

MORPHOMETRIC AND PHYLOGENETIC ANALYSIS OF GOBY FISH (*Glossogobius giuris*) IN THE THREE INTEGRATED LAKES ON SOUTH SULAWESI, INDONESIA

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Abstract

The white goby fish, *Glossogobius giuris* is a germplasm in Tempe Lake that is decreasing due to the genetic barrier. This becomes a threat for its population in the three integrated lakes, namely Sidenreng, Lapompakka, and Tempe. Hence, efforts are needed to preserve its population and habitat by conservation programs to prevent the extinction of this fish. This conservation program is carried out when the basic biology information and population structure of goby fish are known. Therefore, this study aims to analyze the genetic variation of goby fish through morphometric and phylogenetic analysis in the three integrated lakes. The morphometric analysis was carried out using Stepwise Discriminant Analysis, while the difference in genetic distance between populations was analyzed using Predicted Group Membership and Pairwise Group Comparison, and the identifiable characters were analyzed using the Equality of Group Means Test. Similarly, the phylogenetic analysis was conducted based on mitochondrial DNA (mtDNA) targeting Cytochrome C Oxidase subunit I (COI). The morphometric analysis showed that each population has a special character with a very low value of similarity between populations. Furthermore, there were 17 and 18 identifiable characters for males and females, respectively in this study. Based on phylogenetic analysis, three groups of goby fish with low genetic diversity were identified. In addition, there was one haplotype shared by the three populations, but the other 7 haplotypes are unique, which indicated genetic speciation of goby fish from these lakes. Based on these results, goby fish from the three integrated lakes have special characters, adaptive potential, and genetic speciation due to the declining population in the lakes.

Keywords: *Glossogobius giuris*; Morphometric; Phylogenetic; Integrated lakes

Introduction

Glossogobius giuris (Hamilton, 1822) is known as the white goby fish with cylindrical body shape, flathead, superior mouth type, and yellow brownish with black spots body color. Furthermore, there are small longitudinal spots on the dorsal fin and a rounded caudal fin with white, black pattern. The head scales are cycloid, and body scales are ctenoid [1], while the pectoral and caudal fins are gray or hialin [2]. This fish is one of the benthopelagic and amphidromous fish that generally lives in the freshwater, estuary, and coastal area [3-6], and is

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geographically distributed at all Indo-pacific areas [7, 8], from the East Coast of Africa, Red Sea, and India to China [3]. In Indonesia, its local name is Bungo fish, and one of its habitats is Tempe Lake [9, 10].

Tempe Lake is one of the biggest in the middle of South Sulawesi [1] and is integrated with two other lakes, namely Sidenreng and Lapompakka (Buaya Lake) [10]. During the rainy season, these lakes are integrated into a body of water with an area of 35.000ha, which makes the fish composition not to be significantly different, including the *G. giuris* [11, 12]. The *G. giuris* has high economic value and became favorite local food [9] because of its specific taste [10]. This fish also has thick meat, less bone [9], low fat, and high protein [4, 13-15], and is popular in the local and international markets such as Italy, India, Burma, Nepal, and France [14].

The high price market makes this species an important catch for fishermen [3] which influences its decreasing size and population in nature [1] and is on IUCN red list [16]. This population decline is caused by changes in environmental conditions, the introduction of new species, and overexploitation [17]. Since this occurred in Tempe [1, 9] and Lompok Lake [11], therefore, efforts are needed to preserve the population and habitat of this fish from becoming extinct as the endemic germplasm of Tempe Lake [10], which is achieved through a conservation program.

A conservation program is carried out to develop strategies for sustainable management and preservation of biodiversity [17]. This is applicable when the basic knowledge of the biology and population structure of a species is known [4, 18]. Moreover, fish morphology is the main biological basic knowledge for taxonomic characters and their evolution because each species has a shape, size, pigmentation pattern, fin disposition, and other external organs for recognition, identification, and classification [18]. Species with a wide geographical distribution often have morphological variability, which makes distinguishing between species to be difficult [19]. This problem can be solved through morphometric studies and phylogenetic analysis.

Morphometry is a measurable character based on biological form, the covariation between patterns of morphological variation from the interactions between biotic and abiotic factors [20, 21]. This morphometric relationship is important in fisheries management to determine the growth rate of a species and variation within one species in different locations [13, 22, 23]. It becomes a constructive tool used by taxonomists to identify each species and its systematic relationship, ontogenetic traces, and other population parameters [3, 4]. The morphometric character is also used by taxonomists to prepare identification keys [24]. However, the goby fish has a small body size and several morphological variations that make identification and classification difficult [25]. Therefore, the International Union for Conservation of Nature (IUCN) recommended a more detailed study to clarify the identification of this fish [26].

The DNA barcoding technique is a complementary technique compared to morphological methods in identifying species levels [27, 28]. It is a powerful tool for species identification using standard DNA sequences [29]. Currently, the most commonly used DNA barcoding technique is the standard gene sequence, which is the mitochondrial DNA cytochrome C oxidase subunit I (COI) gene, for rapid and accurate identification as well as differentiation between individuals at the species level [30, 31]. Meanwhile, differences in the morphology and morphometric among individuals have led to genetic variation of organisms in a population [32].

Genetic variation is a key characteristic of the adaptability of a species [17] which is indispensable for formulating an appropriate management program [2]. This genetic information is rare for aquatic species that are threatened with extinction and persists in a local population [17], such as the goby fish. Due to the overexploitation in Tempe Lake, a reduction in morphology occurred [26] which affect genetic diversity [9]. Therefore, this study aims to

analyze the genetic variation of goby fish through morphometric characters and phylogenetic analysis using the CO1 gene target in the three integrated lakes, South Sulawesi, Indonesia.

Experimental part

Materials

Goby fish samples used were collected from local collectors in the three integrated lakes area, namely Tempe, Lopompakka, and Sidenreng, South Sulawesi, Indonesia (Fig. 1). For the phylogenetic analysis, 10 fish samples/population were collected from each location. Furthermore, 50mg of fresh fish muscle tissue from each sample was preserved using 70% ethanol solution. The collected samples were stored at room temperature before the DNA extraction process was carried out.

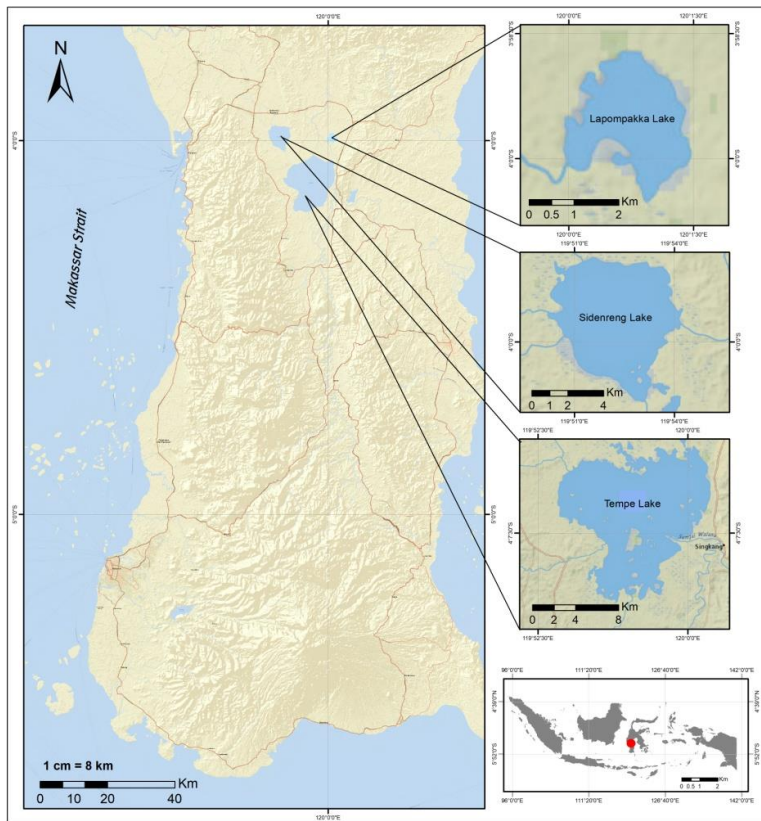


Fig. 1. Sampling location

Methods

Morphometric Analysis

The measurement of morphometric parameters in this study was based on the method of Song *et al.* [33] using the character of the snakehead fish *Channa striata* (Bloch, 1793), which was modified by giving each morphometric character code (Figs. 2-5 and Table 1).

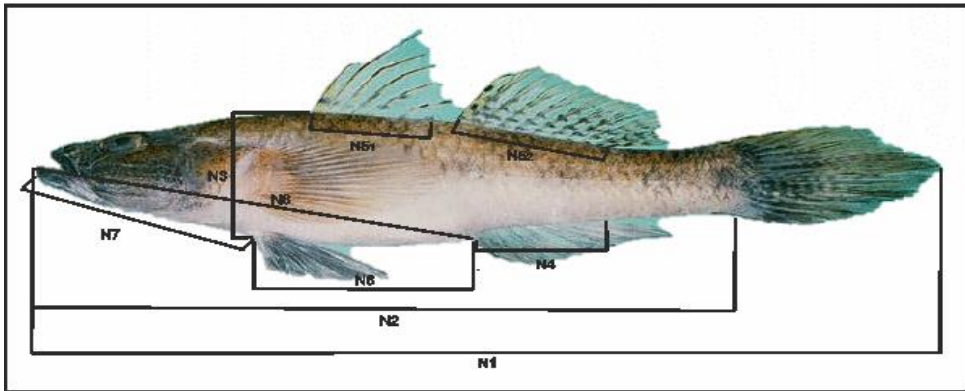


Fig. 2. Fish Schematic Showing Morphometric Characteristics and Measures Used in Identification (left side)
(1. N1; 2. N2; 3. N3; 4. N4; 5. N5₁; 6. N5₂; 7. N6; 8. N7; 9. N8)

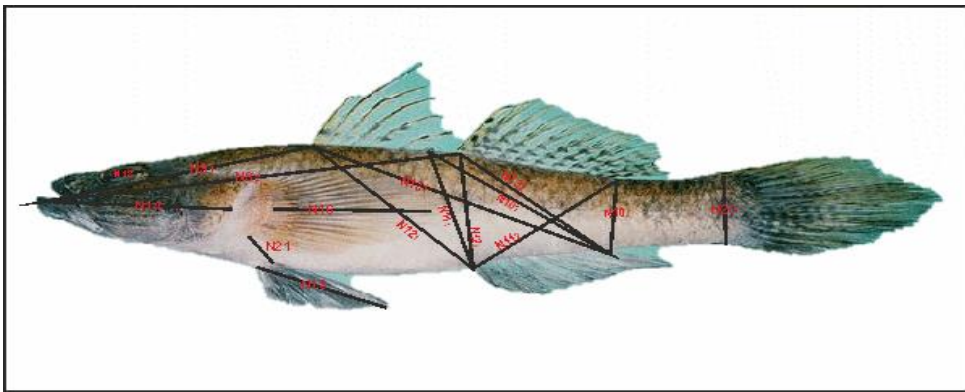


Fig. 3. Fish Schematic Showing Morphometric Characteristics and Measures Used in Identification (left side)
(10. N10₁; 11. N10₂; 12. N11₁; 13. N11₂; 14. N12₁; 15. N12₂; 16. N13₁; 17. N13₂; 20. N14; 22. N16; 24. N18; 26. N20; 27. N21; 29. N23)

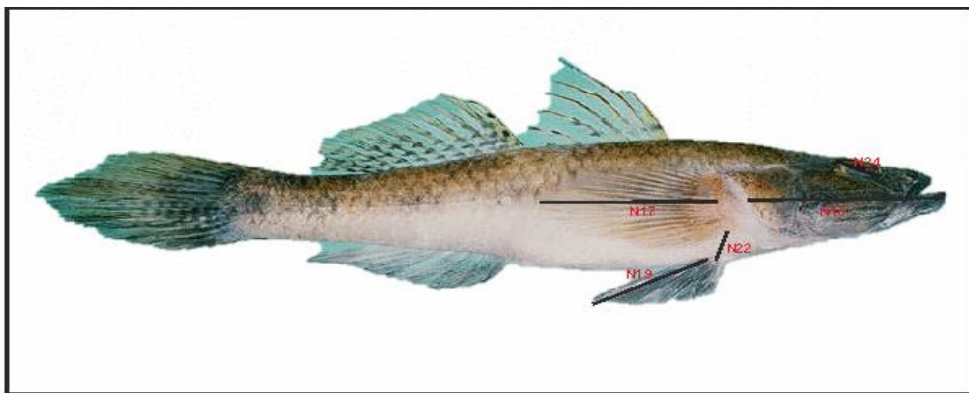


Fig. 4. Fish Schematic Showing Morphometric Characteristics and Measures Used in Identification (right side)
(21. N15; 23. N17; 25. N19; 28. N22; 30. N24)



Fig. 5. Fish Schematic Showing Morphometric Characteristics and Measures Used in Identification (dorsal side) (31. N25; 32. N26; 33. N27; 34. N28)

Table 1. Morphometric Measurement Character Gabus Fish *Channa striata* (Bloch, 1793)

No	Morphometric Characters	Description
1	Total Length (N1)	The distance between the mouth tip and the most posterior tip of the caudal fin
2	Standard Length (N2)	The distance between the mouth tip and the crease of the base of the caudal fin
3	Height (N3)	Measured at the tip of the leading dorsal fin with the pelvic fin
4	The length of the base of the anal fin (N4)	The distance between the base of the first spokes and the place of the fin membrane behind the last fingers
5	The length of the base of the dorsal fin (N5 ₁)	The distance between the base of the first ray and the place of the fin behind the last ray on the first dorsal fin
6	The length of the base of the dorsal fin (N5 ₂)	The distance between the base of the first dorsal fin and the place of the fin behind the last ray of the second dorsal fin
7	Distance between pelvic fin and anal fin (N6)	Measured from the tip of the leading pelvic fin to the base of the first anal fin
8	Distance between mouth tip and pelvic fin (N7)	Measured from the leading edge of the mouth to the leading edge of the pelvic fins
9	Distance between mouth tip and anal fin (N8)	Measured from the tip of the leading edge to the first radius of the anal fin
10	Distance between the base of the leading dorsal fin and the tip of the mouth (N9 ₁)	Measured from the first dorsal fin at the base of the leading fin rays to the tip of the leading mouth
11	Distance between the base of the leading dorsal fin and the mouth tip (N9 ₂)	Measured from the second dorsal fin at the base of the leading fin rays to the tip of the leading mouth
12	Distance between the base of the dorsal fin and the base of the anal fin (N10 ₁)	Measured from the base of the first dorsal fin on the back to the base of the rear anal fin
13	Distance between the base of the dorsal fin and the base of the anal fin (N10 ₂)	Measured from the base of the second dorsal fin on the back to the base of the rear anal fin
14	Distance between the base of dorsal fin and base of anterior anal fin (N11 ₁)	Measured from the base of the first dorsal fin on the back to the base of the front anal fin
15	Distance between the base of hind dorsal fin and base of anterior anal fin (N11 ₂)	Measured from the base of the second dorsal fin on the back to the base of the front anal fin
16	Distance between the base of anterior dorsal fin and base of anterior anal fin (N12 ₁)	Measured from the base of the first dorsal fin on the front to the base of the front anal fin
17	Distance between the base of anterior dorsal fin and base of anterior anal fin (N12 ₂)	Measured from the base of the second dorsal fin on the front to the base of the front anal fin
18	Distance between the base of front dorsal fin and base of hind anal fin (N13 ₁)	Measured from the base of the first dorsal fin on the front to the base of the rear anal fin

No	Morphometric Characters	Description
19	Distance between the base of anterior dorsal fin and base of hind anal fin (N13 ₂)	Measured from the base of the second dorsal fin on the front to the base of the rear anal fin
20	Distance between the tip of the mouth and the left operculum (N14)	Measured from the front end of the mouth to the back of the left operculum
21	Distance between the tip of the mouth and the right operculum (N15)	Measured from the leading edge of the mouth to the back of the right operculum
22	The left pectoral fin length (N16)	The distance from the base of the fin to the longest tip of the left pectoral fin
23	Right pectoral fin length (N17)	The distance from the base of the fin to the longest tip of the right pectoral fin
24	Left pelvic fin length (N18)	The distance from the base of the fin to the longest tip of the left pelvic fin
25	Right pelvic fin length (N19)	The distance from the base of the fin to the longest tip of the right pelvic fin
26	Width of caudal fin base (N20)	Measured from both caudal fin bases
27	Distance between left pectoral fin and left pelvic fin (N21)	Measured from the first radius of the left pectoral fin to the last radius of the left pelvic fin
28	Distance between right pectoral fin and right pelvic fin (N22)	Measured from the first radius of the left pectoral fin to the last radius of the right pelvic fin
29	The left eye height (N23)	Measured from the midline length of the left eye socket
30	The right eye height (N24)	Measured from the midline length of the right eye socket
31	Distance between holes above the mouth (N25)	Measured from the middle of the two holes
32	Distance between nostrils near eyes (N26)	Measured from the middle of the two nostrils
33	Distance between eyes (N27)	Measured from the two upper edges of the eye sockets
34	Body width (N28)	Measured from the distance between the right and left pectoral fins

The data obtained were initially standardized with the formulation proposed by *Elliott et al.* [34], which is $M_s = M_o(L_s/L_o)$ where M_s = standardized size, M_o = length of the measured character, L_s = arithmetic mean of standard length (N2), L_o = standard length (N2), b = regression coefficient from $\log M_o$ to $\log L_o$ estimated for each character measured by the allometric growth equation, namely $M = aL^b$. After standardization, all characters were ratioed or divided by standard length characters. Furthermore, the morphometric character data were analyzed using discriminant analysis to determine characterizing characters between locations.

Phylogenetic Analysis

DNA extraction was carried out using a mini genomic DNA kit (Geneaid), while the genetic diversity analysis was conducted using mt-DNA with primer F (5'-CGC CTG TTT AAC AAA AAC AT - 3') and primer R (5'-CCG GTC TGA ACT CAG ATC ATG T-3') according to *Hadijah et al.* [9]. The amplification of the mt-DNA gene was carried out using PCR with an initial denaturation program at 94°C for 2 minutes after 40 cycles of 94°C for 15 seconds, annealing 60°C for 30 seconds, and elongation of 68°C for 1 minute. Furthermore, the final elongation was carried out in 1 cycle at 68°C for 5 minutes and the PCR amplification products were separated using 2% agarose gel electrophoresis. Agarose gel DNA fragments were documented using the Gel Documentation system (Biometra) and the size was measured using a 100bp plus DNA marker. The electrophoretic product was purified on a 1% agarose (gel agarose) mixture, which consisted of 0.75g, SB buffer 75mL, and 4L ethidium bromide at 100V/400 amps for 30min before sequencing. Meanwhile, the sequencing results are viewed manually using the sequence navigator program.

The data obtained were analyzed using the Mega 7.0 Program [35] to determine nucleotide variation and genetic distance between individuals (phylogenetic tree). To determine the similarity among the sequences, the mt-DNA gene sequences were aligned with the existing ones in the Gene Bank using the BLAST-N (basic local alignment search tool-nucleotide)

program. Furthermore, the DNAsp ver. Program 5.10 [36] was used to determine the haplotype differences between Bungo fish populations from the site.

Results and Discussion

Morphometric Analysis

The morphometric study is one of the tools used to identify species because it is consistent in the description and numerical differences in morphology [20]. It also plays a role in determining individual well-being, evaluating life history, and morphological characteristics of populations from various locations [3], such as growth variability, ontogenetic traces, and various other population parameters [37].

Meanwhile, the analysis of Canonical Discriminant Functions (CDF) results showed distinctive groupings between male and female fish (Fig. 6) from the three lakes. The distribution pattern of male and female fish plots from Lapompakka Lake with significantly different centroid groups showed that they have special characters, however, some females from Tempe Lake have the same character as the females from Lapompakka. This was also shown by the species from Sidenreng, although there were several males from Tempe Lake with the same character as males from Sidenreng lake. Therefore, this plot showed that each population has its character from Lapompakka, Tempe, and Sidenreng lakes. However, few individuals from Sidenreng and Lapompakka lakes have the same character as fish from Tempe Lake. This is presumably due to the migration pattern of goby fish.

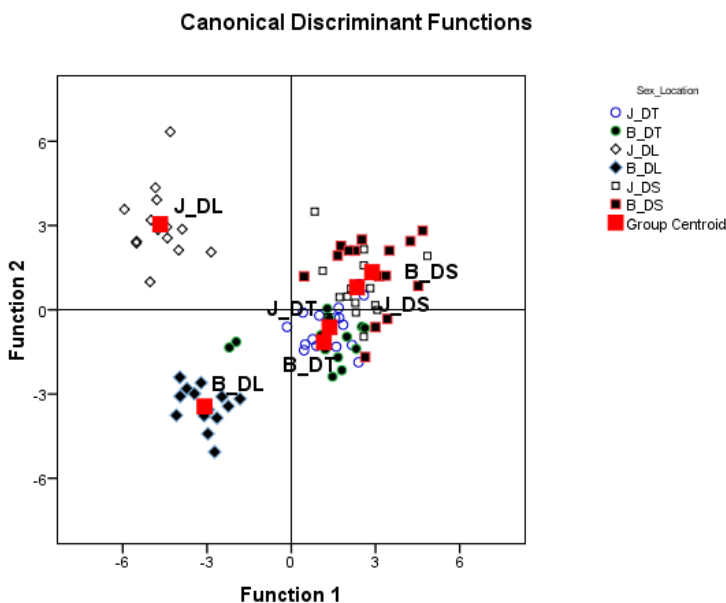


Fig. 6. Scatter plot CDF population of female and male goby fish from Lake Lapompakka, Lake Sidenreng and Lake Tempe. Desc. J: jantan (Male); B: betina (Female); DT: danau Tempe (Lake Tempe); DL: danau Lapompakka (Lake Lampompakka); DS: danau Sidenreng (Lake Sidenreng)

In South Sulawesi, the fish from Bone Bay, which migrates to Tempe Lake via the Pallime and Walanae rivers [38] migrates from Tempe to Sidenreng and Lapompakka lakes through naturally formed waterways. These three lakes are formed in the middle of the integrated Tempe Lake basin in 2 districts, namely Sidrap (Lake Sidenreng) and Wajo Regency

(Lapompakka and Tempe lakes). Lake Tempe has a basin that consists of a flood and a terraced plain, but the boundaries between these two areas are not clear. During the rainy season, the three lakes unite due to flooding and are separated from each other during the dry season [39, 40]. This is supported by the results of the Predicted Group Membership (PGM) analysis which showed that the value of the similarity of characters between the 2 populations and Lake Tempe is very low (Table 2). This migration pattern mostly occurs during the rainy season to prevent an increase in the pattern, which causes a similar character between the population of fish in Sidenreng and Tempe lakes by 14.3%. Similarly, this also occurred to Lapompakka lake which had the same character as the fish population from Tempe Lake with a value of 6.7%.

Table 2. PGM analysis results of goby fish (*Glossogobius giuris*) population and diversity between 3 male and 3 female populations.

Classification	Code	Predicted Group Membership						Total
		J_DT	B_DT	J_DL	B_DL	J_DS	B_DS	
Count	J_DT	9	7	0	0	0	0	16
	B_DT	7	5	0	2	0	0	14
	J_DL	0	0	15	0	0	0	15
	B_DL	0	0	0	15	0	0	15
	J_DS	1	0	0	0	11	3	15
	B_DS	0	0	0	0	1	14	15
Cross-validated	J_DT	56.2	43.8	.0	.0	.0	.0	100.0
	B_DT	50.0	35.7	.0	14.3	.0	.0	100.0
	J_DL	.0	.0	100.0	.0	.0	.0	100.0
	B_DL	.0	.0	.0	100.0	.0	.0	100.0
	J_DS	6.7	.0	.0	.0	73.3	20.0	100.0
	B_DS	.0	.0	.0	.0	6.7	93.3	100.0

Noted: 85.6% original grouped cases correctly classified; 76.7% of cross-validated grouped cases correctly classified. The validation value $\geq 50\%$ was valid. Noted: J: jantan (Male); B: betina (Female); DT: danau Tempe (Lake Tempe); DL: danau Lapompakka (Lake Lampompakka); DS: danau Sidenreng (Lake Sidenreng)

Based on the Pairwise Group Comparison (PGC) analysis results, the three populations of goby fish had a large degree of difference. Meanwhile, the highest degree of difference in male and female fish between the population of Sidenreng and Lapompakka lakes were 50.64 and 51.63, respectively (Table 3).

This showed that the two populations have their special characteristics based on the high value of the degree of difference produced in both male and female fish (≥ 50). These character differences are closely related to genetic and/or environmental differences [15, 18]. Moreover, fish is one of the most sensitive animals to environmental changes that adapt quickly by changing physiology and behavior, which affect their morphological character [13, 18]. According to Islam et al. [15], variations in the aquatic environment changes the feeding habits, availability of food, growth models, and reproductive plans of a species. Meanwhile, the characters that showed these differences are analyzed using Equality of Group Means (EGM) analysis (Table 4).

The Equality of Group Means (EGM) analysis results showed the differences between the three populations. Out of the 33 identifiable characters measured, only 5 were not significant ($P > 0.05$), namely the distance between the base of the hind dorsal and front anal fins (N112), the distance between the base of the front dorsal and front anal fins (N121), the distance between the base of the front dorsal and rear anal fins (N132), left eye height (N23), and right eye height (N24). Furthermore, the characterizing or distinguishing between populations both male and female population are shown in Table 5.

Table 3. PGC analysis results from a genetic distance based on species morphometric *Glossogobius giuris*

Code		J_DT	B_DT	J_DL	B_DL	J_DS	B_DS
J_DT	F		.361	41.634	35.512	23.10	20.041
	Sig.					9	
B_DT	F	.361	.950	.000	.000	.000	.000
	Sig.			40.025	29.397	23.12	20.099
J_DL	F	.950	41.634	40.02	37.722	50.53	51.626
	Sig.			5		8	
B_DL	F	.000	.000	.000	.000	.000	.000
	Sig.	35.512	29.39	37.733		41.30	47.059
J_DS	F	.000	.000	.000	.000	.000	.000
	Sig.	23.109	23.12	50.638	41.301		16.730
B_DS	F	.000	.000	.000	.000	16.73	
	Sig.	20.041	20.09	51.626	47.059	0	
		.000	.000	.000	.000	.000	

Noted: J : jantan (Male); B : betina (Female); DT : danau Tempe (Lake Tempe); DL: danau Lapompakka (Lake Lampompakka); DS : danau Sidenreng (Lake Sidenreng)

Table 4. Analysis result of Test of Equality of Group from Character Traits between 3 goby fish populations

Character Traits	Wilks' Lambda	F	df1	Sig
N1	.192	70.704	5	.000
N3	.166	84.570	5	.000
N4	.446	20.853	5	.000
N5 ₁	.638	9.534	5	.000
N5 ₂	.685	7.719	5	.000
N6	.703	7.085	5	.000
N7	.478	18.341	5	.000
N8	.535	14.586	5	.000
N9 ₁	.763	5.226	5	.000
N9 ₂	.806	4.055	5	.002
N10 ₁	.838	3.242	5	.010
N10 ₂	.654	8.886	5	.000
N11 ₁	.744	5.787	5	.000
N11 ₂	.889	2.095	5	.074
N12 ₁	.929	1.292	5	.275
N12 ₂	.688	7.604	5	.000
N13 ₁	.674	8.112	5	.000
N13 ₂	.920	1.460	5	.212
N14	.453	20.290	5	.000
N15	.441	21.278	5	.000
N16	.492	17.381	5	.000
N17	.489	17.588	5	.000
N18	.718	6.587	5	.000
N19	.726	6.338	5	.000
N20	.637	9.594	5	.000
N21	.579	12.217	5	.000
N22	.558	13.285	5	.000
N23	.932	1.228	5	.303
N24	.936	1.157	5	.337
N25	.797	4.286	5	.002
N26	.796	4.306	5	.002
N27	.618	10.391	5	.000
N28	.524	15.290	5	.000

Table 5. Distinguishing character traits or distinguishing between populations based on the average morphometric characters

Character Traits	Description	Average Distance (Population)					
		J_DT	B_D T	J_DL	B_D L	J_DS	B_DS
N1	Total length	1.298	1.271	1.440	1.108	1.288	1.323
N3	Height	0.470	0.460	0.189	0.134	0.396	0.447
N4	The length of the base of the anal fin	0.164	0.157	0.185	0.144	0.263	0.173
N5 ₁	The length of the base of the dorsal fin	0.124	0.123	0.165	0.129	0.159	0.161
N5 ₂	The length of the base of the dorsal fin	0.189	0.186	0.239	0.160	0.202	0.206
N6	Distance between pelvic fin and anal fin	0.307	0.309	0.328	0.251	0.291	0.360
N7	Distance between mouth tip and pelvic fin	0.350	0.343	0.402	0.300	0.333	0.330
N8	Distance between mouth tip and anal fin	0.634	0.632	0.732	0.523	0.624	0.628
N9 ₁	Distance between the base of the leading dorsal fin and the tip of the mouth	0.414	0.412	0.463	0.396	0.386	0.381
N9 ₂	Distance between the base of the leading dorsal fin and the mouth tip	0.609	0.604	0.684	0.625	0.533	0.518
N10 ₁	Distance between the base of the dorsal fin and the base of the anal fin	0.296	0.295	0.325	0.269	0.275	0.255
N10 ₂	Distance between the base of the dorsal fin and the base of the anal fin	0.118	0.123	0.105	0.095	0.181	0.209
N11 ₁	Distance between the base of dorsal fin and base of anterior anal fin	0.194	0.201	0.259	0.165	0.197	0.196
N12 ₂	Distance between the base of anterior dorsal fin and base of anterior anal fin	0.167	0.162	0.186	0.146	0.216	0.229
N13 ₁	Distance between the base of front dorsal fin and base of hind anal fin	0.407	0.406	0.457	0.363	0.420	0.399
N14	Distance between the tip of the mouth and the left operculum	0.328	0.328	0.377	0.283	0.317	0.327
N15	Distance between the tip of the mouth and the right operculum	0.331	0.331	0.376	0.282	0.317	0.327
N16	The left pectoral fin length	0.228	0.227	0.214	0.184	0.239	0.266
N17	Right pectoral fin length	0.300	0.222	0.215	0.178	0.240	0.244
N18	Left pelvic fin length	0.198	0.191	0.197	0.164	0.153	0.193
N19	Right pelvic fin length	0.198	0.188	0.201	0.161	0.153	0.182
N20	Width of caudal fin base	0.102	0.097	0.129	0.086	0.116	0.153
N21	Distance between left pectoral fin and left pelvic fin	0.055	0.054	0.141	0.110	0.114	0.076
N22	Distance between right pectoral fin and right pelvic fin	0.055	0.052	0.142	0.106	0.110	0.073
N25	Distance between holes above the mouth	0.074	0.073	0.090	0.066	0.066	0.064
N26	Distance between nostrils near eyes	0.046	0.047	0.054	0.045	0.059	0.057
N27	Distance between eyes	0.033	0.031	0.054	0.052	0.057	0.056
N28	Body width	0.146	0.147	0.191	0.144	0.160	0.215

Noted: J: jantan (Male); B: betina (Female); DT: danau Tempe (Lake Tempe); DL: danau Lapompakka (Lake Lampompakka); DS: danau Sidenreng (Lake Sidenreng)

Table 5 showed that 17 male characters from Lapompakka Lake have the longest size, while the females have 18 characters with the shortest size.

In addition, the Table 6 showed that males are generally longer than females in each population. These results were similar to a study by *Unito-Ceniza et al.* [37] which showed that the female has a wider body and abdomen, while the male has a slimmer body than the population of Mainit, Mindanao, and Philippines lakes. This is due to their mechanism in reproducing and maintaining the population. Moreover, male fish plays a role in environmental adaptation to natural changes and maintaining superiority to compete with partners, while females are more adaptive for breeding [20]. The functional adaptations of male fish also exhibit egg-protecting behavior from predators [37] In addition, the length of the morphometric

character of the goby fish body is associated with an increase in the efficiency of swimming fish in the lake for foraging, especially in extreme environments [18].

Adaptation to foraging behavior was also shown from the morphometric size of the head (N25, N26, and N27), where the highest head size was indicated in male fish populations from Lapompakka and Sidenreng lakes. According to *Cabuga et al.* [20], a larger head size aims to maximize mouth opening and suction speed. In addition to influencing differences in eating patterns or behavior, differences in morphometric characters also indicate variations in environmental conditions related to biological, physical, and chemical factors that produce different morphological responses [18].

Table 6. Genetic distance between populations shows in the *pairwise distances* method

Location	Lake Lapompakka	Lake Sidenreng	Lake Tempe
Lake Lapompakka			
Lake Sidenreng	0.00398		
Lake Tempe	0.05882	0.02857	

Out of the 28 identifiable characters in the male and female populations of goby fish, 17 of the longest characters came from the male population of Lapompakka Lake (yellow color), while 18 characters with the smallest size were from the female population of the lake (green).

Phylogenetic Analysis

The electrophoresis results showed that the mt-DNA gene from the sample was 623bp with good quality and the size after editing the sequencing was 598bp. Furthermore, the DNA analysis results showed that the goby fish population showed an insignificant difference between locations included in clade I (red line) combined with goby fish sequences from the gene bank (Fig. 7), except for 5 samples from Tempe Lake. These five samples, namely H7, H8, and H10 were included in clade II, while H4 and H5 were in clade III.

Furthermore, the gene sequencing of Bungo fish samples showed similarities with Goby fish from India (accession numbers: KX373718.1 with 94.9% blast-n and KT878122 with 95.26%). Apart from India, the gene sequences of the Bungo fish samples from this study also showed similarities with the goby fish from Bangladesh (accession number: MF593303.1) and Vietnam (accession number: MH699831.1) with similarity percentages of 94.63% and 94.99%, respectively. These results showed a genetic combination of the three locations. Meanwhile, it is assumed that the genetic combination of goby fish was due to the sampling time, which was carried out in the rainy season. During this season, the waters of the three lakes merged and the population of Bungo fish in the lakes began to search for food, mate, and avoid environmental threats as well as predators.

This is in line with the study of *Omar et al.* [11] which stated that when a major flood occurs, the water from the three lakes merges around the residential areas in Sidenreng Rappang, Soppeng, and Wajo Regencies. Similarly, the fish in these lakes merge into one population for their ichthyofauna species composition to be the same. With the existence of these crocodiles, the distribution and mixing of eggs, as well as larvae in the three locations, is significantly large, which affects the genetic diversity of goby fish in the area. This is also supported by the distance of the three adjacent lakes ranging from 10-20km, namely between Tempe and Sidenreng lakes with approximately 15.4km, and between Tempe and Lapompakka lakes with approximately 20.5km in the dry season. Despite the opportunity for fish distribution between these locations, genetic barriers such as overexploitation, the introduction of new

species, and loss of habitat limit the gene flow of the goby fish population and lead to low genetic diversity [17]. Furthermore, samples from Tempe Lake were different, however, some of the samples were in clade I, II, and III. This showed that the genetic variation of goby fish in Tempe Lake was higher (number of haplotypes 6) compared to the lakes with the number of haplotypes of two each (Table 7).

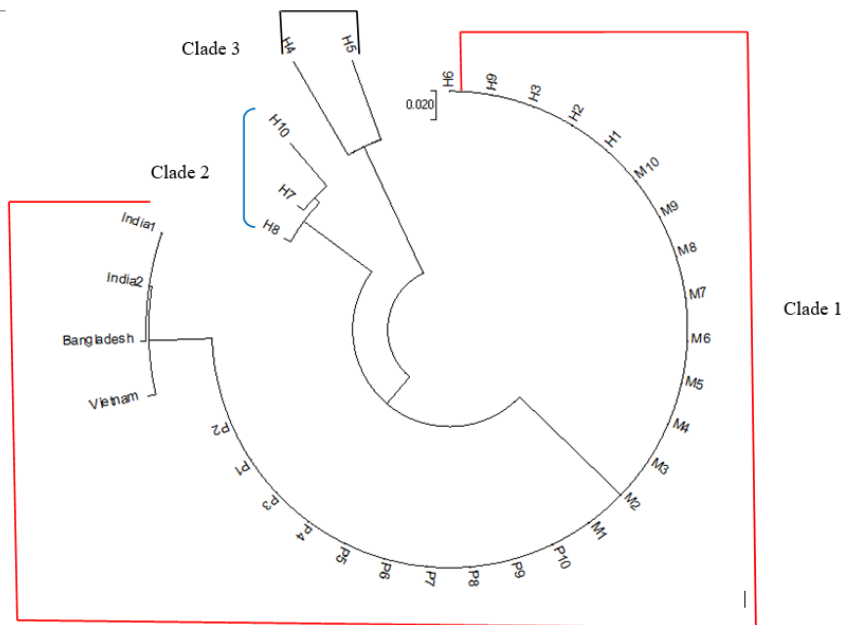


Fig. 7. Phylogenetic tree of Bungo Fish from Tempe-Sidenreng-Lapompakka Lakes

Table 7. Population Parameters of Bungo Fish. Notes: Sample numbers (n), Haplotype numbers (Nh), Polymorphic site (Np), Haplotype diversity (h), and Nucleotide Diversity (π) between population

Population	n	Nh	Np	h	π
Lake Lapompakka	10	2	2	0.200	0.0000
Lake Sidenreng	10	2	1	0.356	0.0007
Lake Tempe	10	6	156	0.778	0.1331

Clade II and III showed limited samples originating only from Tempe Lake because the ecological condition of the lake is very broad when compared to the other two lakes. Furthermore, Tempe Lake has deep waters with varying depths, therefore, there is a possibility for the population of goby fish that never migrates, even during the rainy season or when the lakes have waters together. This is in line with a study by *Ardestani et al.* (17) which showed the presence of a locus that is only obtained in 1 population, but not in the other populations due to differences in the ecological conditions, especially in adjacent areas. *Ardestani et al.* (17) subsequently explained that selection pressure from certain environmental factors and/or genetic drift effect loci substantially more than others. The existence of isolated goby in Tempe Lake also leads to striking phenotypic and genotypic differences between fish populations within a species [18]. This can be indicated based on the genetic distance of the goby fish between the three populations (Table 6).

The genetic distance value was from the pairwise method, meanwhile, samples from Lapompakka and Sidenreng lakes had the lowest genetic distance with 0.00398 (approximately 0), while the sample with the furthest distance was from Lapompakka and Tempe lakes with 0.05882 (Table 6). These results showed that the goby fish species (*Glossogobius giuris*) from the locations have low genetic variation because their values were approximately 0, except for samples from Tempe Lake. Based on the morphometric analysis, the fish population in Lapompakka has similar characteristics to the Tempe Lake population due to its adaptive potential. According to *Teiseira and Huber* [41], the adaptive potential is the ability of an individual to respond to changes in environmental pressure or be selected through phenotypic and/or molecular changes. Hence, species that have successfully colonized in extreme environments can develop substantial and predictable organ changes. This process is usually slow, gradual, and associated with a series of morphological changes known as troglomorphism [42]. In addition, physical isolation due to the extreme environment and the absence of restocking operations has led to the genetic distance being hampered and not be recovered by subsequent gene flow [43]. This is shown in the haplotype distribution in the three integrated lakes (Fig. 8).

The low haplotype diversity was also shown by samples from Lapompakka and Sidenreng Lakes which only had 2 haplotypes, while those from Tempe Lake were significantly high because they had 6 haplotypes (Fig. 8).

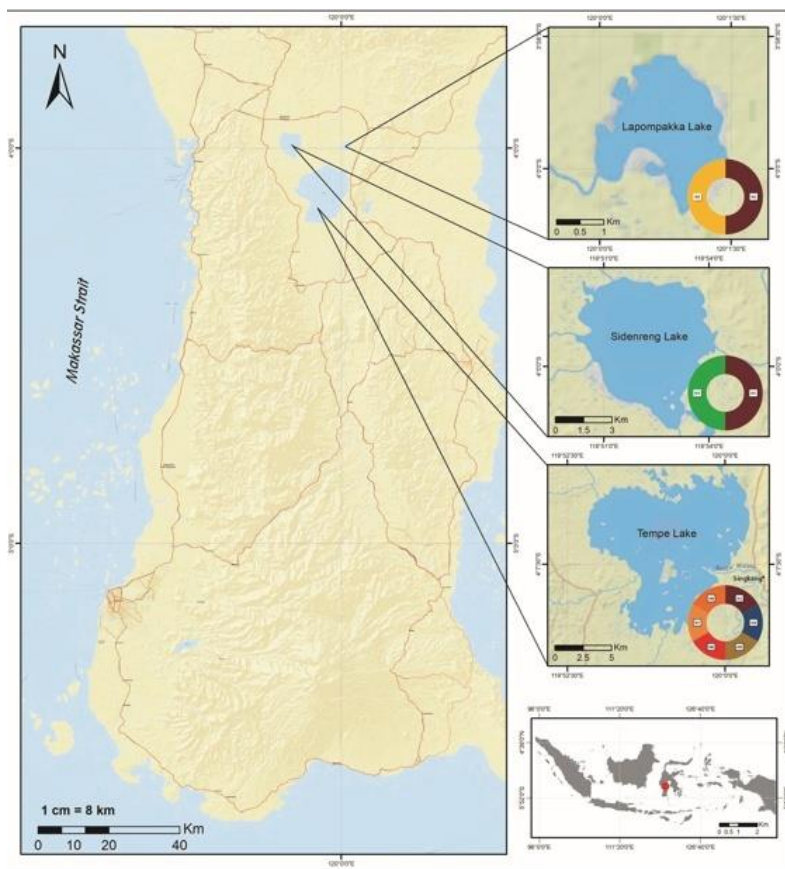


Fig. 8. Goby fish haplotype distribution map (*Glossogobius giuris*) from sampling location

Several haplotypes of the same type were scattered in different locations. Therefore, there were 8 haplotypes were obtained in this study with haplotype and nucleotide diversity values ranging from 0.003-0.05. Meanwhile, Table 7 summarized the population parameters analyzed using DNAsp version 5.

The haplotype distribution showed that there was 1 haplotype in all lakes that supported the adaptive potential of the three lakes. Meanwhile, the other seven haplotypes were only in 1 location which became a special character of the lake. This showed the low gene flow between 1 location and another, therefore, the genetic barriers are unable to migrate too far [9]. Although the number of haplotypes from Tempe Lake is only 5, the haplotype and nucleotide diversity values are significantly higher than the other lakes and have 156 polymorphism sites (Table 7).

Furthermore, it also showed a high haplotype diversity of 0.7 (close to 1), but low nucleotide diversity with a value of 0.1 (close to 0). This showed that the genetic speciation of goby fish in Tempe Lake is due to variations in population expansion. Differences in ecological variables also encouraged individuals to adapt towards ecological, physiological, and behavioral differences which leads to assortative mating and speciation [44].

This is in line with *A.L. McMillen-Jackson and T.M. Bert* [45] which stated that a sudden population expansion influences the genetic diversity of a species. Meanwhile, this occurred when the haplotypes that appeared are closely related and others are at a higher frequency, even in a small population. In addition, there are genetic barriers that occur both when there is separation (dry season) and population expansion (rainy season) which is repeated annually. This leads to high mortality and causes the loss of several genotypes as well as haplotypes [43] faster than heterozygosity due to decreased effective population size [17]. Therefore, when a population is to be lost, there is a possibility that the genetic information contained in that population is not present in others [46].

Conclusions

Based on morphometric results, the *Glossogobius giuris* has a special character for each of the three integrated lakes in South Sulawesi, Indonesia, however, some fish have similar characters between one lake to another. These character differences are closely related to genetic and environmental variations, which led to functional adaptations in both males and females as well as different morphological responses from each goby.

The genetic diversity of the *Glossogobius giuris* from the three integrated lakes area was low. Furthermore, the three genetic clades of goby fish showed the potential for adaptive and genetic speciation due to expansion that occurred when populations declined and were also supported by genetic barriers.

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