Research Article

Check for updates   OPEN ACCESS

Genetic Diversity of Mudskipper Species (*Periophthalmus* spp.) from the Southern Coast of Java, Indonesia, Based on the Mitochondrial *16S* Gene

Tuty Arisuryanti[1], Tasya Nuraliyah[1], Vindi Dwijayanti[1], Katon Waskito Aji[1], Dwi Sendi Priyono[2], Budi Setiadi Daryono[1*]

[1]*Laboratory of Genetics and Breeding, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*
[2]*Laboratory of Animal Systematics, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia*

ABSTRACT

The genus *Periophthalmus* (Bloch & Schneider, 1801) comprises a diverse group of mudskippers within the family Oxudercidae and subfamily Oxudercinae. It includes 20 verified species globally, with 11 of these species originating from Indonesia. Among them, *P. kalolo* and *P. argentilineatus* are prevalent across seven major islands. However, *P. novemradiatus* is less documented, with limited distribution records. The morphological similarities among *Periophthalmus* spp. complicate recognition, often leading to misidentification. To address this challenge, this study investigated the phylogenetic relationships and genetic diversity among these three species, utilizing the *16S* rRNA gene as a marker. PCR amplification of the DNA from samples collected from seven regions along Java's southern coast employed primers 16Sar and 16Sbr. Phylogenetic analysis revealed three monophyletic clades corresponding to each species, with genetic divergences ranging from 3.66 to 5.40%. *P. argentilineatus* displayed a relatively high intraspecific divergence of 0.21-2.26%, suggesting the potential identification of cryptic species within this lineage, even with a conserved gene like *16S* rRNA. We identified 47 variable sites within the 552-bp *16S* rRNA sequence, including four singleton sites and 43 parsimony-informative sites. We discovered a unique marker at position 172 that could serve as a genetic identifier for distinguishing these species. These findings suggest that the *16S* rRNA gene has potential as an alternative marker for species identification while also revealing genetic diversity, thereby complementing or even serving as an alternative to the commonly used *COI* gene in *Periophthalmus* spp.

1. Introduction

Periophthalmus (Bloch and Schneider, 1801), commonly known as mudskippers, is a genus of amphibious gobies belonging to the family Oxudercidae and the subfamily Oxudercinae (Nelson *et al.* 2016; Kuang *et al.* 2018; McCraney *et al.* 2020). It is among the most diverse groups, comprising 20 species validated to ddate(Murdy 1989; Murdy and Takita 1999; Darumas and Tantichodok 2002; Larson and Takita 2004; Jaafar and Larson 2008; Jaafar *et al.* 2016; Fricke and Eschmeyer 2024). They inhabit a wide range of intertidal environments, including open tidal flats, bare mud banks, mangrove forests, and freshwater swamps, illustrating their adaptability to various coastal ecosystems (Baeck *et al.* 2008; Larson 2008; Polgar and Crosa 2009; Polgar *et al.* 2010; Polgar and Bartolino 2010; Takita *et al.* 2011). They are particularly well-suited for terrestrial living, with specialized adaptations in vertebral, respiratory, appendicular, epithelial, and locomotory systems that enable them to survive on land for extended periods, up to 14 hours (You *et al.* 2018; Hidayat *et al.* 2022; Steppan *et al.* 2022). Additionally, they possess the capability to

* Corresponding Author
  E-mail Address: bs_daryono@ugm.ac.id

bioaccumulate heavy metals, which positions them as essential bioindicators for assessing heavy metal levels in coastal waters (Ansari *et al.* 2014; Santoso *et al.* 2020; Sangur *et al.* 2021).

In Indonesia, 11 *Periophthalmus* spp. have been identified, spanning the major islands from Sumatra to Papua (Pormansyah *et al.* 2019). However, their taxonomic identification poses challenges due to morphological similarities, often leading to confusion and misidentifications (Murdy 1989; Jaafar *et al.* 2006; Jaafar & Larson 2008; Jaafar *et al.* 2009). Polgar *et al.* (2014) and Arisuryanti *et al.* (2024b) have demonstrated the effectiveness of molecular techniques in identifying cryptic species within *Periophthalmus* populations, which traditional morphology-based methods often overlook. For this, Polgar *et al.* (2014) utilized mitochondrial and nuclear markers, including the D-loop, *16S* rRNA, and rag1, in *Periophthalmus* spp., specifically *P. argentilineatus*. In contrast, Arisuryanti *et al.* (2024b) employed DNA barcoding. *P. kalolo* and *P. argentilineatus* are among Indonesia's most prevalent mudskippers, typically overlapping across geographical regions (Polgar 2014). These species are widespread across the seven major Indonesian islands (Pormansyah *et al.* 2019). However, records of species such as *P. novemradiatus* in Indonesia are limited, with only a few studies in these areas (Dahruddin *et al.* 2017; Aji and Arisuryanti 2021). Focusing on these three species (*P. kalolo, P. argentilineatus,* and *P. novemradiatus*) is crucial for understanding the genetic diversity within Periophthalmus in Indonesia, as they encompass both common and less-documented species.

Despite advancements in population genetics, investigations on *Periophthalmus* in Indonesia have primarily focused on single or two-species analyses. Most studies, including those on *P. argentilineatus* and *P. kalolo*, have employed the COI (Arisuryanti *et al.* 2018; Rha'ifa *et al.* 2021; Febrianti *et al.* 2023; Arisuryanti *et al.* 2024a, 2024b). Aji and Arisuryanti (2021) examined all three target species in Indonesian waters; however, this study was limited to the *COI* marker and focused on a single population. The *COI* gene remains a foundational marker in fish genetics, complemented by other mitochondrial markers such as the *16S* rRNA gene in phylogenetics and DNA barcoding. The *16S* rRNA gene has been utilized to enhance the resolution provided by the *COI* gene, aiding in species identification and differentiation in various taxa, including amphibians and mollusks (Vences *et al.* 2005; Feng *et al.* 2011). Moreover, the partial mitochondrial *16S* rRNA gene is valuable for identifying and assessing genetic diversity across a wide range of taxa, including mammals, birds, and shrimp (Kitano *et al.* 2007; Yang *et al.* 2014), as well as fish (Saha *et al.* 2019; Saad *et al.* 2019; Arisuryanti *et al.* 2020; Akhtar *et al.* 2020; Thi *et al.* 2023). Therefore, our current study incorporates the *16S* rRNA gene as a marker to complement previous studies and provide a more comprehensive interspecific genetic analysis.

This study focused on seven regions along the southern coast of Java, an area characterized by extensive mangrove ecosystems. These habitats are vital for sustaining biodiversity and coastal resilience, but they face increasing threats from land conversion, pollution, and habitat fragmentation (Nordhaus *et al.* 2019; Rudianto *et al.* 2020). Genetic studies in these regions are crucial for informing conservation strategies and maintaining the ecological balance of this rapidly changing coastal environment. Thus, this study examined genetic interrelationships and characterized three mudskipper species (*Periophthalmus* spp.) from seven distinct regions along Java's southern coast, using the *16S* rRNA gene as a marker. The data obtained may enhance the understanding of genetic relationships and diversity within these Indonesian mudskipper species.

2. Materials and Methods

2.1. Sample Collection

In total, 55 mudskipper specimens, representing three species, were collected from seven sites along the southern coast of Java (Table 1; Figure 1). Each specimen was cleaned, labeled with a population code, placed in individual Ziplock bags, transported on ice to the Genetics and Breeding Laboratory, Faculty of Biology, UGM, and then stored at -20°C.

2.2. DNA Extraction, DNA Amplification, Electrophoresis, and Sequencing

The total genomic DNA of each mudskipper specimen was extracted from 50-100 mg of dorsal muscle tissue located near the gill region above the pectoral fins using the DNeasy Blood and Tissue Kit (QIAGEN, Valencia, CA, USA), following manufacturer'ser's instructions. The mitochondrial *16S* rRNA gene was amplified with the primers 16Sar (5'-CGCCTGTTTATCAAAAAC'T-3') and 16Sbr (5'-CCGGTCTGAACTCAGA TCAC'T-3') (Palumbi 1996) on a T100 Thermal Cycler (Bio-Rad). PCR reactions were prepared in 25 µL volumes containing 5–50 ng of genomic DNA, 12.5 µL MyTaq HS Red Mix (Bioline), 1 mM $MgCl_2$, 0.6 µM each

Table 1. Sampling location, sample code, geographic reference, and sample size of mudskippers

Location	Sample code	Latitude (S)	Longitude (E)	Sample size (N)
Santolo Beach, Garut, West Java	MGR	7°39'31.58"	107°41'20.05"	9
Cikabuyutan Beach, Tasikmalaya, West Java	MTS	7°48'5.71"	108°15'19.73"	8
Cilacap Mangrove Forest, Central Java	MCL	7°41′2.08″	109°00′36.38″	9
Bogowonto Lagoon, Yogyakarta	MSB	7°53'58.1"	110°01'54.2"	6
Baros Beach, Yogyakarta	MBR	8°00'27.4"	110°17'02.2"	10
Kondang Bandung Beach, East Java	MSK	8°22'20.0"	112°23'19.5"	7
Clungup Beach, East Java	MSC	8°26'15.1"	112°40'07.0"	6
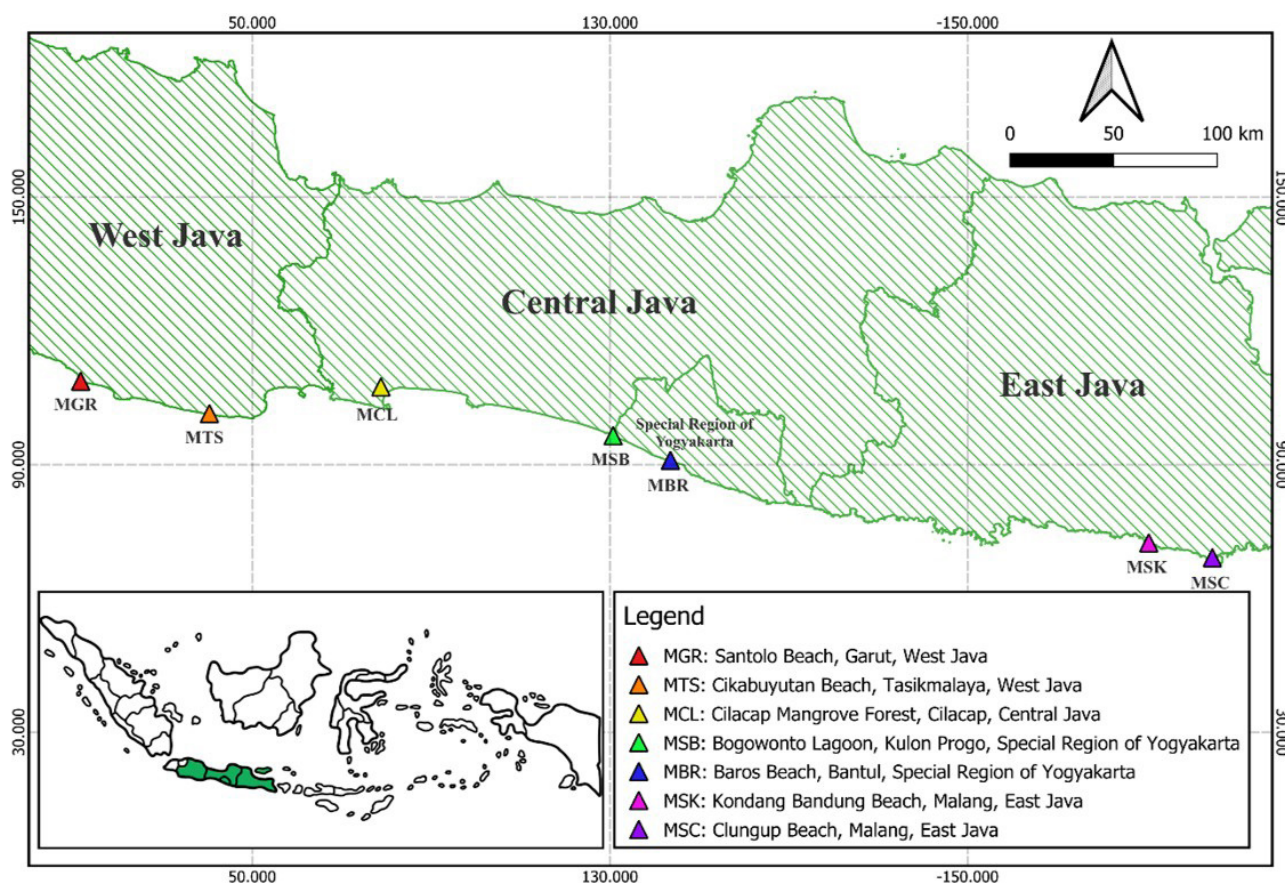
Figure 1. Map sampling sites for the three *Periophthalmus* species investigated in this study

of forward and reverse primers, and 5.5 µL double-distilled water (ddH$_2$O). A negative control, without template DNA, was included to confirm the absence of contamination and ensure the reliability of the results. The PCR program consisted of a 2 min pre-denaturation at 95°C, followed by 35 cycles at 95°C for 15 s, 50°C for 30 s, and 72°C for 30 s, with a final extension at 72°C for 5 min. The PCR products were separated using 1% agarose gel electrophoresis at 100 V for 25 minutes, stained with Florosafe (Bioline), and then buffered with Tris-acetate-EDTA (TAE). The bands were visualized under UV light and documented for image capture and analysis utilizing a GelDoc connected to a PC. All amplicons were transported to the First Base Sdn Bhd (Selangor, Malaysia) through PT Genetika Science (Jakarta) and LPPT UGM (Universitas Gadjah Mada, Indonesia) for purification and bidirectional sequencing.

2.3. Sequence Editing and Alignment of the *16S* Gene

The resulting chromatograms (ab1 format) of the *16S* gene from mudskippers were manually edited employing GeneStudio and validated with the EditSeq program (DNASTAR Inc., Madison, USA) to obtain the consensus sequences. These were cross-referenced with GenBank data via Nucleotide BLAST (https://blast.

ncbi.nlm.nih.gov/Blast.cgi) for species confirmation. The 55 samples were aligned in Opal of Mesquite v.3.51 (Maddison and Maddison 2018) and trimmed at both ends to produce clean consensus sequences for interspecific genetic analysis.

2.4. Genetic Diversity and Genetic Distance

The interspecific genetic diversity was assessed by examining the number of variable, singleton, and parsimony-informative sites, as well as haplotype and nucleotide diversity, using DnaSP ver. 6 (Rozas *et al.* 2017). Genetic distances between samples were estimated using the Kimura 2-parameter substitution model with 1,000 bootstraps in the MEGAX program (Kumar *et al.* 2018).

2.5. Phylogenetic Relationship

The 72 sequences, including 15 additional comparative samples and two outgroup samples obtained from the GenBank: *Periophthalmus kalolo* (PQ119848-52), *Periophthalmus argentilineatus* (OQ996258-66), *Periophthalmus novemradiatus* (NC038266), *Periophthalmus schlosseri* (NC030766), and *Boleophthalmus pectinirostris* (JN631352), were phylogenetically analyzed. Neighbor-joining (NJ) and Maximum Likelihood (ML) trees were constructed using the Kimura-2-parameter (K2P) substitution model with 1,000 bootstrap replications in MEGA X (Kumar *et al.* 2018). In addition, Bayesian Inference (BI) analysis was conducted using BEAST (Suchard *et al.* 2018), employing the GTR + I + G substitution model, which was identified as optimal based on the Akaike Information Criterion (AIC) in jModelTest 2.1.10 (Darriba *et al.* 2012). The analysis ran for 10 million generations, with a 25% burn-in, and sampled every 1,000 generations to estimate posterior probability distributions. Final consensus trees were visualized in FigTree 1.4.4 (Rambaut 2019).

3. Results

3.1. Phylogenetic Tree and Genetic Distance

The phylogenetic trees displayed identical topologies across the NJ, ML, and BI methods. Therefore, we present only the BI tree (Figure 2), as Bayesian inference provides more reliable branch support by incorporating prior probability distributions and using Markov Chain Monte Carlo (MCMC) sampling. The phylogenetic tree revealed three distinct clades corresponding to *P.

argentilineatus (yellow), *P. kalolo* (green), and *P. novemradiatus* (blue). The mean K2P interspecies genetic divergence of 16S rRNA was 3.66% between *P. kalolo* and *P. argentilineatus* and 5.4% between *P. argentilineatus* and *P. novemradiatus* (Table 2). Moreover, the mean genetic distances among species within their respective populations ranged from 3.4% between *P. argentilineatus* from Baros Beach and *P. kalolo* from Bogowonto Lagoon to 6.0% observed between *P. novemradiatus* from Baros Beach and *P. argentilineatus* from Clungup Beach (Table 3).

3.2. Genetic Diversity of Three mudskipper Species Based on the *16S* rRNA Gene

We identified 47 variable sites, including four singleton and 43 parsimony-informative sites. Among these, a notable gap in overall sequence alignment was detected at site number 237. The mean haplotype diversity (Hd) and nucleotide diversity (π) across the three mudskipper species were 0.891±0.027 and 0.02385±0.00215, respectively. We employed distinct colors for each species: *P. kalolo* in green, *P. argentilineatus* in yellow, and *P. novemradiatus* in blue (Tables 4 and 5). Furthermore, we discovered a unique variable site at position 172 that could serve as a genetic marker for distinguishing between these species.

4. Discussion

Molecular genetic tools are essential in fish for distinguishing between species and elucidating their genetic interrelationships and diversity (Chauhan and Rajiv 2010; Amoussou *et al.* 2019; Abdalwahhab *et al.* 2020). Within the genus *Periophthalmus*, these genetic approaches are particularly beneficial, as the interspecific morphological similarity frequently challenges traditional identification methods (Murdy 1989; Jaafar *et al.* 2006; Jaafar and Larson 2008; Jaafar *et al.* 2009). To the best of our knowledge, this study is the first to examine three *Periophthalmus* species: *P. kalolo, P. argentilineatus,* and *P. novemradiatus* across seven regions along the southern coast of Java by employing the *16S* rRNA gene to assess their genetic correlations and diversity.

The phylogenetic tree constructed by applying BI, NJ, and ML methods revealed well-supported monophyletic clades for *P. kalolo, P. argentilineatus,* and *P. novemradiatus*. Although the posterior probability at the branching point between *P. kalolo*
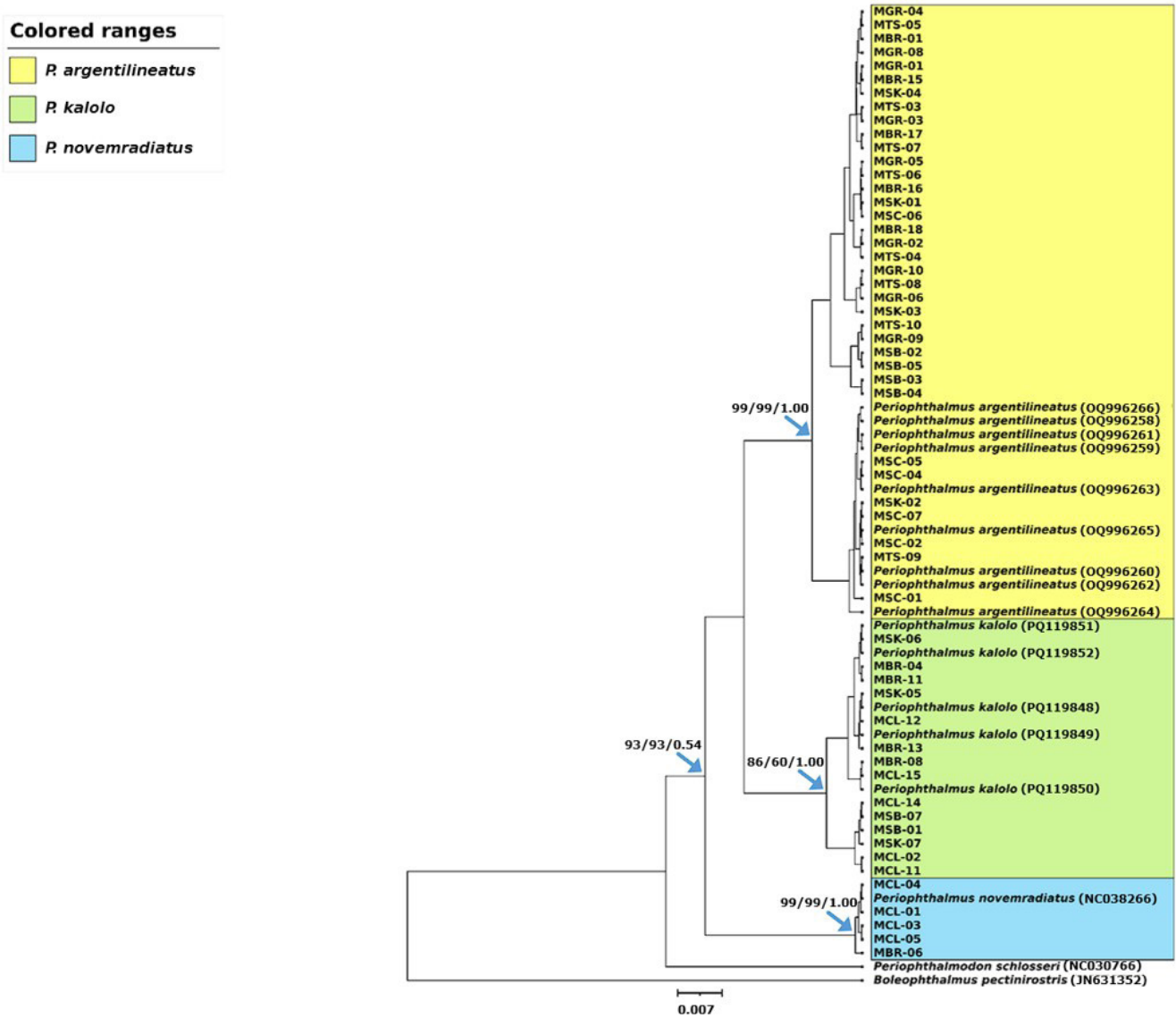
Figure 2. Bayesian phylogenetic tree inferred from mitochondrial 16S rRNA sequences. This tree was generated from 10 million generations using the GTR + I + G model. Each node is marked with three values: bootstrap values for the NJ method, bootstrap values for the ML method, and posterior probabilities for the BI method. The scale bar indicates substitutions per site. The tree illustrates three distinct species, represented by different colors: *P. argentilineatus* (yellow), *P. kalolo* (green), and *P. novemradiatus* (blue)

Table 2. Mean percentage nucleotide divergence of *16S* rRNA among three species of *Periophthalmus* spp.

	P. kalolo	*P. argentilinieatus*	*P. novemradiatus*
P. kalolo			
P. argentilinieatus	3.66		
P. novemradiatus	3.77	5.40	

and *P. argentilineatus* with *P. novemradiatus* was relatively low (0.54), bootstrap values in both NJ and ML analyses provided strong support (>90%) for the tree's structure. Each species clade demonstrated robust support (NJ and ML = 99, BI = 1.00), except for the *P. kalolo* clade in the ML tree, which had comparatively weaker support (bootstrap = 60), indicating some uncertainty. The remaining high bootstrap values suggested that our analysis effectively captured the essential evolutionary relationships among these species. The 15 additional sequences from GenBank, including *P. kalolo* (PQ119848-52), *P. argentilineatus* (OQ996258-66), and *P. novemradiatus* (NC038266), clustered within the clades of their respective species, forming well-supported monophyletic groups

Table 3. Mean percentage nucleotide divergence of *16S* rRNA among three species of Periophthalmus spp. across different populations

	MCL	MSB	MBR	MSK	MGR	MTS	MSB	MBR	MSC	MSK	MCL	MBR
MCL												
MSB	0.44											
MBR	0.64	0.82										
MSK	0.54	0.49	0.33									
MGR	3.57	3.47	3.62	3.50								
MTS	3.58	3.49	3.64	3.52	0.45							
MSB	4.18	3.94	4.44	4.21	1.19	1.31						
MBR	3.48	3.40	3.50	3.41	0.21	0.35	1.33					
MSC	3.80	3.72	3.83	3.73	1.29	1.16	2.26	1.14				
MSK	3.59	3.51	3.61	3.51	0.55	0.61	1.62	0.40	0.94			
MCL	3.77	3.54	3.83	3.67	5.16	5.25	5.69	5.13	5.80	5.35		
MBR	3.97	3.74	4.02	3.86	5.36	5.44	5.89	5.33	6.00	5.54	0.18	

Table 4. Polymorphic sites of three mudskippers species inferred from the *16S* rRNA gene (sites 01 to 237)

Nucleotide sites number	01	02	20	22	54	70	85	114	132	149	150	152	170	171	172	174	175	210	218	219	229	231	235	237
MCL-02	T	C	C	T	A	C	G	C	C	C	T	T	T	A	T	G	A	A	A	C	A	T	C	A
MCL-11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A
MCL-12	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MCL-14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
MCL-15	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MSB-01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
MSB-07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
MBR-04	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MBR-08	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MBR-11	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MBR-13	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MSK-05	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MSK-06	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	A	.	C	C	.	.	.	C	A
MSK-07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	A
MGR-01	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-02	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-03	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-04	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-05	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-06	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-08	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MGR-09	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	.	.	C	T	T	.	A	T	-
MGR-10	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-03	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-04	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-05	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-06	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-07	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-08	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MTS-09	.	.	.	.	.	T	A	.	.	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MTS-10	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	.	.	C	T	T	.	A	T	-
MSB-02	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	.	.	C	T	T	.	A	T	-
MSB-03	G	T	.	G	.	T	A	.	.	T	C	C	.	C	C	.	.	C	T	T	.	A	T	-
MSB-04	G	T	.	G	.	T	A	.	.	T	C	C	.	C	C	.	.	C	T	T	.	A	T	-
MSB-05	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	.	.	C	T	T	.	A	T	-
MBR-01	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MBR-15	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MBR-16	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MBR-17	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MBR-18	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-

Table 4. Continued

Nucleotide sites number	01	02	20	22	54	70	85	114	132	149	150	152	170	171	172	174	175	210	218	219	229	231	235	237
MSC-01	.	.	.	.	.	T	A	.	T	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MSC-02	.	.	.	.	.	T	A	.	.	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MSC-04	.	.	.	.	.	T	A	.	.	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MSC-05	.	.	.	.	.	T	A	.	.	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MSC-06	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MSC-07	.	.	.	.	.	T	A	.	.	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MSK-01	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MSK-02	.	.	.	.	.	T	A	.	.	.	C	C	C	.	C	A	.	C	T	T	.	A	T	-
MSK-03	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MSK-04	.	.	.	.	.	T	A	.	.	T	.	C	.	C	C	A	.	C	T	T	.	A	T	-
MCL-01	.	.	.	.	T	.	A	T	.	T	.	C	.	.	A	C	C	.	.	.	G	C	.	-
MCL-03	.	.	.	.	T	.	A	T	.	T	.	C	.	.	A	C	C	.	.	.	G	C	.	-
MCL-04	.	.	.	.	T	.	A	T	.	T	.	C	.	.	A	C	C	.	.	.	G	C	.	-
MCL-05	.	.	.	.	T	.	A	T	.	T	.	C	.	.	A	C	C	.	.	.	G	C	.	-
MBR-06	.	.	.	.	T	.	A	T	.	T	.	C	.	.	A	C	C	.	.	.	G	C	.	-

Table 5. Polymorphic sites of Indonesian mudskippers inferred from the *16S* rRNA gene (site 239 to 507)

Nucleotide sites number	239	247	248	258	261	269	270	291	327	329	338	341	357	362	367	372	376	390	391	392	406	407	492	507
MCL-02	T	A	A	G	T	T	C	A	T	A	A	A	G	C	C	G	T	A	G	A	C	G	C	C
MCL-11	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MCL-12	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MCL-14	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MCL-15	C	.	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
MSB-01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MSB-07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MBR-04	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MBR-08	C	.	.	.	.	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.
MBR-11	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MBR-13	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MSK-05	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MSK-06	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MSK-07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
MGR-01	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MGR-02	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	T
MGR-03	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MGR-04	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MGR-05	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MGR-06	.	.	.	.	C	.	T	.	C	G	.	C	A	.	T	.	.	.	.	.	.	.	.	.
MGR-08	.	G	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MGR-09	.	.	.	.	C	.	T	.	.	G	.	C	A	T	T	A	.	.	.	.	.	.	.	.
MGR-10	.	.	.	.	C	.	T	.	C	G	.	C	A	.	T	.	.	.	.	.	.	.	.	.
MTS-03	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MTS-04	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	T
MTS-05	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MTS-06	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MTS-07	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MTS-08	.	.	.	.	C	.	T	.	C	G	.	C	A	.	T	.	.	.	.	.	.	.	.	.
MTS-09	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MTS-10	.	.	.	.	C	.	T	.	.	G	.	C	A	T	T	A	.	.	.	.	.	.	.	.
MSB-02	.	.	.	.	C	.	T	.	.	G	.	C	A	T	T	A	.	.	.	.	.	.	.	.
MSB-03	.	.	.	.	C	.	T	.	.	G	.	C	A	T	T	A	.	.	.	.	.	.	.	.
MSB-04	.	.	.	.	C	.	T	.	.	G	.	C	A	T	T	A	.	.	.	.	.	.	.	.

Table 5. Continued

Nucleotide sites number	239	247	248	258	261	269	270	291	327	329	338	341	357	362	367	372	376	390	391	392	406	407	492	507
MSB-05	.	.	.	.	C	.	T	.	.	G	.	C	A	T	T	A	.	.	.	.	.	.	.	.
MBR-01	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MBR-15	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MBR-16	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MBR-17	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MBR-18	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	T
MSC-01	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MSC-02	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MSC-04	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MSC-05	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MSC-06	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MSC-07	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MSK-01	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MSK-02	.	.	G	A	C	.	T	.	C	G	.	T	A	.	.	.	.	.	.	.	.	.	.	.
MSK-03	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	T	.	.	.
MSK-04	.	.	.	.	C	.	T	.	C	G	.	C	A	.	.	.	.	.	.	.	.	.	.	.
MCL-01	.	.	T	.	.	C	.	G	.	.	.	C	.	.	G	.	C	C	A	G	.	.	T	.
MCL-03	.	.	T	.	.	C	.	G	.	.	.	C	.	.	G	.	C	C	A	G	.	.	T	.
MCL-04	.	.	T	.	.	C	.	G	.	.	.	C	.	.	G	.	C	C	A	G	.	.	T	.
MCL-05	.	.	T	.	.	C	.	G	.	.	.	C	.	.	G	.	C	C	A	G	.	.	T	.
MBR-06	.	.	T	.	.	C	.	G	.	.	.	C	.	.	G	.	C	C	A	G	.	A	T	.

alongside our original sequences. The clade structure aligned with Aji and Arisuryanti (2021), who also indicated a closer relationship between *P. kalolo* and *P. argentilineatus* than with *P. novemradiatus* based on *COI* mitochondrial gene analysis. The *COI* gene is commonly used for species identification due to its high mutation rate, which effectively distinguishes closely related taxa (Hebert *et al.* 2003; Ward *et al.* 2005). In contrast, the *16S* rRNA gene evolves more slowly, with numerous conserved regions that facilitate primer design, making it especially useful for broader sample coverage, such as metabarcoding (Elbrecht *et al.* 2016). Based on our study, the *16S* gene could serve as a viable alternative to *COI* for species identification. However, these results are specific to the three species studied, and further research is needed to determine the gene'sNA gene's applicability to other mudskipper species. A contributing factor to this uncertainty is that 16S sequences are less frequently available in public databases compared to *COI* sequences, which are more widely used and better represented across various species (Ratnasingham and Hebert 2007). This may limit the broader use of 16S for species identification, especially when database resources are limited.

The genetic relationship observed in the phylogenetic tree is further supported by the morphological similarities between *P. kalolo* and *P. argentilineatus*.

Both species share key traits, including similar first and second dorsal fin (D1 and D2) structures and comparable pelvic fin characteristics. They exhibit united pelvic fins. However, *P. kalolo* possesses a strong frenum, whereas *P. argentilineatus* lacks one. Additionally, *P. kalolo* has a convex D1 margin, while *P. argentilineatus* has a concave or straight D1 margin, contrasting with *P. novemradiatus*, which has a different structure compared to both species, including a lack of spots on the D1 and distinct dark stripes on the D2. Furthermore, *P. kalolo* and *P. argentilineatus* show similar longitudinal scale counts, although *P. argentilineatus* exhibits a wider range (Murdy 1989). Polgar (2014) even suggests that due to their similarities, specimens of these two species must be observed from a close distance in a ventral view for proper identification. In contrast to *P. kalolo* and *P. argentilineatus, P. novemradiatus* exhibits more distinct morphological traits. These include a more prominent pelvic frenum, a basal membrane that unites the inner rays of its pelvic fins for over half their length, a shorter anal fin base, and a longer D1 spine in males, which is a sexually dimorphic feature not found in *P. kalolo* or *P. argentilineatus* (Jaafar *et al.* 2009). These morphological differences align with the genetic divergence observed in our study, further confirming the phylogenetic distinction between *P. novemradiatus* and

the other two species. Furthermore, the phylogenetic arrangement was consistent with the Oxudercinae cladogram proposed by Murdy (1989), suggesting that *Periophthalmus* taxa are more closely related to *Periophthalmodon* than to *Boleophthalmus*. Recently, Steppan *et al.* (2022) indicated that *Boleophthalmus* is considerably more distantly related to these genera.

We observed interspecific genetic divergence values ranging from 3.66% (*P. kalolo* and *P. argentilineatus*) to 5.4% (*P. argentilineatus* and *P. novemradiatus*). This finding suggested a more recent common ancestry or shared environmental adaptations, as these two species frequently partake in habitats (Polgar 2014). Interestingly, the intraspecific genetic divergence observed in *P. argentilineatus* was higher than that typically anticipated, ranging from 0.21% to 2.26%, supporting previous findings that identify P. argentilineatus as a cryptic species (Polgar *et al.* 2014; Arisuryanti *et al.* 2024b), demonstrating that even a conserved marker like the *16S* rRNA gene can unravel cryptic diversity. In contrast, *P. kalolo* and *P. novemradiatus* exhibited more conventional intraspecific genetic distances, remaining at <1%. The genetic divergence observed in *Periophthalmus* falls within a moderate range compared to other fish genera. It is higher than the low variability reported in *Stellifer* (1.2%) (Vinson *et al.* 2004) but does not reach the extensive divergence seen in *Channa* (2.24-11.55%) (Lakra *et al.* 2010). Instead, its genetic variability is more comparable to that of *Trichiurus* (5.5%–6.5%) (Chakraborty *et al.* 2006) and *Apogon* (5.4%) (Mabuchi *et al.* 2003), indicating that *Periophthalmus* exhibits an intermediate level of genetic divergence within this spectrum.

We identified 47 variable sites, including four singleton and 43 parsimony-informative sites. Among them, position 172 had unique holding potential as a distinguishing marker for the three species. Additionally, we observed an indel at site 237, mar'e' by a '-' in *P. argentilineatus* and *P. novemradiatus* and an adenine (A) in *P. kalolo*. Given the close evolutionary relationship between *P. kalolo* and *P. argentilineatus*, this indel might have primarily resulted from mechanisms such as polymerase slippage (Montgomery *et al.* 2013) or from unequal crossovers in repeat sequences or insertion of mobile elements. These explanations hold true, especially if the mutation was randomly fixed in *P. argentilineatus* and *P. novemradiatus* but not in *P. kalolo*. Britten (2003) supports the significance of these findings,

indicating that a substantial portion of divergence between closely related DNA sequences is attributable to indels. This observation highlights the evolutionary role of indels, particularly in facilitating rapid genetic diversification. Furthermore, Väli *et al.* (2008) demonstrated that indels could serve as effective genetic markers in natural populations, highlighting their potential for distinguishing species. However, our findings are based on a limited sample size and are confined to the southern coast of Java. Future studies with broader sampling across varying Indonesian regions could enhance their robustness and provide deeper insights into intraspecific genetic diversity.

In summary, this study highlights the potential of the *16S* rRNA gene as an alternative genetic marker for species identification, while also revealing the genetic diversity among the three *Periophthalmus* species examined. The findings suggest that *16S* rRNA may serve as a viable complement to, or even an alternative for, the widely used *COI* gene in these species. However, further research is necessary to determine whether the *16S* rRNA gene can provide similar benefits for other mudskipper species. Future studies should include broader sampling and additional genetic markers to gain a more comprehensive understanding of the genetic diversity within these three mudskipper species.

Acknowledgements

References

Abdalwahhab, O., Galal-Khallaf, A., Saber, S.A.E.L., Osman, A.G., Mohammed-Geba, K., 2020. A case study for application of DNA barcoding in identifying species and genetic diversity of fish from the Suez city market, Egypt. *Aquat. Living Resour.* 33, 1-9. https://doi.org/10.1051/alr/2020012

Aji, K.W., Arisuryanti, T., 2021. Molecular identification of mudskipper fish (*Periophthalmus* spp.) from Baros Beach, Bantul, Yogyakarta. *J. Trop. Biodivers. Biotechnol.* 6, 1-13. https://doi.org/10.22146/jtbb.66391

Akhtar, T., Ghazanfar, A., Shafi, N., Rauf, A., 2020. Molecular characterization of subfamily Schizothoracinae (Teleostei: Cyprinidae) using complete sequence of mitochondrial *16S* rRNA gene. *Pak. J. Zool.* 52, 273-282. https://doi.org/10.17582/journal.pjz/2020.52.1.273.282

Amoussou, T.O., Karim, I.Y.A., Dayo, G.K., Kareem, N., Toko, I.I., Chikou, A., Toguyéni, A., 2019. An insight into advances in fisheries biology, genetics and genomics of *African tilapia* species of interest in aquaculture. *Aquac. Rep.* 14, 1-15. https://doi.org/10.1016/j.aqrep.2019.100188

Ansari, A.A., Trivedi, S., Saggu, S., Rehman, H., 2014. Mudskipper: a biological indicator for environmental monitoring and assessment of coastal waters. *J. Entomol. Zool. Stud.* 2, 22-33.

Arisuryanti, T., Aji, K.W., Herawati, H., Sari, I.P., Rha'ifa, F.A., Febriyanti, D., Priyono, D.S., 2024b. Cryptic diversity of barred mudskippers, *Periophthalmus argentilineatus* (Valenciennes, 1837), from the Southern Coast of Java and East Lombok, Indonesia inferred by COI mitochondrial gene. *J. Trop. Biodivers. Biotechnol.* 9, 1-15. https://doi.org/10.22146/jtbb.84328

Arisuryanti, T., Aji, K.W., Shabrina, F.N., Febriyanti, D., Daryono, B.S., Priyono, D.S., 2024a. Phylogenetic and genetic variation of common mudskippers (*Periophthalmus kalolo* Lesson, 1831) from the southern coast of Java, Indonesia inferred from the COI mitochondrial gene. *J. Genet. Eng. Biotechnol.* 22, 1-10. https://doi.org/10.1016/j.jgeb.2023.100335

Arisuryanti, T., Alfianti, A., Nida'Firdaus, N.U., Hakim, L., 2020. Detection of 16S mitochondrial gene polymorphism on barb fish (*Barbodes binotatus* Valenciennes, 1842) from Lake Lebo Taliwang, West Nusa Tenggara. *J. Perikanan Univ. Gadjah Mada.* 22, 123-126. https://doi.org/10.22146/jfs.54064

Arisuryanti, T., Hasan, R.L., Koentjana, J.P., 2018. Genetic identification of two mudskipper species (Pisces: Gobiidae) from Bogowonto Lagoon (Yogyakarta, Indonesia) using COI mitochondrial gene as a DNA barcoding marker. *AIP Conf. Proc.* 2002, 020068. https://doi.org/10.1063/1.5050164

Baeck, G.W., Takita, T., Yoon, Y.H., 2008. Lifestyle of Korean mudskipper *Periophthalmus magnuspinnatus* with reference to a congeneric species *Periophthalmus modestus*. *Ichthyol. Res.* 55, 43-52. https://doi.org/10.1007/s10228-007-0009-y

Britten, R.J., Rowen, L., Williams, J., Cameron, R.A., 2003. Majority of divergence between closely related DNA samples is due to indels. *Proc. Natl. Acad. Sci.* 100, 4661-4665. https://doi.org/10.1073/pnas.0330964100

Chakraborty, A., Aranishi, F., Iwatsuki, Y., 2006. Genetic differences among three species of the genus *Trichiurus* (Perciformes: Trichiuridae) based on mitochondrial DNA analysis. *Ichthyol. Res.* 53, 93-96. https://doi.org/10.1007/s10228-005-0313-3

Chauhan, T., Rajiv, K., 2010. Molecular markers and their applications in fisheries and aquaculture. *Adv. Biosci. Biotechnol.* 1, 281-291. https://doi.org/10.4236/abb.2010.14037

Dahruddin, H., Hutama, A., Busson, F., Sauri, S., Hanner, R., Keith, P., Hadiaty, R., Hubert, N., 2017. Revisiting the ichthyodiversity of Java and Bali through DNA barcodes: taxonomic coverage, identification accuracy, cryptic diversity and identification of exotic species. *Mol. Ecol. Resour.* 17, 288-299. https://doi.org/10.1111/1755-0998.12528

Darriba, D., Taboada, G.L., Doallo, R., Posada, D., 2012. jModelTest 2: more models, new heuristics and parallel computing. *Nat. Methods.* 9, 772-772. https://doi.org/10.1038/nmeth.2109

Darumas, U., Tantichodok, P., 2002. A new species of Mudskipper (Gobbidae: Oxudercinae) from Southern Thailand. *Phuket Mar. Biol. Cent. Res. Bull.* 64, 101-107.

Elbrecht, V., Taberlet, P., Dejean, T., Valentini, A., Usseglio-Polatera, P., Beisel, J.-N., … Leese, F., 2016. Testing the potential of a ribosomal *16S* marker for DNA metabarcoding of insects. *PeerJ.* 4, e1966. https://doi.org/10.7717/peerj.1966

Febrianti, D., Aji, K.W., Priyono, D.S., Arisuryanti, T., 2023. Genetic identification of two mudskipper species (Oxudercidae: Periophthalmus) from Kulon Progo, Special Region of Yogyakarta, Indonesia. *J. Trop. Biodivers. Biotechnol.* 8, 1-15. https://doi.org/10.22146/jtbb.78161

Feng, Y., Li, Q., Kong, L., Zheng, X., 2011. DNA barcoding and phylogenetic analysis of Pectinidae (Mollusca: Bivalvia) based on mitochondrial COI and *16S* rRNA genes. *Mol. Biol. Rep.* 38, 291-299. https://doi.org/10.1007/s11033-010-0107-1

Fricke, R., Eschmeyer, W.N., 2024. Eschmeyer'schmeyer's Catalog Of Fishes: Genera. Available at: http://researcharchive.calacademy.org/research/ichthyology/catalog/fishcatmaiDatep. [Date accessed: 31 October 2024].

Hebert, P.D.N., Ratnasingham, S., deWaard, J.R., 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proc. R. Soc. Lond. B: Biol. Sci.* 270, S96-S99. https://doi.org/10.1098/rsbl.2003.0025

Hidayat, S., Wicaksono, A., Raharjeng, A., Jin, D.S.M., Alam, P., Retnoaji, B., 2022. The morphologies of mudskipper pelvic fins in relation to terrestrial and climbing behaviour. *Proc. Zool. Soc.* 75, 83-93. https://doi.org/10.1007/s12595-021-00422-1

Jaafar, Z., Larson, H.K., 2008. A new species of mudskipper, *Periophthalmus takita* (Teleostei: Gobiidae: Oxudercinae), from Australia, with a key to the genus. *Zool. Sci.* 25, 946-952. https://doi.org/10.2108/zsj.25.946

Jaafar, Z., Lim, K.K., Chou, L.M., 2006. Taxonomical and morphological notes on two species of mudskippers, *Periophthalmus walailakae* and *Periophthalmodon schlosseri* (Teleostei: Gobiidae) from Singapore. *Zool. Sci.* 23, 1043-1047. https://doi.org/10.2108/zsj.23.1043

Jaafar, Z., Perrig, M., Chou, L.M., 2009. *Periophthalmus variabilis* (Teleostei: Gobiidae: Oxudercinae), a valid species of mudskipper, and a re-diagnosis of *Periophthalmus novemradiatus*. *Zool. Sci.* 26, 309-314. https://doi.org/10.2108/zsj.26.309

Jaafar, Z., Polgar, G., Zamroni, Y., 2016. Description of a new species of *Periophthalmus* (Teleostei: Gobiidae) from the Lesser Sunda Islands. *Raffles Bull. Zool.* 64, 278-283.

Kitano, T., Umetsu, K., Tian, W., Osawa, M., 2007. Two universal primer sets for species identification among vertebrates. *Int. J. Leg. Med.* 121, 423-427. https://doi.org/10.1007/s00414-006-0113-y

Kuang, T., Tornabene, L., Li, J., Jiang, J., Chakrabarty, P., Sparks, J.S., Naylor, G.J.P., Li, C., 2018. Phylogenomic analysis on the exceptionally diverse fish clade Gobioidei (Actinopterygii: Gobiiformes) and data-filtering based on molecular clocklikeness. *Mol. Phylogenetics Evol.* 128, 192-202. https://doi.org/10.1016/j.ympev.2018.07.018

Kumar, S., Stecher, G., Li, M., Knyaz, C., Tamura, K., 2018. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547-1549. https://doi.org/10.1093/molbev/msy096

Lakra, W.S., Goswami, M., Gopalakrishnan, A., Singh, D.P., Singh, A., Nagpure, N.S., 2010. Genetic relatedness among fish species of genus *Channa* using mitochondrial DNA genes. *Biochem. Syst. Ecol.* 38, 1212-1219. https://doi.org/10.1016/j.bse.2010.12.012

Larson, H.K., 2008. Weber's mudskipper *Periophthalmus weberi*: new record for the Daly River. *North. Territ. Nat.* 20, 19-21. https://doi.org/10.5962/p.295507

Larson, H.K., Takita, T., 2004. Two new species of *Periophthalmus* (Teleostei: Gobiidae: Oxudercinae) from northern Australia, and a re-diagnosis of *Periophthalmus novaeguineaensis*. *The Beagle: Records of the Museums and Art Galleries of the Northern Territory*. 20, 175-185. https://doi.org/10.5962/p.286325

Mabuchi, K., Okuda, N., Kokita, T., Nishida, M., 2003. Genetic comparison of two color-morphs of *Apogon properuptus* from southern Japan. *Ichthyol. Res.* 50, 293-296. https://doi.org/10.1007/s10228-003-0171-9

Maddison, W.P., Maddison, D.R., 2018. Mesquite: a modular system for evolutionary analysis. *Evolution.* 62, 1103-1118.

McCraney, W.T., Thacker, C.E., Alfaro, M.E., 2020. Supermatrix phylogeny resolves goby lineages and reveals unstable root of Gobiaria. *Mol. Phylogenetics Evol.* 151, 1-15. https://doi.org/10.1016/j.ympev.2020.106862

Montgomery, S.B., Goode, D.L., Kvikstad, E., Albers, C.A., Zhang, Z.D., Mu, X.J., Ananda, G., Howie, B., Karczewski, K.J., Smith, K.S., Anaya, V., Richardson, R., Davis, J., 1000 Genomes Project Consortium, MacArthur, D.G., Sidow, A., Duret, L., Gerstein, M., Makova, K.D., Marchini, J., McVean, G., Lunter, G., 2013. The origin, evolution, and functional impact of short insertion–deletion variants identified in 179 human genomes. *Genome Res.* 23, 749-761. https://doi.org/10.1101/gr.148718.112

Murdy, E.O., 1989. A taxonomic revision and cladistic analysis of the oxudercine gobies (Gobiidae: Oxudercinae). *Rec. Aust. Mus. Supplement.* 11, 1-93. https://doi.org/10.3853/j.0812-7387.11.1989.93

Murdy, E.O., Takita, T., 1999. *Periophthalmus spilotus*, a new species of mudskipper from Sumatra (Gobiidae: Oxudercinae). *Ichthyol. Res.* 46, 367-370. https://doi.org/10.1007/bf02673979

Nelson, J.S., Grande, T.C., Wilson, M.V., 2016. *Fishes of the World.* John Wiley & Sons, Hoboken, New Jersey.

Nordhaus, I., Toben, M., Fauziyah, A., 2019. Impact of deforestation on mangrove tree diversity, biomass and community dynamics in the Segara Anakan lagoon, Java, Indonesia: a ten-year perspective. *Estuar. Coast. Shelf Sci.* 227, 1-12. https://doi.org/10.1016/j.ecss.2019.106300

Palumbi, S.R., 1996. Nucleic acids II: the polymerase chain reaction. Molecular systematics, 205-247.

Polgar, G., 2014. The mudskipper. Available at: http://www.themudskipper.org. [Data accessed 31 October 2024]

Polgar, G., Bartolino, V., 2010. Size variation of six species of oxudercine gobies along the intertidal zone in a Malayan coastal swamp. *Mar. Ecol. Prog. Ser.* 409, 199-212. https://doi.org/10.3354/meps08597

Polgar, G., Crosa, G., 2009. Multivariate characterisation of the habitats of seven species of *Malayan mudskippers* (Gobiidae: Oxudercinae). *Mar. Biol.* 156, 1475-1486. https://doi.org/10.1007/s00227-009-1187-0

Polgar, G., Sacchetti, A., Galli, P., 2010. Differentiation and adaptive radiation of amphibious gobies (Gobiidae: Oxudercinae) in semi-terrestrial habitats. *J. Fish Biol.* 77, 1645-1664. https://doi.org/10.1111/j.1095-8649.2010.02807.x

Polgar, G., Zane, L., Babbucci, M., Barbisan, F., Patarnello, T., Rüber, L., Papetti, C., 2014. Phylogeography and demographic history of two widespread Indo-Pacific mudskippers (Gobiidae: Periophthalmus). *Mol. Phylogenetics Evol.* 73, 161-176. https://doi.org/10.1016/j.ympev.2014.01.014

Pormansyah, I.M., Arum, S., Indra, Y., Hilda, Z., 2019. A review of recent status on Mudskippers (Oxudercine Gobies) in Indonesian Waters. *Oceanogr. Fish Open Access J.* 9, 1-4. https://doi.org/10.19080/OFOAJ.2019.09.555769

Rambaut, A., 2019. FigTree v 1.4.4. Available at: http://tree.bio.ed.ac.uk/software/figtree/. [Data accessed 31 October 2024]

Ratnasingham, S., Hebert, P., 2007. BOLD: The barcode of life data system. *Mol. Ecol. Notes.* 7, 355-364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Rha'ifa, F.A., Audrea, D.J., Hakim, L., Arisuryanti,, T., 2021. DNA barcode of barred mudskipper (*Periophthalmus argentilineatus* Valenciennes, 1837) from Tekolok Estuary (West Nusa Tenggara, Indonesia) and their phylogenetic relationship with other Indonesian barred mudskippers. *J. Trop. Biodivers. Biotechnol.* 6, 1-13. https://doi.org/10.22146/jtbb.59702

Rozas, J., Ferrer-Mata, A., Sánchez-DelBarrio, J.C., Guirao-Rico, S., Librado, P., Ramos-Onsins, S.E., Sánchez-Gracia, A., 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol. Biol. Evol.* 34, 3299-3302. https://doi.org/10.1093/molbev/msx248

Rudianto, R., Bengen, D.G., Kurniawan, F., 2020. Causes and effects of mangrove ecosystem damage on carbon stocks and absorption in East Java, Indonesia. *Sustainability.* 12, 1-17. https://doi.org/10.3390/su122410319

Saad, Y.M., Shaikh, O.A.K.M., Gharbawi, W.M., 2019. Evaluation of molecular diversity in some Red sea parrotfish species based on mitochondrial *16S* ribosomal RNA gene sequence variations. *Res. J. Biotechnol.* 14, 8-21.

Saha, S., Ferdous, Z., Jahan, H., Khandaker, A.M., Shahjahan, R.M., Begum, R.A., 2019. Polymorphic loci analysis of *16S* ribosomal RNA gene of economically important marine lizardfish Bombay duck (*Harpadon nehereus*). *Bangladesh J. Zool.* 47, 49-57. https://doi.org/10.3329/bjz.v47i1.42020

Sangur, K., Leiwakabessy, F., Tuaputty, H., Tuwankotta, L.V., Samloy, S.V., Ratila, C., Salakory, O.B., Matulessy, C., Rumahlatu, D., 2021. Mudskipper as an indicator species for lead, cadmium and cuprum heavy metal pollution in the Mangrove, Ambon, Indonesia. *J. Ecol. Eng.* 22, 1-19. https://doi.org/10.12911/22998993/134077

Santoso, H.B., Suhartono, E., Yunita, R., Biyatmoko, D., 2020. Mudskipper fish as a bio-indicator for heavy metals pollution in a coastal wetland. Egypt. *J. Aquat. Biol. Fish.* 24, 1073-1095. https://doi.org/10.21608/ejabf.2020.144402

Steppan, S.J., Meyer, A.A., Barrow, L.N., Alhajeri, B.H., Al-Zaidan, A.S., Gignac, P.M., Erickson, G.M., 2022. Phylogenetics and the evolution of terrestriality in mudskippers (Gobiidae: Oxudercinae). *Mol. Phylogenetics Evol.* 169, 1-9. https://doi.org/10.1016/j.ympev.2022.107416

Suchard, M.A., Lemey, P., Baele, G., Ayres, D.L., Drummond, A.J., Rambaut, A., 2018. Bayesian phylogenetic and phylodynamic data integration using BEAST 1.10. *Virus Evol.* 4, 1-5. https://doi.org/10.1093/ve/vey016

Takita, T., Larson, H.K., Ishimatsu, A., 2011. The natural history of mudskippers in northern Australia, with field identification characters. *The Beagle, Records of the Museums and Art Galleries of the Northern Territory.* 27, 189-204. https://doi.org/10.5962/p.287482

Thi, H.K., Thanh, L.D., Quang, L.N., 2023. General diversity of marbled eel (*Anguilla marmorata*) population in central of Vietnam, based on 16S rRNA sequences by barcode DNA. *Res. J. Biotechnol.* 18, 41-48. https://doi.org/10.25303/1804rjbt041048

Väli, Ü., Brandström, M., Johansson, M., Ellegren, H., 2008. Insertion-deletion polymorphisms (indels) as genetic markers in natural populations. *BMC Genetics.* 9, 1-8. https://doi.org/10.1186/1471-2156-9-8

Vences, M., Thomas, M., Van der Meijden, A., Chiari, Y., Vieites, D.R., 2005. Comparative performance of the *16S* rRNA gene in DNA barcoding of amphibians. *Front. Zool.* 2, 1-12. https://doi.org/10.1186/1742-9994-2-5

Vinson, C., Gomes, G., Schneider, H., Sampaio, I., 2004. Sciaenidae fish of the Caeté River estuary, Northern Brazil: mitochondrial DNA suggests explosive radiation for the Western Atlantic assemblage. *Genet. Mol. Biol.* 27, 174-180. https://doi.org/10.1590/S1415-47572004000200008

Ward, R.D., Zemlak, T.S., Innes, B.H., Last, P.R., Hebert, P.D.N., 2005. DNA bAustralia'sstralia's fish species. *Philos. Trans. R. Soc. B: Biol. Sci.* 360, 1847-1857. https://doi.org/10.1098/rstb.2005.1716

Yang, L., Tan, Z., Wang, D., Xue, L., Guan, M.X., Huang, T., Li, R., 2014. Species identification through mitochondrial rRNA genetic analysis. *Sci. Rep.* 4, 1-11. https://doi.org/10.1038/srep04089

You, X., Sun, M., Li, J., Bian, C., Chen, J., Yi, Y., Yu, H., Shi, Q., 2018. Mudskippers and their genetic adaptations to an amphibious lifestyle. *Animals.* 8, 1-12. https://doi.org/10.3390/ani8020024