two slightly white bands on the tail peduncle, the tip of the first dorsal fin is yellow. This species is a goby sleeper with an elongated body with a cylindrical anterior part and a compressed posterior. It has a brownish to mahogany body color. The characteristic of *B. belobranchus* is that it has a darker, thicker body color and extends from the chest to the base of the tail fin along the scale line (Keith et al. 2012). The *B. belobranchus* species in this study shows that males have darker and more striking color patterns than females which tend to be young. Distance between head-dorsal body border point and the anterior base of the anal fin is the only character truss in *B. belobranchus* which does not recommended to differentiate males and females. Keith et al. (2012) reported the standard length of *B. belobranchus* adults in several locations, which varied compared to results in current research with a range of 53.04-54.84 mm. In Leililef Waibulen Halmahera, Indonesia, *B. belobranchus* reaches a standard length of 70 mm, in Manado Celebes, it ranges from 94-123 mm, in Pehavohori River Vanuatu, it ranges from 32-65 mm, in Penaorou River Santo island Vanuatu 102-165 mm, and at Kumafa River Camp, Papua Province, it ranges from 43-58 mm.

Awaous grammepomus is characterized by small spots on the cheeks and the upper part of the operculum, varying in size. There is a distinct dark spot on the top of the pectoral fin, accompanied by a smaller spot inside, though it may not always be clearly visible. Rows of scales along the body, particularly those in front of the dorsal fin, exhibit ctenoid scales. The operculum and cheeks are usually scaly. In male fish, the body color is light brown, while female fish have a paler body color. Male fish also tend to have a slimmer body shape compared to the rounder body shape of females. Almost all truss morphometric characters can be considered a distinguishing feature for determining the sex of this species. The thirteen morphological trusses which are not recommended to be used to differentiate sexuality in *A. grammepomus* are distance between posterior base of the dorsal fin and the anterior base of the second dorsal fin, distance between anterior base of the anal fin and the anterior base of the ventral fin, distance between outer tip of the snout and the anterior base of the ventral fin, distance between head-dorsal body border point and the anterior base of the anal fin, distance between head-dorsal body border point and the anterior base of the ventral fin, distance between anterior starting point of the dorsal fin and anterior base of the ventral fin, distance between posterior starting point of dorsal fin and anterior base of the ventral fin, distance between anterior base of ventral fin and posterior base of anal fin, distance between anterior base of the second dorsal fin and anterior base of anal fin, distance between posterior base of the second dorsal fin and anterior base of ventral fin, distance between posterior base of the second dorsal fin and anterior base of anal fin, distance between anterior base of dorsal caudal fin and posterior base of anal fin, and distance between anterior base of dorsal caudal fin and midpoint of anterior base of the caudal fin. Standard length of *A. grammepomus* reported by Jahan et al. (2017) is 82 mm. This size is longer than the fish samples in this research which only measured 53.04 to 54.84 mm. The differentiating characteristics of *A. grammepomus* sexes as a result of this research are new data that can be used to support species conservation movements and management of amphidromous fish resources. Some recent studies on *A. grammepomus* do not focus on morphology and morphometric aspects but rather study the genetics side (Kushwaha et al. 2023; Kader et al. 2023), biochemical composition (Hossain et al. 2015) and product processing (Mahmud et al. 2019).

According to Matthews (1998), morphological variation in fish is influenced by genetic factors inherited from their parents, which differentiate them from other species. Additionally, as mentioned by Brezky and Doyle (1988), male and female fish typically exhibit different growth patterns, leading to variations in various body parts or truss distances. Conducting a thorough analysis allows for the identification of these differences, forming the basis for employing truss morphometric techniques. Various studies have been conducted that apply the use of morphometry and morphology as differentiators in fish (Kitano et al. 2007; Uba 2019; Bakhshalizadeh et al. 2022; Yurtseva and Uspenskiy 2022), however the use of similar methods to differentiate males and females has been reported in this research. In the future, it is important to combine this study results with tracing genetic aspects to gain confirmation of genetic variations that could influence the phenotypic characteristics of amphidromous goby fish due to the dynamics of the habitat they experience.

In summary, this study successfully identified distinct morphological differences between males and females of *S. longifilis*, characterized by a slimmer body shape in males compared to the rounded shape in females. For *B. belobranchus*, male individuals exhibited more striking coloration than their female counterparts. In *A. grammepomus*, males were characterized by a light brown body color, while females displayed a paler coloration. Regarding truss morphometric characters, on the head part of body, all trusses can be used to differentiate between males and females in these three goby species. All morphometric characaters can be used to differentiate males and females of *S. longifilis*. In the other two gobies, *B. belobranchus* and *A. grammepomus*, there are several characters that still overlap between males and females so that they cannot be relied upon to differentiate between sexes.

ACKNOWLEDGEMENTS

We would like to thank Musdalifah Mahua, and Miranti Khairunisa Abudi, for their contribution during the research. We also extend gratitude to the reviewers who have contributed valuable input to enhance and sharpen the delivery of research results on this manuscript.

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