



Identification of gelodok fish (Perciformes, Gobiidae) in a coastal mangrove ecosystem, based on morphological and molecular characteristics

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Gelodok fish (mudskippers) are a subfamily of amphibian fish commonly found living in mangrove ecosystems, one of which is in Tabongo Village, Dulupi District, Boalemo Regency, Gorontalo Province. It is difficult to distinguish between species of this fish because they have very similar characteristics. This study aims to (1) determine the detailed morphological characteristics that distinguish gelodok fish species; and (2) determine the molecular characteristics of gelodok fish species found in coastal mangrove ecosystem of Tomini Bay, Tabongo Village, Boalemo Regency, Gorontalo. This research was conducted from 2022 to 2023 using purposive sampling method. Morphological characteristics were performed for 39 characters. Geometric-morphometric analysis was performed using ImageJ software with 10 land marks. Amplification of COI sequences was carried out with F1R1 and F2R2 primers. Based on morphological characteristics, it is known that two species of gelodok fish are found in the area, namely *Periophthalmus argentilineatus* and *P. malaccensis*. COI sequencing results of the samples confirmed one species of gelodok fish found in the area, namely *P. argentilineatus*. The other species cannot be definitively identified as *P. malaccensis* due to the absence of COI sequences for that species in GeneBank.

Keywords: mangrove ecosystem; Tomini Bay; gelodok fish; Tabongo Village; Boalemo District; Gorontalo.

Introduction

Tomini Bay has a large area of mangrove ecosystems. Landsat image interpretation results in 2009–2010 showed that the area of the Tomini Bay mangrove ecosystem amounted to 161.05 km² (Ilman et al., 2011). Good mangrove development in Tomini Bay is supported by a variety of coastal morphological types that vary in the coastal area. Some coastal areas of Tomini Bay have relatively flat and straight beaches while other coastal areas are indented, craggy, in the form of small closed bays, or even cliffs (Damanik & Djamaludin, 2012). In addition, some areas of the Tomini Bay coast are the estuaries of permanent rivers, allowing mangroves to grow optimally (Ilman et al., 2011).

Mangroves are woody plants that grow in the border area between land and ocean. Mangroves are unique because they have morphological and physiological capabilities that allow them to tolerate high salinity, wave, wind, temperature, and mud conditions and anaerobic substrates (Kathiresan & Bingham, 2001). Because of their uniqueness, mangrove ecosystems have a special species composition. Many species from the phytoplankton group (Hasan et al., 2022; Xu et al., 2022), zooplankton (Balqis et al., 2022; Hasan et al., 2022), arthropods (Sebastian & Jessy Joseph Kavumkal, 2016; Tran et al., 2021; Yeo et al., 2021; Fazhan et al., 2022), echinoderms (Ibrahim et al., 2015; Bahri et al., 2021), mollusks (Zvonareva et al., 2019; Ebadzadeh et al., 2021; Jahid & Singh, 2021), and fishes (Zhao et al., 2022; Hewindati et al., 2023; Qiu et al., 2023) live in mangrove ecosystems.

One of the subfamilies of fish that live in mangrove areas is gelodok fish (mudskippers). This fish has different local names in Indonesia. Some communities refer to them as belodok, tembakul, or timpul, while others call them gabus laut or lunjat (Muhtadi et al., 2016). Based on taxonomic studies, the fish belongs to the Order Perciformes, Family Gobiidae. The Family Gobiidae group consists of 321 genera and approximately 2,167 species (Nelson et al., 2016). Gobies are widely geographically distributed in the world, ranging from the Indo Pacific region to the Atlantic coast of Africa (Jaafar & Murdy, 2017). In Indonesia, gobies are found in

the region of Sumatra (Maeda & Tan, 2013; Ridho et al., 2021; Harefa & Chen, 2022; Khairul & Lubis, 2022; Hernawati et al., 2023), Java (Arisuryanti et al., 2018; Ningsih & Santoso, 2020; Aji & Arisuryanti, 2021; Baihaqi et al., 2022; Febrianti et al., 2023), Maluku (Taniwel et al., 2020), Sulawesi (Gani et al., 2020, 2021; Bandjolu et al., 2021; Hasan et al., 2021; Astuti et al., 2022; Baderan et al., 2023; Zakiyah Darajat et al., 2023), Nusa Tenggara (Rhaifa et al., 2021), and Papua (Greenfield & Tor-nabene, 2014; Putra et al., 2022).

Gelodok fish have distinctive distinguishing characteristics when compared to other fish groups in the order Perciformes. Generally, this group of fish has pelvic fins that are fused together like a disk (Kottelat & Whitten, 1996). In addition, gelodok fish have only 5 branchiostegal rays (Akihito Prince, 1986). Other diagnostic features for this group of fish are a small body (maximum 10 cm); a short and broad head; a rounded snout; small, sharp, conical teeth, arranged in 1 row; a head with sensory canals, pores, and cutaneous papillae; separate dorsal fins, the first dorsal fin with 4–8 spines and the second dorsal fin with one spina followed by 9–18 rays; and a body without a lateral line (Murdy & Hoese, 2002).

Generally, identification of gelodok fish is done based on morphological characters. However, the challenge that then arises is the difficulty of determining the correct species name for the identified specimen due to convergence and divergence adaptations that can cause changes in the morphological characters of the fish (Bingpeng et al., 2018). One of the methods that can be used to confirm the accuracy of species identification is to use COI as a DNA barcoding marker. This method is very widely used and has proven effective for fish identification and classification (Arisuryanti et al., 2018; Panprommin et al., 2020; Ude et al., 2020; Aji & Arisuryanti, 2021; Febrianti et al., 2023).

Material and methods

This research was conducted from May to June 2023. Sampling was conducted in the mangrove ecosystem area of Tabongo Village, Dulupi District, Boalemo Regency, Gorontalo Province (Fig. 1). Identification of

morphological characters and preservation of DNA samples were carried out at the Agrotechnology Laboratory, Faculty of Agriculture, Gorontalo State University. DNA isolation and amplification were carried out at PT Griya Sains East Java. DNA sequencing was conducted at 1stBASE Laboratories Sdn Bhd.

The tools used in this study were fishing nets, fish scrapings, jars, gauze, rubber bands, scissors, binocular stereo microscopes, sewing

needles, petri dishes, digital caliper, environmental factor measuring instrument (thermometer and pH meter), brushes, tweezers, stationary freezer (Thermo Scientific™ Forma™ 7000 Series -40°C Upright Ultra-Low Temperature Freezers), Camera (Nikon DX VR, AF-S NIKKON 18-55 mm with additional lens, Pro Tama Digital PRO Macro Lens 0.45X HD WIDE LENS SDW-045 52 mm), A4 millimeter block, and ruler.

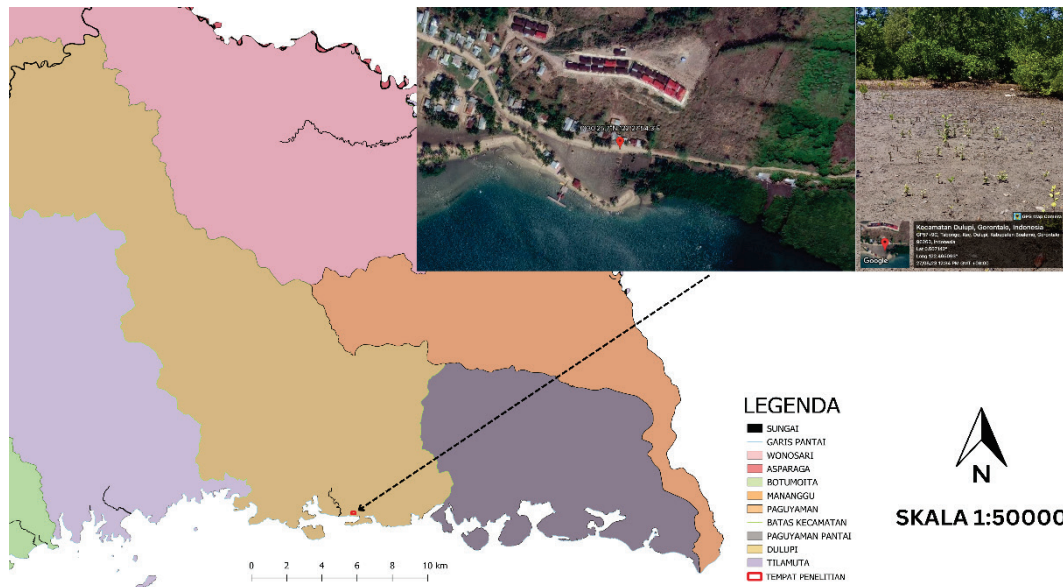


Fig. 1. Research Location Tabongo Village Dulupi Sub-district (0.507142° N 122.465093° E)

Materials used for sample collection and preservation include 70% alcohol, absolute ethanol, gloves, labels, and 2 mL cryovial.

Fish sampling in the mangrove ecosystem area was carried out at low tide in the morning to evening using a net or rake. The collected samples were then transferred to the Agrotechnology Laboratory of the Faculty of Agriculture, Gorontalo State University. Samples were then stored in 70% alcohol. DNA sample collection was carried out at the Faculty of Agriculture Laboratory, Gorontalo State University. DNA samples were obtained from the median to posterior muscles of the fish using a sterile scalpel. Samples were then stored in a cryotube containing absolute ethanol and frozen at -80 °C before being transferred to the PT Griya Sains laboratory for DNA extraction and amplification.

Observations were made on morphological, meristic, morphometric, and geometric-morphometric characters. Morphological observations focused on the color and external structure of the body (Murdy, 1989; Murdy & Hoes, 2002; Nelson et al., 2016; Jaafar & Murdy, 2017). Morphometric character measurements were made using ImageJ. Meristic character observations were made on 7 characters, morphometric character observations were made on 39 characters, and geometric-morphometric observations were made on 10 landmarks (Aydalina, 2016).

The whole genome DNA extraction procedure refers to the NexPrep Tissue Kit protocol. The COI gene from the extracted sample DNA was then amplified using 2 pairs of primers (FishF1-5'TCAACCAACCACAAAGACATTGGCAC3' + FishR1-5'TAGACTTCTGGGTGGCCA AAGAATCA3' and FishF2-5'TCGACTAATCATAAAGATATCG GCAC3' + FishR2-5'ACTTCAGGGTGACCGAAGAATCAGAA3') (Ward et al., 2005).

The extracted DNA were then amplified using a Biorad PCR machine with a total reaction volume of 30 uL consisting of 15 uL PCR Master Mix Nexpro, 1 uL sample DNA Template (100 ng/uL), 10 uL water, 1.5 uL primers (5 pmol for each primer). PCR thermocycling was carried out with pre-denaturation conditions at 95 °C for 2 minutes, then continued with 35 cycles consisting of denaturation at 94 °C for 30 seconds, annealing at 52.6 °C for 30 seconds, and extension at 72 °C for 1 minute. Furthermore, the post elongation process is carried out at 72 °C for 10 minutes. PCR products were then electrophoresed on 1% agarose. All amplification samples were then bidirectional sequenced using 1stBASE Laboratories Sdn Bhd sequencing services.

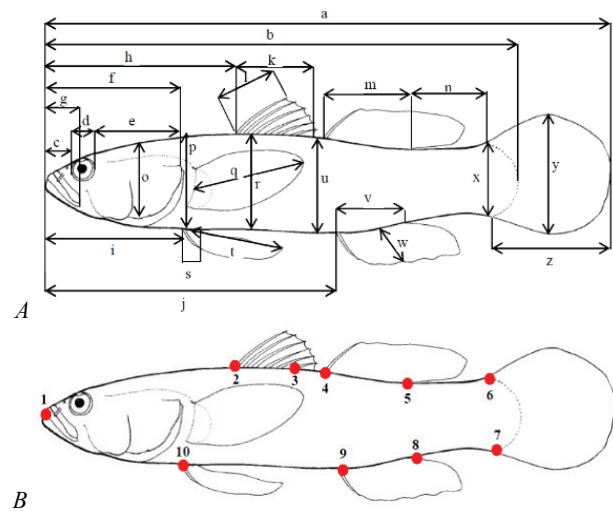


Fig 2. Schematic of morphometric and geometric-morphometric character observation: (a) total length, (b) standard length, (c) pre-orbital length, (d) eye diameter, (e) post-orbital length, (f) post-orbital length, (g) mouth length, (h) pre-dorsal length, (i) pro ventral length, (j) pre-anal length, (k) first dorsal fin base length, (l) height of the longest first spiny dorsal fin, (m) second dorsal fin base length, (n) length of caudal peduncle, (o) head height, (p) front body height, (q) length of the longest spiny pectoral fin, (r) middle body height, (s) middle body height, (t) length of the longest spiny pelvic spin, (u) caudal fin height, (v) anal fin base length, (w) length of the longest spiny anal fin, (x) caudal peduncle depth, (y) caudal fin height, (z) caudal fin length; B – schematic of geometric-morphometric landmark (Aydalina, 2016; Murdy & Hoes, 2002)

Data on ecological conditions and morphological characteristics were described descriptively quantitatively. Geometric-morphometric data were analyzed using MorphoJ (Klingenberg lab, United Kingdom, 2023). Consensus DNA sequences of the COI gene were obtained by combining forward reverse strands using BioEdit 7.2 (Informer Technologies, Inc., 2017). Each sample was analyzed with NCBI BLAST to confirm species

identity. Next, all samples were aligned with ClustalW using MEGA 11 (The Pennsylvania State University, USA, 2021). The Neighbor-Joining (NJ) tree of Kimura 2-parameter distance was constructed with additional mitochondrial COI sequences obtained from GeneBank NCBI using MEGA 11 to show species divergence patterns. Phylograms were created by bootstrapping 1000 replicates.

Results

Ecological condition of research location. The research was conducted in the mangrove area of Tabongo Village, Dulupi District, Boalemo Regency, Gorontalo Province. The condition of the mangrove ecosystem in Tabongo Village is dense mangrove growth with muddy and sandy soil substrates. The mangrove area is adjacent to settlements or community residences that have the potential to damage the mangrove ecosystem environment with various community activities, especially in the disposal of household waste, which has an impact on mangrove aquatic biota including gelodok fish species. Table 1 shows the physicochemical condition of the mangrove ecosystem of the study site.

Table 1
Physicochemical condition of mangrove ecosystem of Tabongo Village, Boalemo Regency

Environmental Parameters	Measurement results
Temperature, °C	28–30
Water pH	7.6
Substrate pH	7.0

Gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency. The research in the mangrove ecosystem of Tabongo Village, Boalemo Regency resulted in the finding of two species of gelodok fish, namely *Periophthalmus malaccensis* and *P. argentilineatus*. A comparison of the number of individuals of each species can be seen in Figure 2. The appearance of the fish can be seen in Figure 3.

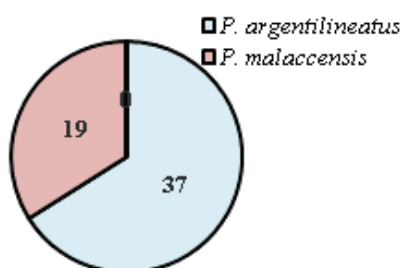


Fig. 3. Comparison of the number of *P. malaccensis* and *P. argentilineatus* individuals found

Periophthalmus malaccensis has a prominent pelvic frenum; some of the pelvic fins are joined by a basal membrane; D1 is medium in height with slightly rounded margins, inframarginal brown lines and white spots on the proximal part, the first spine is elongated; D2 with a single line fading slightly towards the center; and the dorsal fins are not connected by a membrane; 60 longitudinal scales; head height 5.69–10.84% SL; pelvic fin length 5.97–9.97% SL; anal fin basal length 5.15–10.71% SL; D1 basal length 9.15–23.32% SL; D2 basal length 6.20–20.67% SL; D1 element XV; D2 element I,14; and anal fin element I,11.

Periophthalmus argentilineatus lacks pelvic frenum; D1 moderate in height, margin usually curved, sometimes straight, with a black stripe along the lower margin and many small white spots on the back of the fin, no elongate spines; D2 with a single blackish stripe in the middle; dorsal fin not connected by a membrane; head height 5.69–10.84% SL; pelvic fin length 5.18–9.73% SL; anal fin base length 7.93–14.13% SL; D1 basal length 10.93–26.31% SL; D2 basal length 7.14–17.48% SL; D1 element XVI; D2 element I,12; and anal fin element I,11.

Morphological characteristics of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency. Comparison of the pelvic fins of *P. argentilineatus* and *P. malaccensis* is shown in Figure 4. Comparison of morphological, morphometric and meristic characters of *P. argentilineatus* and *P. malaccensis* found in the mangrove ecosystem

of Tabongo Village, Boalemo Regency is shown in Tables 2, 3, and 4, respectively.



Fig. 4. Gelodok fish found in the mangrove ecosystem of Tabongo Village, Boalemo Regency: a – *P. argentilineatus*; b – *P. malaccensis*; 1 cm size bar

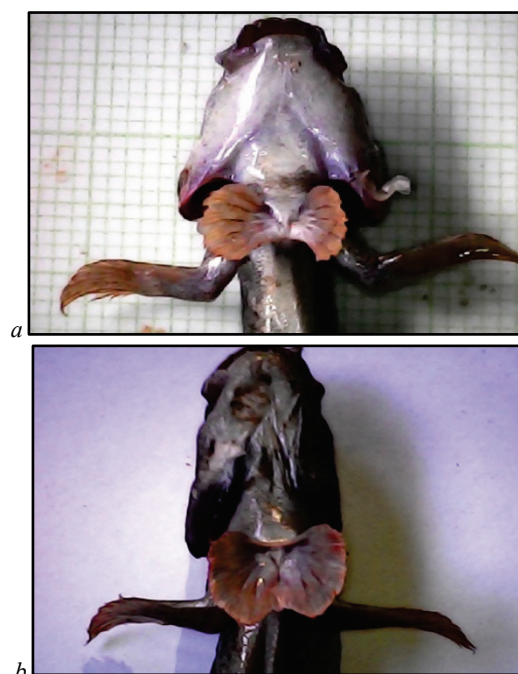


Fig. 5. Comparison of the pelvic fins of gelodok fish: a – *P. argentilineatus*, b – *P. malaccensis*

Table 2
Comparison of morphological characters of *P. argentilineatus* and *P. malaccensis* found in the mangrove ecosystem of Tabongo Village, Boalemo Regency

No.	Morphological characters	Species	
		<i>P. argentilineatus</i>	<i>P. malaccensis</i>
1.	Scale type:		
	– head	cycloid	cycloid
	– predorsal body	cycloid	cycloid
	– preanal body	cycloid	cycloid
	– front body	cycloid	cycloid
	– mid-body	cycloid	cycloid
	– back of the body	cycloid	cycloid
	– trunk tail	cycloid	cycloid
2.	Mouth location type	sub-terminal	sub-terminal
3.	The part of the head that is covered with scales	partially preoperculum without scales	partially preoperculum without scales

Geometric-morphometric analysis of gelodok fish. The results of geometric-morphometric analysis of *P. argentilineatus* and *P. malaccensis*

found in the mangrove ecosystem of Tabongo Village, Boalemo Regency are shown in Figure 6.

Table 3

Comparison of morphometric characters of *P. argentilineatus* and *P. malaccensis* found in the mangrove ecosystem of Desa Tabongo, Kabupaten Boalemo

Character	<i>P. argentilineatus</i>				<i>P. malaccensis</i>			
	\bar{x}	min	max	SD	\bar{x}	min	max	SD
Pre-orbital length	4.65	2.27	7.69	1.38	5.43	1.67	8.24	1.51
Eye diameter	6.63	4.79	8.88	0.98	6.36	1.67	7.73	0.88
Head length	14.24	10.56	19.86	2.38	13.03	6.83	16.75	2.70
Snout length	22.73	8.52	20.02	3.95	22.15	10.95	26.12	3.72
Post-orbital length	10.21	5.20	13.51	1.55	11.09	7.18	14.50	1.92
Pre-dorsal length	20.57	14.30	23.78	2.91	20.04	14.09	23.33	2.85
Pre-ventral length	18.61	12.81	21.99	2.49	18.21	11.13	22.53	3.01
Pre-anal length	24.16	20.15	29.47	2.98	24.69	18.02	29.15	3.68
D1 base length	18.91	10.93	26.31	4.04	17.89	9.15	23.32	3.66
D1 longest spine length	16.39	11.88	24.61	2.81	10.08	8.12	24.39	3.97
D2 base length	11.80	6.20	20.67	2.95	10.91	7.14	17.48	2.23
Length of caudal pedunculus	10.12	5.70	21.63	3.31	8.71	6.53	16.32	2.21
Head height	15.26	5.69	10.84	2.52	14.68	6.94	18.75	3.24
Front body height	14.28	5.69	20.49	2.81	16.21	12.62	20.64	2.22
Pectoral fin's longest ray length	12.59	7.05	17.70	2.71	13.20	8.76	16.93	2.48
Middle body height	14.55	6.62	19.32	2.42	14.48	7.04	17.03	2.62
Pelvic fin base length	7.03	4.00	9.61	1.69	7.40	3.76	9.62	1.56
Pelvic fins' longest ray length	8.49	5.97	9.97	1.13	8.14	5.18	9.73	1.37
Caudal base height	13.91	1.24	19.97	2.74	14.00	9.62	16.00	1.86
Anal fin length	10.95	7.93	14.13	1.52	8.93	5.15	10.71	1.28
Anal fin's longest ray length	4.74	1.93	7.67	1.54	5.31	2.91	8.45	1.54
Caudal pedunculus height	8.60	5.16	14.33	1.57	8.16	6.59	10.84	1.05
Caudal fin height	9.12	5.48	13.59	2.11	8.68	6.39	11.23	1.30
Caudal fin length	19.80	15.48	23.64	2.39	19.63	14.28	23.45	2.38

Table 4

Comparison of meristic characters of *P. argentilineatus* and *P. malaccensis* found in the mangrove ecosystem of Tabongo Village, Boalemo District

Species	Character meristics												LS
	D1		D2		A		P		V		C		
	S	R	S	R	S	R	S	R	S	R	S	R	
<i>P. argentilineatus</i>	16	0	1	12	1	11	0	13	0	6	0	16	70
<i>P. malaccensis</i>	13	0	1	14	1	12	0	13	0	6	0	16	60

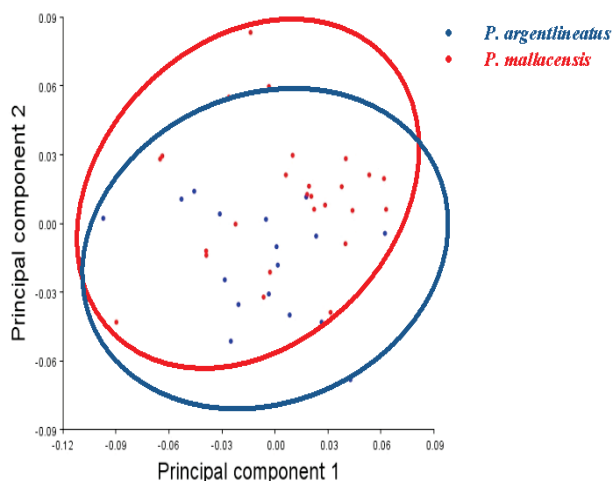


Fig. 6. Results of geometric-morphometric analysis of *P. argentilineatus* and *P. malaccensis* found in the mangrove ecosystem of Tabongo Village, Boalemo Regency

Genetic distance calculation of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency. The results of the calculation of the genetic distance of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency, Gorontalo Province are shown in Tables 5 and 6.

Phylogram of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency. Phylogram of COI sequences of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency, Gorontalo Province based on Neighbor-Joining calculation is shown in Figure 4.

Discussion

The results of the study found two species of spadefish in the mangrove ecosystem of Tabongo Village, Boalemo Regency, Gorontalo Province (*P. argentilineatus* and *P. malaccensis*). In general, the two species found have the same morphological characteristics as *P. argentilineatus* and *P. malaccensis* in previous studies although there are some differences in morphometric and meristic characteristics. *Periophthalmus argentilineatus* was found to have pelvic fin, anal fin basal, and second dorsal fin basal sizes of 8.49% SL, 10.95% SL, and 11.80% SL, respectively. These sizes were slightly smaller than previous studies of 11–15% SL (mean = 13.2%); 14–19% SL (mean = 16.8%); and 18–24% SL (mean = 20.8%); for pelvic fin, anal fin basal, and second dorsal fin basal, respectively. (Jaafar & Murdy, 2017). Similarly, in *Periophthalmus malaccensis*, different morphometric characters were observed for pelvic fin, head length, anal fin basal length and second dorsal fin basal length with sizes of 8.14% SL, 22.15%, 8.93% SL, 10.91% SL, respectively. These sizes are slightly smaller than previous studies, namely 14–16% SL (mean = 14.5%); 14–20% SL (mean = 16.6%); 14–16% SL (mean = 14.5%), and 17–23% SL (mean = 19.8%); for pelvic fin, head length, anal fin basal length and second dorsal fin basal length, respectively (Jaafar & Murdy, 2017). Factors that may affect these morphometric results are due to differences in physicochemical factors in the mangrove ecosystem of Tabongo Village, Gorontalo Regency and other locations (Ghanbarifardi et al., 2014). Different meristic characteristics are only found in *P. malaccensis*. The specimen found has more second dorsal fin elements 3 spines than *P. malaccensis* in the study conducted by Jaafar & Murdy (2017) and 1 spina from the same species found in Kalimantan (Polgar, 2016).

Another factor that can affect the presence of the studied fish species in the Tabongo Village mangrove area is environmental factors. The condition and quality of the environment in the mangrove ecosystem is known by observing temperature, water pH and soil pH. The environmen-

tal temperature contained in the Tabongo Village mangrove area ranges from 28 to 30 °C. This temperature range indicates that the mangrove water environment has a fairly stable temperature variation. This is sup-

ported by the previous study showing that the temperature of mangrove waters ranging from 28–30 °C, which can be said to be the optimal water temperature (Arofah et al., 2021).

Table 5

The genetic distance matrix of COI sequences of *Periophthalmus argentilineatus* in the mangrove ecosystem of Tabongo Village, Boalemo Regency based on Kimura 2 parameter calculation

COI sequences	1	2	3	4	5	6	7	8
TB02005 <i>P. argentilineatus</i>	–	–	–	–	–	–	–	–
TB01007 <i>P. argentilineatus</i>	0.0028	–	–	–	–	–	–	–
MW514017.1 <i>P. argentilineatus</i>	0.0000	0.0029	–	–	–	–	–	–
MW514019.1 <i>P. argentilineatus</i>	0.0000	0.0029	0.0000	–	–	–	–	–
MW514015.1 <i>P. argentilineatus</i>	0.0000	0.0030	0.0000	0.0000	–	–	–	–
OR053787.1 <i>P. argentilineatus</i>	0.0000	0.0031	0.0000	0.0000	0.0000	–	–	–
KU692743.1 <i>P. argentilineatus</i>	0.0000	0.0031	0.0000	0.0000	0.0000	0.0000	–	–
MZ606687.1 <i>P. argentilineatus</i>	0.0000	0.0031	0.0000	0.0000	0.0000	0.0000	0.0000	–
KU944887.1 <i>P. modestus</i>	0.0000	0.0036	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000

Table 6

The genetic distance matrix of COI sequences of *Periophthalmus malaccensis* in the mangrove ecosystem of Tabongo Village, Boalemo Regency based on Kimura 2 parameter calculation

No.	COI sequences	1	2	3	4	5	6	7	8	9
1	TB01005 <i>P. malaccensis</i>	–	–	–	–	–	–	–	–	–
2	TB02004 <i>P. malaccensis</i>	0.0014	–	–	–	–	–	–	–	–
3	TB01006 <i>P. malaccensis</i>	0.0000	0.0000	–	–	–	–	–	–	–
4	MW514017.1 <i>P. argentilineatus</i>	0.1124	0.1126	0.1124	–	–	–	–	–	–
5	MW514019.1 <i>P. argentilineatus</i>	0.1135	0.1136	0.1135	0.0000	–	–	–	–	–
6	MW514023.1 <i>P. argentilineatus</i>	0.1141	0.1143	0.1141	0.0014	0.0015	–	–	–	–
7	MW514024.1 <i>P. argentilineatus</i>	0.1314	0.1316	0.1314	0.0072	0.0058	0.0087	–	–	–
8	MW514015.1 <i>P. argentilineatus</i>	0.1157	0.1157	0.1157	0.0000	0.0000	0.0015	0.0015	–	–
9	MW388787.1 <i>T. lepturus</i>	0.1256	0.1258	0.1256	0.1221	0.1216	0.1239	0.1197	0.1170	–
10	MW388783.1 <i>T. lepturus</i>	0.1256	0.1258	0.1256	0.1221	0.1216	0.1239	0.1197	0.1170	0.0000

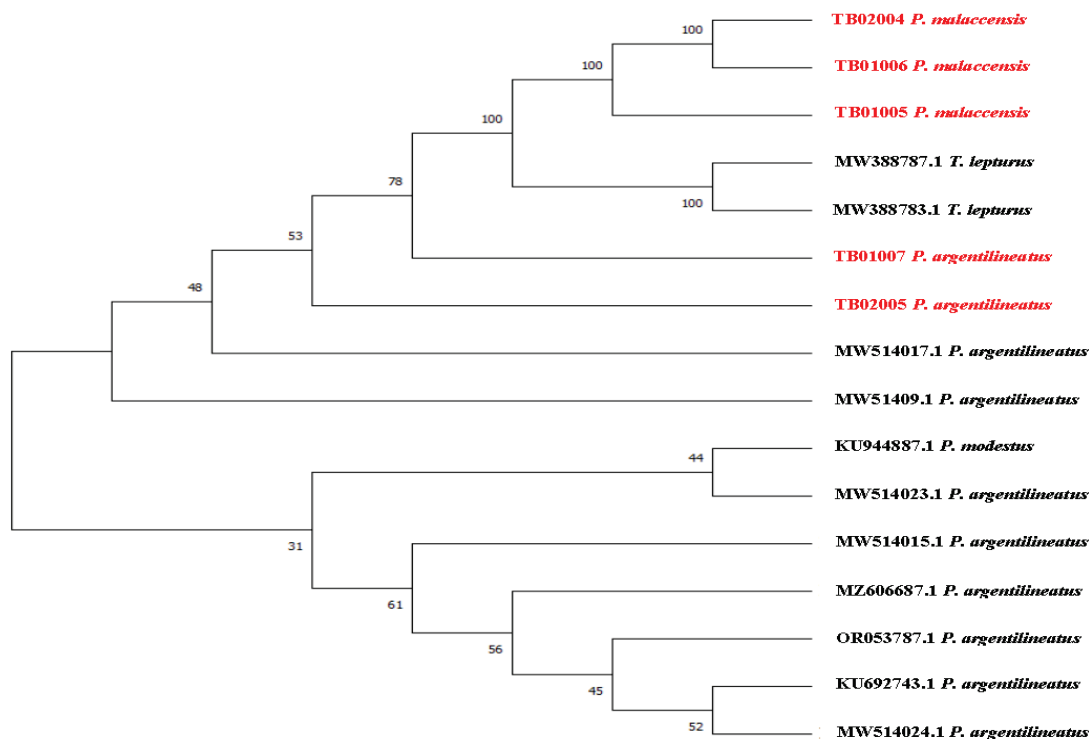


Fig. 7. Phylogram of COI sequences of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency, Gorontalo Province based on neighbor-joining calculation

The pH of the water in the mangrove area of Tabongo Village is 7.6. This indicates that the environmental conditions are neutral and support the life of the studied species. Water acidity with a pH value of 7.5–8.5 includes waters with high productivity. The pH value of the soil in the mangrove area of Tabongo Village is 7.0, which indicates that the environmental conditions are neutral and optimal. This is supported by the previous study that states that the most optimal soil pH value ranges from 6.6 to 7.5 (Setiawan, 2013).

The results of geometric-morphometric analysis of *P. argentilineatus* and *P. malaccensis* found in the mangrove ecosystem of Tabongo Village showed no significant differences for geometric-morphometric characters between the two. This is shown by the Principal Component Analysis graph which overlaps between the two populations.

COI sequence identification performed on two samples of *P. argentilineatus* obtained at the study site showed that the samples were genetically similar to *P. argentilineatus* and *P. modestus*. Sample TB01007 was

confirmed as *P. argentilineatus*. The sample shares percentage identity in the range of 98.69–99.71% with *P. argentilineatus* from Tekolok Estuary, West Nusa Tenggara (MW514017.1; MW514019.1; and MW514015.1); West Oesapa Beach, Kupang (OR053787.1); Java and Bali (KU692743.1). Sample TB02005 shares 100% identity with *P. modestus* from Taiwan (KU944887.1) and 99.85–99.86% with *P. argentilineatus* from Tekolok Estuary, West Nusa Tenggara, Indonesia (MW514017.1; MW514019.1; and MW514015.1); West Oesapa Beach, Kupang (OR053787.1); Java and Bali (KU692743.1; MZ606687.1). This is also supported by the calculation of genetic distance of COI sequences of *P. argentilineatus* with Kimura 2 parameters, where Sample TB01007 has no genetic distance (0%) with all samples compared, while the genetic distance of Sample TB02005 with other *P. argentilineatus* samples ranges from 0.0028–0.0031%. The genetic distance between Sample TB02005 and *P. modestus* from Taiwan was 0.0036%. Although this value is still further away when compared to *P. argentilineatus*, it is still below the species discrimination threshold for vertebrates (Hebert et al., 2003). Nonetheless, there is no possibility that Sample TB02005 is *P. modestus* as the species is endemic to the Northwestern Pacific Ocean region which includes the China Sea, Korean Peninsula, and Japan (Murphy, 1989; He et al., 2015; Jaafar & Murphy, 2017).

Samples TB01005, TB02004, and TB01006, which were identified as *P. malaccensis* based on morphological characteristics, could not be confirmed as *P. malaccensis* based on COI sequence data. This is due to the unavailability of COI sequence data for *P. malaccensis* in the NCBI GeneBank. Available sequences are limited to 16S rRNA, D-loop mitochondrial DNA, and Rag1 gene. The results of DNA sequence matching on GeneBank data show that Sample TB01005 has a percent identity of 89.67–89.81% with *P. argentilineatus* from Tekolok Estuary, West Nusa Tenggara, Indonesia (MW514017.1; MW514019.1; and MW514023.1); and 89.56% with *Trichiurus lepturus* Shanghai, China (MW388787.1; MW388783.1). These results show similarity with Sample TB01006, which has a percentage identity of 89.67–89.81% with *P. argentilineatus* from Tekolok Estuary, West Nusa Tenggara, Indonesia (MW514024.1; MW514017.1; and MW514023.1); and 89.56% with *Trichiurus lepturus* Shanghai, China (MW388787.1; MW388783.1). Unlike these two samples, Sample TB02004 has a percentage identity of 89.54–89.77% with *P. argentilineatus* from Tekolok Estuary, West Nusa Tenggara, Indonesia (MW514017.1; MW514019.1; MW514024.1; MW514023.1; and MW514015.1). This is also supported by the calculation of the genetic distance of the COI sequences of *Periophthalmus malaccensis* with Kimura 2 parameters, where the genetic distance of the three samples compared with *P. argentilineatus* ranged from 11.24–13.16%. This distance is still greater when compared to *Trichiurus lepturus*, which ranges from 12.56–12.58%. When compared to their neighbors, the genetic distance between the three samples only ranged from 0.00% to 0.14%. This indicates that the three samples belong to one species and are genetically different from *P. argentilineatus* and *Trichiurus lepturus*. This is based on the difference in genetic distance between the two that exceeds 2% (Hebert et al., 2003).

The phylogram of COI sequences of gelodok fish in the mangrove ecosystem of Tabongo Village, Boalemo Regency, Gorontalo Province constructed by Neighbor-Joining is consistent with the results of the calculation of the genetic distance of the samples. The phylogram shows that the three samples of *P. malaccensis* are clustered together. This is also the case with *P. argentilineatus*.

Conclusion

Based on the research conducted, it is concluded that based on morphological data, there are two species from the *Periophthalmus* genus, namely *P. argentilineatus* and *P. malaccensis*, in the mangrove ecosystem of Tabongo Village, Boalemo Regency. COI sequencing results of the samples could only confirm one species of gelodok fish found in the area, namely *P. argentilineatus*. The other species cannot be definitively identified as *P. malaccensis* due to the absence of COI sequences for that species in GeneBank.

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