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Genetic variation of the Mudskipper (*Periophthalmus argentilineatus*) in the mangrove ecosystem, North Coast of Aceh, Aceh Province, Indonesia

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Abstract. *Dewiyanti I, Siregar PKA, Octavina C, Nurfadillah, Nurfadli, Marimuthu K. 2025. Genetic variation of the Mudskipper* (Periophthalmus argentilineatus) *in the mangrove ecosystem, North Coast of Aceh, Aceh Province, Indonesia. Biodiversitas 26: 6399-6409*. Mudskipper (*Periophthalmus argentilineatus*), a species belonging to the Gobiidae family of the Perciformes order, is an important ecological indicator of mangrove health, yet information on its genetic diversity in Aceh remains limited. This study analyzes genetic variation in mudskipper populations from the northern coast of Aceh using mitochondrial Cytochrome Oxidase Subunit I (COI) gene sequencing. Although the species has been widely studied in other regions of Indonesia, no molecular genetic baseline has ever been reported for Aceh populations, creating a critical gap in understanding their genetic structure and conservation status. Twenty samples were collected in Aceh Besar and Banda Aceh populations and combined with 19 additional COI sequences obtained from GenBank. The results showed that the mudskipper species in the research locations were *P. argentilineatus*. In addition, *P. argentilineatus* populations from Aceh Besar and Banda Aceh showed no detectable genetic variation, as reflected by low genetic distance values indicating close genetic relatedness. This pattern suggests that similar environmental pressure may be contributing to genetic homogeneity between the two populations. However, genetic variation of *P. argentilineatus* was moderate in the Bali population, and there was a high genetic distance between the Aceh Besar and Bali populations, as well as Banda Aceh and Bali (0.393). These findings highlight the need for conservation strategies that maintain mangrove habitat integrity and promote connectivity among regional mudskipper populations. Maintaining mangrove habitat connectivity and implementing periodic genomic monitoring are critical future steps to preserve gene flow in *P. argentilineatus* populations along the North Coast of Aceh.

Keywords: COI, genetic distance, Gobiidae, haplotype, population

INTRODUCTION

Mangrove forests are highly productive coastal ecosystems that provide essential nursery, feeding, and breeding habitats for a wide range of aquatic organisms. Their high nutrient content supports fish, shrimp, crab, and other fauna (Naresh et al. 2019; Tresnati et al. 2021). The structural complexity of mangroves also enhances habitat availability and supports diverse aquatic communities (Muhtadi et al. 2016; Jimenez et al. 2021). Among the organisms closely associated with mangroves, mudskippers (*Periophthalmus* spp.) fish from the Gobiidae family are particularly important due to their dual adaptation to terrestrial and aquatic environments and their sensitivity to habitat quality (Santoso et al. 2020; Baderan et al. 2023). These characteristics make mudskippers valuable bioindicators for assessing mangrove ecosystem health (Ansari et al. 2014). They also contribute substantially to food web dynamics within these habitats (Bidawi et al. 2017). Despite their ecological importance, information on the biology, population genetics, and population dynamics of mudskippers in Indonesia remains limited.

Mudskipper belongs to the order Perciformes, which consists of 2,167 species and 321 genera (Nelson et al. 2016). Species identification of mudskippers is challenging because members of the order Perciformes, especially the genus *Periophthalmus*, exhibit high morphological similarity, leading to frequent misidentifications (Jafar et al. 2009). However, the similarities between species within this genus have led to misidentifications, such as between *Periophthalmus kalolo* and *Periophthalmus argentilineatu*s (Polgar et al. 2014). Therefore, in recent years, molecular biology approaches have been applied to identify and understand the genetic variation of the fish species due to the limitations of morphological identification. The most widely used method currently is DNA barcoding using the

COI gene as a DNA code marker, and an efficient approach (Bhattacharya et al. 2016; Marnis et al. 2024). Furthermore, species identification using DNA barcoding that has very similar morphology between species and species with incomplete morphology is more accurate than morphological identification (Ali et al. 2020; Raharinaivo et al. 2020). In particular, the mitochondrial (COI) gene is an effective marker for fish species identification and assessing intraspecific genetic variation (Imtiaz et al. 2017; Khusna et al. 2024). In addition, genetic variation is the response of living organisms to various environmental conditions that cause species with different ecological systems to have varying patterns (Freudiger et al. 2021). This shows that the genetic variation of species can be analyzed using molecular markers that accurately estimate diversity (Pino-Querid et al. 2015; Dias et al. 2018). The segment near the 5' end has a length of approximately 650 bases and is a region widely used as a DNA barcode for fauna, such as fish (Hebert et al. 2003).

Several studies related to the molecular identification of mudskipper have been conducted, such as Aji and Arisuryanti (2021), who reported that there were 3 species, namely *P. kalolo*, *P. argentilineatus*, and *P. novemradiatus*, in Baros Beach, Bantul, Yogyakarta, Indonesia. In addition, DNA barcoding of mudskipper was conducted by Rha'ifa et al. (2021), who reported that the fish in Muara Tekolok belongs to the species *P. argentilineatus*. There were 4 species, namely *P. argentilineatus*, *P. novemradiatus*, *P. barbarus* and *Boleophthalmus boddarti* in Abonnema, Nigeria (Sokefun et al. 2022). Arisuryanti et al. (2018) showed that haplotype diversity of *P. argentilineatus* was 1.0, and its nucleotide diversity was 0.0077 from Bogowonto Lagoon, Indonesia. Dewiyanti et al. (2022) conducted a study in the Banda Aceh and Aceh Besar areas regarding the growth and condition factors of mudskipper in the mangrove ecosystem of Aceh Province, Indonesia, and morphologically identified the species *P. gracilis*. Despite these studies, no research has examined the molecular identity or genetic variation of mudskipper populations in the mangrove ecosystems of Banda Aceh and Aceh Besar on the north coast of Aceh. This gap hinders accurate conservation planning, as genetic diversity strongly influences population resilience and adaptive capacity. Given the ecological role of *P. argentilineatus* in mangrove food webs and its function as a bioindicator species, conserving its genetic resources is essential. Accordingly, this study aims to analyze the genetic variation of mudskippers (*Periophthalmus* sp.) in the mangrove ecosystem along the north coast of Aceh, using COI gene.

MATERIALS AND METHODS

Study area

The research was conducted between March and August 2024. Mudskipper samples were obtained from mangrove habitats along the northern coast of Aceh, Indonesia, and analyzed at the Genetics and Aquatic Biodiversity Laboratory, Faculty of Marine and Fisheries, Universitas Syiah Kuala, Indonesia. The sampling sites are presented in Figure 1.

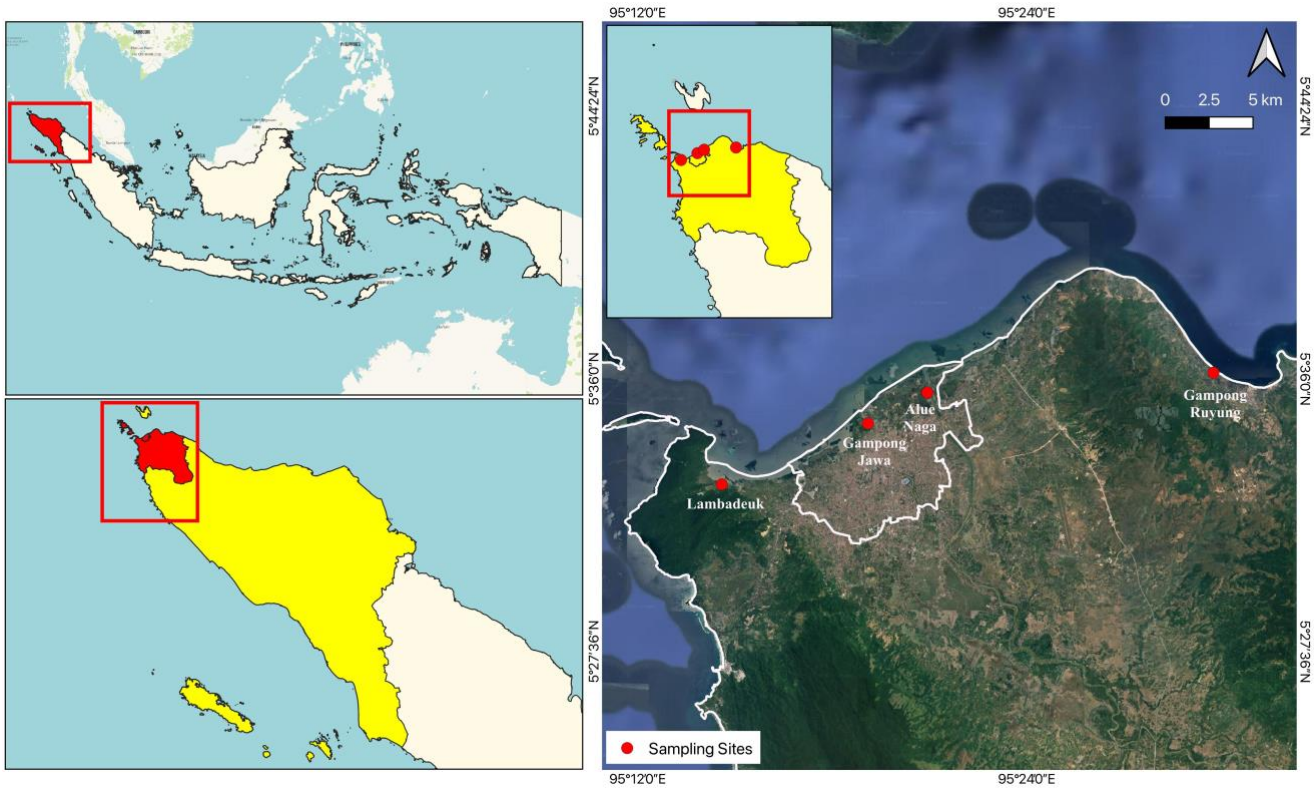

Figure 1. Location of specimen sampling on the northern coast of Aceh, Indonesia

Procedures

Collection of samples

The fish sampling locations were determined based on a survey in which mudskipper fish were found. Sampling was performed during low tide using a scoop net. The sampling sites consisted of Aceh Besar and Banda Aceh locations. Gampong Ruyung (5°36'11.5"N, 95°29'46.0"E) and Lambadeuk (5°32'45.0"N, 95°14'36.2"E), representing the Aceh Besar location, and Gampong Jawa (5°34'37.7"N, 95°19'06.7"E) and Alue Naga (5°35'35.0"N, 95°20'56.6"E), representing the Banda Aceh location within the mangrove ecosystem of the north coast of Aceh. All specimens were transported to the Genetics and Aquatic Biodiversity Laboratory, cleaned, and their fins removed before being stored in 1.5 mL tubes containing 96% alcohol for preservation. Each sample was labelled individually. Ethical approval for animal handling procedures was granted under Universitas Syiah Kuala guidelines (Code No. 958/2015).

Sample size justification

A total of 20 mudskipper samples were collected from each location, 10 samples in Aceh Besar and 10 samples in Banda Aceh for molecular analysis, a commonly accepted minimum for preliminary population genetic studies. Although larger sample sizes can provide better estimates of genetic parameters, this number is sufficient to detect general patterns of genetic variation, particularly when ecological conditions and sampling constraints limit specimen collection. Similar sample sizes have been used in previous studies by Ye et al. (2022) and Octavina et al. (2023) for genetic variation and diversity analyses.

Fish were collected using a convenience sampling approach within predefined sampling sites. Convenience sampling was applied because mudskippers are highly mobile and only accessible during specific tidal conditions, making transect based sampling impractical.

Sequence extraction from GenBank

To broaden the analysis, 19 additional COI sequences of *P. argentilineatus* from various coastal locations in Indonesia were retrieved from GenBank. Sequences were selected based on adequate fragment length and well documented sampling sites to ensure reliability. These sequences served as reference data for genetic analyses and enabled comparison of genetic variation among populations. One sequence, *Epinephelus areolatus,* was included as an outgroup to root the phylogenetic tree and to clarify evolutionary relationships by providing a point of comparison external to the ingroup taxa. The sources of these sequences are presented in Table 1.

DNA extraction, amplification, electrophoresis, and sequencing of COI

Genomic DNA was extracted from mudskipper pectoral fin tissue using the Cetyl Trimethyl Ammonium Bromide (C-TAB) protocol (Grewe et al. 1993; Quang et al. 2018), following standard molecular procedures (Sambrook and Russell 2001). The procedure involved three main steps: i) cell lysis and membrane disruption, ii) removal of proteins and RNA using proteinase K, and iii) assessment of DNA concentration, purity, and quantity (Kalender et al. 2021).

Table 1. Data sequencing from GenBank based on various coastal locations used for COI-based analysis for *Periophthalmus argentilineatus*

Species	Location	Total number of sequences	Accession numbers	References
Periophthalmus argentilineatus	Bali	4	KU692745	(Dahruddin et al. 2017)
			KU692746	
			KU692744	
			KU692743	
Periophthalmus argentilineatus	Bantul	5	MZ606687	(Aji and Arisuryanti 2021)
			MZ606686	
			MZ606685	
			MZ606684	
			MZ606683	
Periophthalmus argentilineatus	Kupang	5	OR053787	(Baderan et al. 2023)
			OR053788	
			OR053789	
			OR053790	
			OR053791	
Periophthalmus argentilineatus	Lombok	5	MW514019	(Rha'ifa et al. 2021)
			MW514020	
			MW514021	
			MW514022	
			MW514023	
Epinephelus areolatus	Egypt	Out group	MH707292	(Osman et al. 2018)

The Polymerase Chain Reaction (PCR) technique was conducted in the amplification process using each pair (two universal fish primers) of forward and reverse primers from the COI gene. A total of 1 µL of primer COI Fish F1 (5-TCA AAC AAC CAC AAA GAC ATT GGG AC-3) was combined with 1 µL of CO1 Fish R1 (5- TAG ACT TCT GGG TGG CCA AAG AAT CA-3 (Ward et al. 2005), along with CO1 Fish F2 and CO1 Fish R2, 2 µL of template DNA, 8.5 µL of ddH_2O, and 12.5 µL of red master mix. The PCR product size confirmation was consistent across all samples. The amplification process was carried out using a Senso Quest LabCycler Basic machine, which included a pre-denaturation stage for 2 minutes at 95°C. That was followed by 35 cycles consisting of denaturation at 95°C for 15 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 30 seconds, and a final extension at 72°C for 5 minutes (Arisuryanti et al. 2018). Amplified products were separated by electrophoresis on a 1.2% agarose gel prepared with 60 mL of TAE buffer and allowed to set for 40 minutes. Electrophoresis was conducted at 100 V for 30 minutes, and DNA bands were visualized under a UV transilluminator (UVITEC Fire-Reader V10-Plus). Positive samples were identified by clear bands of 400-600 base pairs (Lee et al. 2012).

Subsequently, the sequencing process was carried out by sending positive samples to Apical Scientific, Malaysia, using the Sanger method (Walker et al. 2013) to obtain the nucleotide base sequence. The Sanger sequencing method was commonly used, producing a chromatogram (electropherogram) from which the DNA sequence was derived. These results could be seen using a UV transducer that produced DNA bands.

Molecular data analysis

The COI gene sequences were analyzed and edited using the Molecular Evolutionary Genetic Analysis (MEGA X) to confirm species identity by comparison with GenBank data (Tamura et al. 2013; Habib et al. 2021). Sequence similarity was further verified using the BLAST database (https://blast.ncbi.nlm.nih.gov) and the BOLD Identification System (www.boldsystems.org) (Tamura et al. 2013). Genetic distances were calculated, and a phylogenetic tree was reconstructed in MEGA X using the Neighbor-Joining method with 1,000 bootstrap replicates, considering values ≥70% as strong support for nodes (Hillis and Bull 1993). A substitution saturation test was performed using Data Analysis and Molecular Biology and Evolution DAMBE software to assess sequence suitability for phylogenetic analysis. Haplotype distribution was analyzed using DnaSP 5.10 (Librado and Rozas 2009; Bilandžija et al. 2013), while geographic connectivity among populations was examined using Network software. Population structure, haplotype diversity (Hd), nucleotide diversity (π), and total haplotypes were calculated in ARLEQUIN (Rozas et al. 2017). Moreover, the population genetic structure of *P. argentilineatus* was assessed through Analysis of Molecular Variance (AMOVA), which was used to apportion genetic variation across hierarchical levels. Fixation indices (Fst) derived from this analysis were employed to evaluate genetic differentiation within and between populations. Fst values interpreted according to Wright (1978): <0.05 indicates low, 0.05<Fst<0.15 moderate, 0.15<Fst<0.25 high, and >0.25 very high differentiation.

RESULTS AND DISCUSSION

Species composition

Based on the results, amplification of the mitochondrial COI gene in mudskipper produced a fragment length of approximately 639 base pairs (bp). Electrophoresis visualization identified 20 individuals of *Periophthalmus argentilineatus*. The identified COI gene sequence of *P. argentilineatus* exhibited a similarity score of 100% in BLAST and BOLD results. Table 2 presents the similarity for each observed species.

Nucleotide composition of COI sequences

A total of 39 samples (20 sequences from the study and 19 sequences obtained from Genbank had a nucleotide base length of 639 base pairs (bp), the nucleotide composition differences among two common mudskipper populations (Aceh Besar and Banda Aceh. The average values of nucleotide bases T (Thymine), Cytosine (C), Adenine (A), and Guanine (G) were 30.98%, 26.67%, 25.27%, and 17.07%, respectively. AT had a higher average value of 56.25% compared to GC, which exhibited an average value of 43.74% and decreased at each post (Table 3).

Phylogenetic and genetic distance

In the phylogenetic analysis, a total of 20 specimens of *Periophthalmus argentilineatus* mudskipper were utilized from 2 populations, namely Gampong Ruyung and Lambadeuk (Aceh Besar population), as well as Gampong Jawa and Alue Naga (Banda Aceh population). This phylogenetic tree also used 19 sequences obtained from GenBank to examine interspecific relationships and one sequence as an outgroup (*Epinephelus areolatus*). Totally, phylogenetic tree analysis of 39 samples obtained from 6 different populations in Indonesia, namely Banda Aceh, Aceh Besar, Bali, Bantul, Lombok, and Kupang, yielded a bootstrap value of 70% to 100%. Figure 2 shows the results of the reconstructed phylogenetic tree, which was divided into 2 main clades: one clade from the samples and one clade as an outgroup. However, the main clade can also be divided into 5 clades, namely Banda Aceh and Aceh Besar population (1st clade, n: 20), Bali population (2nd clade, n: 4), Bantul, Kupang, and Lombok population as 3rd, 4th, and 5th clades with n: 5, respectively. Those clades show no overlapping populations based on geographic separation.

The highest value between populations (interspecies) was Aceh Besar and Bali, Banda Aceh and Bali at 0.393, and the lowest is in Banda Aceh and Aceh Besar at 0. The two populations of *P. argentilineatus* originating from the northern coast of Aceh were in a cluster due to their close genetic distance value. The genetic distance values within the populations of Aceh Besar, Banda Aceh, Bantu, Bali, Kupang, and Lombok had low genetic distances. Genetic

distances between populations (interspecies) and within populations of the same species (intraspecies) were obtained based on the results of the MEGA X software (Table 4).

Table 2. Comparison of mudskipper identification based on morphological data and DNA barcoding data

Location/ population	Total sample	Species		Similarity %	
		Morphology	DNA barcode	BLAST	BOLD
1 (Aceh Besar)	10	*Periophthalmus argentilineatus*	*Periophthalmus argentilineatus*	100	100
2 (Banda Aceh)	10	*Periophthalmus malaccensis*	*Periophthalmus argentilineatus*	100	100

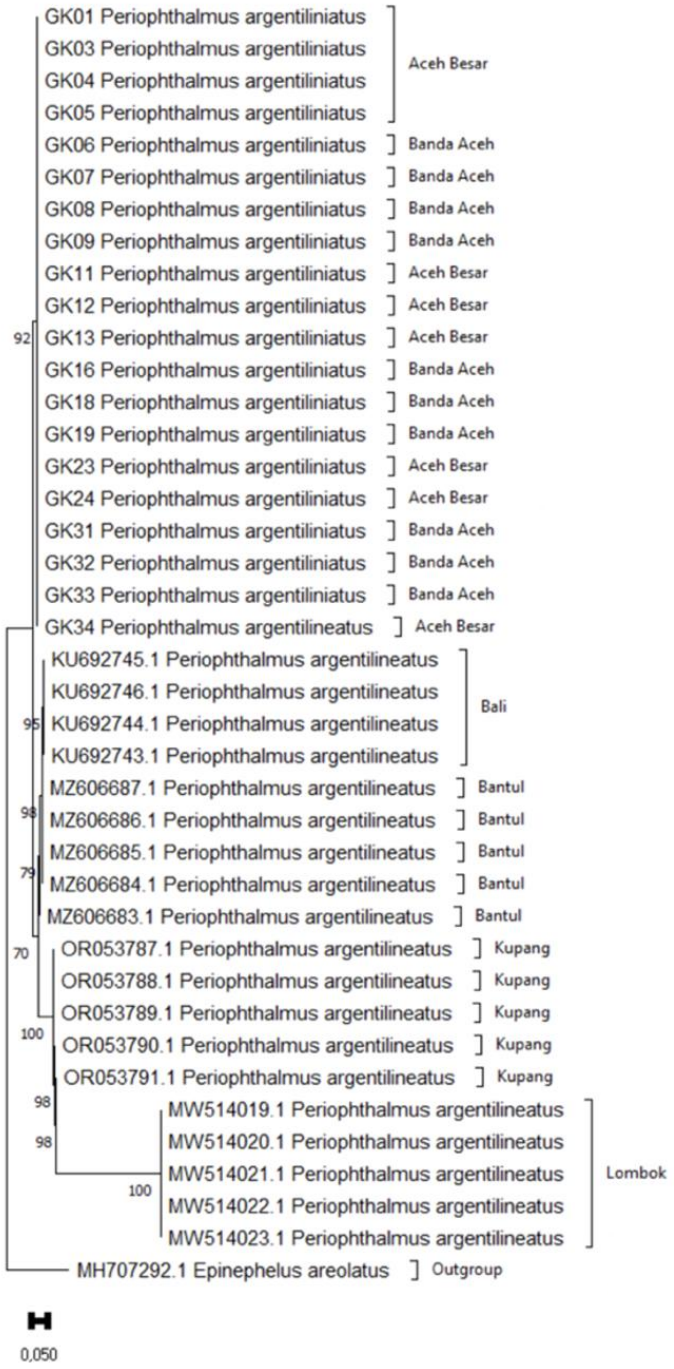

Figure 2. A phylogenetic tree using the Neighbor-Joining (NJ) method, K2P distance with 1000 bootstrap in the MEGA software

Genetic variation and genetic differentiation

Genetic variation within a population can be evaluated using nucleotide diversity (π) and haplotype diversity (Hd). Higher values of π and Hd indicate greater genetic variability, while lower values reflect reduced diversity. In this study, the overall mean values of Hd and π in the Banda Aceh and Aceh Besar populations were low, indicating the absence of detectable genetic variation. The highest π was recorded in the Bantul and Bali populations (0.01), which is classified as low nucleotide diversity. Meanwhile, the highest Hd values were observed in Bantul (0.40) and Bali (0.50), both categorized as moderate haplotype diversity. Table 5 presents these values across six populations.

Genetic differentiation among *P. argentilineatus* populations was assessed using the Fst index, analyzed with an AMOVA, which measures how much genetic variation is shared among populations. Low Fst values indicate little genetic differentiation, whereas higher values suggest greater population structuring. The very high Fst value (0.98) with variation 98.29% between population indicates that populations are almost completely genetically distinct from each other, and 1.71% present within population.

Population connectivity

Connectivity between populations along the coasts of Banda Aceh, Aceh Besar, Bantul, Bali, Kupang, and Lombok was analyzed using sequence data to examine data patterns from the haplotype network and haplotype distribution map. Haplotype distribution of 39 sequenced samples of *P. argentilineatus* mudskipper yielded 7 haplotypes from 6 populations. Haplotype 1 originated from populations on the northern coasts of Banda Aceh and Aceh Besar, haplotype 2 from the Bali population, haplotype 3 from Lombok, haplotypes 4 to 6 from Bantul, and haplotype 7 from the Kupang population (Table 6). Furthermore, Figures 3 and 4 present the haplotype network and distribution map of *P. argentilineatus*, with each circle representing a population, and circle size proportional to sample size.

Discussion

This study recorded 20 mudskipper fish belonging to *P. argentilineatus* using the COI gene sequences yielded a high similarity to reference sequences 100%. According to Triandiza and Madduppa (2018), the most similar GenBank sequences had the same maximum and total scores, namely, query coverage approaching 100%. Sequence similarity comparisons using the National Center for Biotechnology Information (NCBI) BLAST and the Barcode of Life Data System (BOLD) yielded comparable percentage identities. The use of these two complementary tools as intended to improve the accuracy of species identification (Bolaji et al. 2023). Comparison of identifications carried out morphologically and molecularly showed species differences. The results of morphological identification showed that 10 individuals were the species *P. malaccensis*. However, the results of the sequence in molecular analysis suggested that the 10 individuals were *P. argentilineatus*. This was due to the similarities between the 2 species, namely in terms of color, size, eye shape, and snout shape. Baderan et al. (2023) reported that there were morphological similarities between *P. malaccensis* and *P. argentilineatus*, namely a single row of teeth in the upper jaw, a white spot proximally, and a dark median line (mesially). Furthermore, discrepancies between morphological and molecular identification arise because external traits are often influenced by environmental or phenotypic variation, which may lead to misidentification. In contrast, genetic analysis is more stable and can distinguish species that appear morphologically similar (cryptic species) (Wang et al. 2020).

Table 3. Frequency distribution data of the COI sequence nucleotides of mudskipper *Periophthalmus argentilineatus* samples

Nucleotide base	Average	Minimum	Maximum
T %	30.98	29.10	32.39
C %	26.67	26.60	26.91
A %	25.27	23.94	27.54
G %	17.07	16.43	17.21
AT %	56.25	56.02	56.65
GC %	43.74	43.34	43.97
GC % Codon Pos 1	57.11	56.80	57.27
GC % Codon Pos 2	43.66	43.66	43.66
GC % Codon Pos 3	30.45	29.57	31.45

Table 4. Genetic distance between population/interspecies and within the population/intraspecies (bold**)** of Mudskipper (*Periophthalmus argentilineatus*) in the mitochondrial COI gene

Location	Aceh Besar	Banda Aceh	Bantul	Bali	Kupang	Lombok
Aceh Besar	**0**	0	0.043	0.393	0.030	0.072
Banda Aceh	0	**0**	0.043	0.393	0.030	0.072
Bantul	0.043	0.043	**0**	0.375	0.011	0.056
Bali	0.393	0.393	0.375	**0**	0.370	0.298
Kupang	0.030	0.030	0.011	0.370	**0.001**	0.054
Lombok	0.072	0.072	0.056	0.298	0.054	**0**

Table 5. Haplotype diversity (Hd) and nucleotide diversity (π) of mudskipper fish *Periophthalmus argentilineatus*

Species	Population	n	Hn	Hd	π
Periophthalmus argentilineatus	Aceh Besar	10	1	0.00	0.00
Periophthalmus argentilineatus	Banda Aceh	10	1	0.00	0.00
Periophthalmus argentilineatus	Bantul	5	2	0.40	0.01
Periophthalmus argentilineatus	Bali	4	2	0.50	0.01
Periophthalmus argentilineatus	Kupang	5	1	0.00	0.00
Periophthalmus argentilineatus	Lombok	5	1	0.00	0.00

Notes: n: Number of Samples, Hn: Number of Haplotypes, Hd: Haplotype Diversity, π: Nucleotide Diversity

Table 6. Haplotype distribution on COI of *Periophthalmus argentilineatus*

Haplotype	Banda Aceh	Aceh Besar	Lombok	Kupang	Bali	Bantul	Total
1	10	10					20
2					4		4
3			5				5
4						1	1
5						3	3
6						1	1
7				5			5

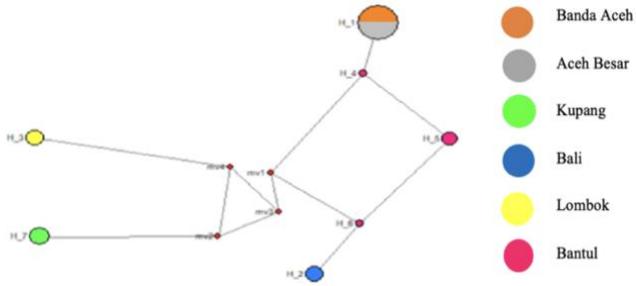

Figure 3. Haplotype network

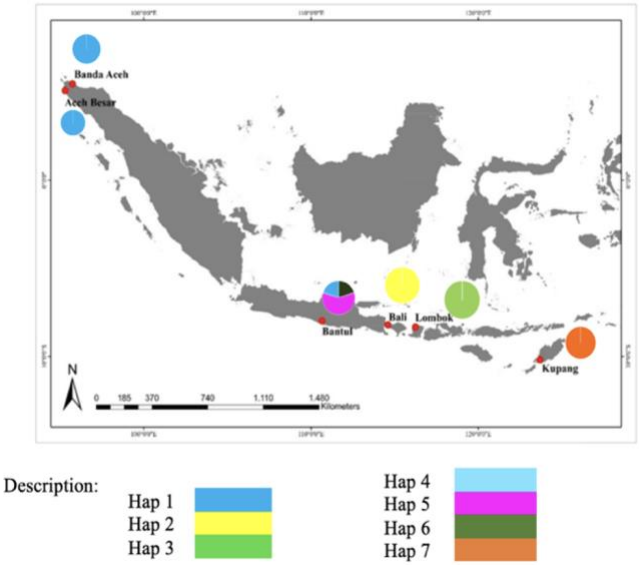

Figure 4. Haplotype distribution map of *Periophthalmus argentilineatus* mudskipper populations

Sequence analysis showed that the nucleotide base length of *P. argentilineatus* was 639 bp (base pair). AT had a higher average value of 56.25% compared to GC, which exhibited an average value of 43.74% and decreased at each post. High AT nucleotide content showed that the mitochondrial genome tended to be rich in AT. Aji and Arisuryanti's (2021) study on several mudskipper species, including *P. argentilineatus*, showed that AT values were higher than GC, with the AT range ranging from 42.14% to 42.66%. Li et al. (2016) reported that GC content generally decreased in the third codon position. Low Guanine (G) and Cytosine (C) content could cause DNA denaturation to occur more easily. (Assal and Lin 2021). DNA repair genes influenced the number of mutations that caused an increase in AT, ensuring that base mutations tended to lead to AT nucleotides (Li et al. 2016). Furthermore, mutations that occurred in mtDNA caused high genetic variation (Allio et al. 2017). According to Ramadhaniaty et al. (2018), when the AT nucleotide base composition was higher than GC, then the species had many similarities due to independent parallel substitutions. Furthermore, Arisuryanti et al. (2024a) identified a high AT nucleotide content in this study, which was a common characteristic in fully sequenced mtDNA.

Phylogenetic analysis showed the evolutionary origins of species that could be arranged into a phylogenetic tree based on the alignment of protein or gene sequences by assuming proximity. This was based on the total nucleotide length of the sample between homologous sequences (Temereva et al. 2015) to show phylogenetic relationships between species (Schreiber et al. 2013). Phylogenetic relationships between species *P. argentilineatus* were observed through geographic distance between sequences. Geographic distance between populations affected low gene flow, which was considered sporadic (Cheng et al. 2020; Oh and Oh-un 2022). Furthermore, phylogenetic analysis provides insights into the evolutionary relationships among *P. argentilineatus* populations, which are critical for

conservation planning. Recognizing these evolutionary lineages ensures that conservation strategies not only preserve current genetic variation but also the presence of the species in the long term. Bootstrap values indicate the stability and reliability of branching patterns in a phylogenetic tree. Moderate to high bootstrap scores reflect well-supported clades and stable tree topology, whereas low values indicate weak support and potential instability (Saleky et al. 2020). Saleky and Dailami (2021) stated that a high bootstrap value resulted in a better phylogenetic tree. In general, bootstrap values below 70% suggest that the corresponding clades may change under different analytical conditions (Rebman and Simpson 2006). As stated by Soltis and Douglas (2003) the numbers above each branch represent bootstrap support, with values approaching 100% indicating very strong confidence in the inferred evolutionary relationships.

Analysis of kinship relationships between species could be seen from the genetic distance between each individual and population (Basith et al. 2021; Xin et al. 2024). The two populations *of P. argentilineatus* originating from the northern coast of Aceh were in a cluster due to their close genetic distance value. The *P. argentilineatus* populations within the same clade exhibited relatively close genetic distance values. Low genetic distances between Aceh populations suggest limited divergence, which may reflect ongoing or recent gene flow, or a historical bottleneck leading to genetic homogenization. In contrast, higher genetic distances observed between Aceh and Bali populations indicate greater differentiation, consistent with historical isolation and restricted gene flow across regions. Hakim et al. (2022) stated that high and low genetic distance values could influence the level of kinship. A high genetic distance value showed a more distant relationship between populations due to a high heterosis effect when crossed. Meanwhile, a low genetic distance value showed a closer relationship and smaller genetic variation between populations, suggesting that the similarities between the 2 populations were greater (Hefzi et al. 2023).

Genetic diversity, defined as variation among individuals within a population, reflects the extent of gene flow within and between populations (Akbar et al. 2014; Kusuma et al. 2016). It can be assessed using haplotype diversity (Hd) and nucleotide diversity (π) (Li et al. 2013). In this study, Aceh Besar and Banda Aceh populations of *P. argentilineatus* showed low Hd and π, indicating limited genetic variation, likely due to shared lineage, reduced connectivity, and mangrove habitat degradation. In contrast, Bali and Bantul populations exhibited moderate Hd and low π, which may reflect larger population sizes and greater connectivity via ocean currents. Similar results were reported by Arisuryanti et al. (2024a), where high haplotype diversity ($Hd \geq 0.5$) and low nucleotide diversity ($\pi \leq 0.01$) were observed in each clade, the high Hd in mudskipper fishes at their study site indicates high genetic variation. Low nucleotide diversity combined with high haplotype diversity often indicates large population sizes, recent expansion, and the presence of unique genetic variants (Binashikhbubkr et al. 2024). The genetic homogeneity observed in Aceh populations reduces adaptive capacity and increases vulnerability to environmental pressures, such as habitat loss and water quality changes (Wellband et al. 2021). Conversely, genetic heterogeneity, reflected in diverse haplotypes, enhances resilience in dynamic mangrove ecosystems. The genetic patterns in Aceh differ from those reported for mudskipper populations in Java and Sulawesi, which generally show high haplotype diversity but low nucleotide diversity, suggesting past population bottlenecks followed by expansion (Polgar et al. 2014; Arisuryanti et al. 2024b). In Aceh, low diversity without signs of expansion indicates that habitat degradation rather than natural oceanographic barriers is the main driver of reduced genetic variation, emphasizing the need for habitat protection and connectivity restoration to maintain population resilience.

Anthropogenic pressures on the northern coast of Aceh are likely contributing to the reduced genetic variation observed. Extensive mangrove conversion for aquaculture, coastal settlement expansion (Natsir et al. 2022), charcoal and firewood (Dewiyanti et al. 2023) have driven substantial habitat loss and fragmentation along the Aceh coastline. Since *P. argentilineatus* relies on continuous mangrove channels for spawning and juvenile development, this fragmentation restricts movement among subpopulations and limits gene flow. The zero haplotype and nucleotide diversity in Aceh Besar and Banda Aceh populations, therefore, may reflect a combination of historical oceanographic isolation and recent degradation of mangrove habitats. Conservation strategies should prioritize maintaining genetic heterogeneity and habitat connectivity to ensure gene flow and prevent population fragmentation. According to Wright's (1978) classification, high Fst values suggest strong population structure, often resulting from historical isolation and limited gene flow, though extremely high values may also arise from low within-population variation or small sample sizes (Hedrick 2005). Overall, genetic diversity is a key indicator of a population's capacity to adapt to environmental changes and long-term resilience (Zizka et al. 2020; Hohenlohe et al. 2021).

Luo et al. (2015) stated that species positioned closely on a phylogenetic tree are often geographically close. This pattern was evident in haplotype 1, which occurred in both Banda Aceh and Aceh Besar, two populations located along the northern coast of Aceh with similar hydrological characteristics, including tidal and current regimes. Ocean current direction affects water circulation, which can influence biological variation and species distribution (Chan et al. 2022). Moreover, ocean circulation in the western Strait of Malacca is dominated by a long shore current with weak exchange from the south, limiting connectivity with other regions (Rizal et al. 2012). Such oceanographic barriers are known to constrain larval dispersal and promote population divergence in coastal fishes (Chan et al. 2022). Haplotype network analysis also showed that, aside from haplotype 1, the remaining haplotypes were population-specific, occurring only in a single location. Population-specific haplotypes suggest limited or absent gene flow and indicate partial or complete genetic isolation. In contrast, the shared haplotype 1 between Banda Aceh and Aceh Besar likely reflects mixing

driven by geographic proximity. Haplotypes are essential for tracing population origins, identifying genes linked to particular traits, and understanding genetic polymorphism (Leitwein et al. 2020; Shipilina et al. 2023).

In conclusion, this study confirmed that all mudskipper samples collected from the mangrove ecosystems of Banda Aceh and Aceh Besar belong to *P. argentilineatus*, supported by 100% similarity in mitochondrial COI gene sequences based on BLAST and BOLD databases. Genetic diversity analysis revealed no variation between the Banda Aceh and Aceh Besar populations, indicating high genetic homogeneity likely shaped by similar environmental conditions and shared lineage. Low genetic diversity in Aceh populations highlights the need for conservation actions focused on protecting and restoring mangrove habitats to maintain gene flow and population stability. However, this study is limited by the small sample size, unequal sampling between regions, and reliance on a single mitochondrial marker, which may not fully capture genome wide variation. Future studies should include larger sample sizes, whole genome analyses, and expanded geographic coverage to capture seasonal and environmental influences on genetic structure. Integrating molecular findings with ecological monitoring will enhance management strategies for sustaining *P. argentilineatus* populations and mangrove ecosystem resilience. Comparative studies across additional Indonesian regions are also needed to establish a broader genetic baseline for *P. argentilineatus* and to support more effective conservation and management strategies.

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