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Phylogenetic Analysis of Groupers at Labuhan Lombok Based on CO1 Gene

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Abstract: Groupers (family Serranidae) are high-value reef fish that are often prone to misidentification due to their morphological similarities. This research examined the phylogenetic relationships of grouper species landed at the Pondok Sampan Fish Landing Site in Labuhan Lombok using the Cytochrome Oxidase Subunit I (CO1) gene. One specific sample (K5) was analyzed to verify its taxonomic status. DNA isolation was performed through the CTAB technique, followed by PCR amplification and sequencing. Morphological identification initially suggested the sample belonged to the genus *Epinephelus*. However, molecular results showed that K5 is *Aethaloperca rogaa* (100% similarity). The phylogenetic analysis was conducted using the Neighbor-Joining approach with 10,000 bootstrap iterations and the Kimura 2-Parameter model in MEGA XI, which placed the specimen within a monophyletic group alongside A. rogaa sequences from Egypt, showing a genetic distance of 0.00. The nucleotide composition (T: 29.8%, C: 27.3%, A: 25.5%, G: 17.4%) was identical to the reference sequence, confirming the anti-G bias typical of teleost mitochondrial DNA. This research illustrates the critical importance of DNA barcoding to correct morphology-based misclassifications. It emphasizes the need for genetic confirmation in fisheries diversity evaluations and in the sustainable management of grouper populations across the Indo-Pacific.

Keywords: *Aethaloperca rogaa*; DNA Barcoding; CO1; Labuhan Lombok; Phylogenetics.

Introduction

Indonesia is recognized as a global hub of grouper species richness, harboring approximately 40–45 species in the family Serranidae (Mujiyanto & Syam, 2015) and distributed across major fishing zones in the Indo-Pacific. Groupers, belonging to the subfamily Epinephelinae, occupy critical ecological niches in coral reef ecosystems as apex predators, and simultaneously represent a high-value commodity in the national and international fish trade. (Pinheiro *et al*., 2016). The genus *Epinephelus* dominates Indonesian waters; however, other genera, including *Plectropomus, Variola, Cephalopholis, Cromileptes,* and *Aethaloperca,* also play important ecological roles (Ayu *et al*., 2024). High exploitation levels, habitat degradation from overfishing, and inadequate species verification mechanisms pose significant threats to the sustainability of grouper populations (Agustina *et al*., 2018).

Traditional morphology-based identification is often unreliable for groupers due to high phenotypic variation and the presence of morphologically cryptic species—taxa that share similar external features but belong to distinct genetic lineages (Pham *et al*., 2024). In international trade, cases of species mislabeling and substitution are common, where lower-value fish are fraudulently sold as premium groupers (Putri & Madduppa, 2020). Misidentification at the landing-site level, such as at the Pondok

Sampan Fish landing Site in Labuhan Lombok, can lead to erroneous biodiversity data and flawed fisheries management decisions.

The importance of this study lies in its contribution to enhancing the species diversity database of fish found in the Labuhan Lombok region within genetic repositories (Rahayu & Jannah, 2019). By using the *Cytochrome c Oxidase subunit I* (CO1) gene as a standardized genetic marker, this research provides high-resolution discrimination of morphologically cryptic species (Ward *et al.*, 2005). This accuracy ensures the validity of biodiversity data, which is crucial for future fisheries management and the protection of local germplasm (Kamal *et al.*, 2019). Understanding phylogenetic relationships among grouper species (*Epinephelus* sp.) is therefore essential to determine their degree of genetic relatedness (Rodemann *et al*., 2023). This study aims to analyze the phylogenetic relationships of grouper (*Epinephelus* sp.) landed at the Pondok Sampan Fish Landing Site in Labuhan Lombok, based on CO1 gene sequences.

Materials and Methods

Study Site and Sampling

This study employed a descriptive, exploratory research design using a case study approach, focusing on grouper specimens (K5) obtained from fishermen, with random sampling conducted at the Pondok Sampan Fish Landing in Labuhan Lombok (**Figure 1**). The specimen was selected through purposive sampling based on morphological ambiguity during initial field identification. A sample collection was conducted between September and October 2025 at the Pondok Sampan Fish Landing Site in Labuhan Lombok, Indonesia. Sample K5 was initially identified morphologically as *Epinephelus* sp. based on field observations. A sample collection was conducted between September and October 2025 at the Pondok Sampan Fish Landing Site in Labuhan Lombok, Indonesia. Sample K5 was initially identified morphologically as *Epinephelus* sp. based on field observations.

Morphological Identification

Initial morphological identification was conducted by examining external features, including body profile, coloration patterns, fin ray counts (dorsal spines and soft rays), caudal fin shape, and head profile. Characters were compared with reference descriptions from FishBase (www.fishbase.se) and literature on Serranidae taxonomy (Tawari *et al*., 2024). Sample K5 initially appeared to be consistent with the genus Epinephelus due to its brownish coloration and body shape; however, closer examination revealed a deeper body profile and distinct white spot patterning, warranting molecular verification.

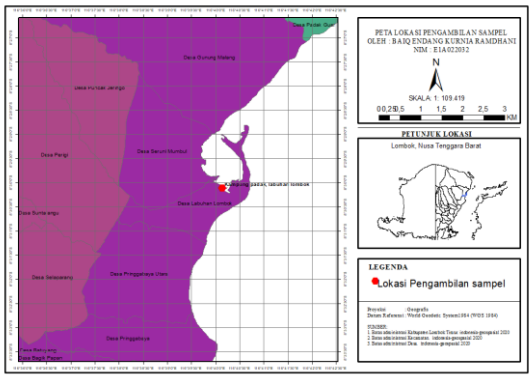

Figure 1. Map of the sampling location at Pondok Sampan Fish Landing Site, Labuhan Lombok, Indonesia (8°30'9.96"S; 116°40'9.74"E).

DNA Extraction and PCR Amplification

Genomic DNA was isolated from muscle samples using the adapted CTAB protocol. The CO1 gene was amplified using universal FishF1 and FishR1 primer sequences (Setyawati & Zubaidah, 2021). Tissue specimens were pulverized in liquid nitrogen to obtain fine particles, then transferred into 1.5 mL microcentrifuge tubes and treated with 1 mL of CTAB buffer solution (containing CTAB, PVP-40, NaCl, β-mercaptoethanol) for 10 minutes at 36°C with sonication. Following centrifugation at 13,000 rpm for 10 minutes, the resulting supernatant was subjected to chloroform-isoamyl alcohol (1:1) purification and isopropanol precipitation procedures. The resulting DNA precipitate was washed with 70% ethanol, air-dried, and reconstituted in 60 µL of sterile water.

DNA quantity and quality were evaluated using NanoDrop spectrophotometry (Karim *et al*., 2022). Amplification reactions were conducted in 10.0 µL reaction mixtures containing 5.0 µL GoTaq Green Master Mix (Promega), 0.5 µL of each primer (10 µM

concentration), 1.0 µL DNA template, and 3.0 µL sterile water. Thermocycling was executed on a Sensoquest thermal cycler using the following parameters: initial denaturation at 94°C (2 min); 35 repetitive cycles involving denaturation at 94°C (30 s), primer binding at 52°C (45 s), and elongation at 72°C (40 s); concluding extension at 72°C (10 min); followed by cooling to 10°C (Santosa *et al*., 2021). Amplification products were analyzed by 1% agarose gel electrophoresis at 90 V for 35 minutes, with ethidium bromide staining, using a 100 bp DNA ladder for size comparison (Anggreni *et al*., 2022). Samples producing sharp, single bands in the 650–700 bp range were submitted for Sanger sequencing at a DNA sequencing service in Jakarta (Persada *et al*., 2021).

Data Analysis

Sequence editing and alignment were conducted using ClustalW. Evolutionary tree construction was carried out employing the Neighbor-Joining approach with the Kimura 2-Parameter substitution model in MEGA XI software (Tamura *et al*., 2021).

Results and Discussion

Morphological Characteristics

Morphological examination of sample K5 revealed key diagnostic characters. The specimen exhibited a robust, brownish-black body with a slightly rounded caudal fin, a deeply arched dorsal profile, and a relatively straight to slightly convex head shape. Fin ray counts recorded were: XI dorsal spines, 17 soft dorsal rays, III anal spines, and 9 soft anal rays. Body depth was notably greater than that typically observed in *Epinephelus*, warranting molecular verification (**Figure 2**).

The morphological characters observed in sample K5 are consistent with *Aethaloperca rogaa* (the Redmouth Grouper), rather than *Epinephelus*, as documented in FishBase and corroborated by Tawari *et al*. (2024). The deeper body profile, relatively straight-to-slightly convex head shape, and distinct white spot patterning are diagnostic features consistent with the Redmouth Grouper. These findings highlight that morphological convergence between Aethaloperca and Epinephelus is a well-recognized source of misidentification in market surveys and landing-site assessments (Putri & Madduppa, 2020).

(a) **(b)**

Figure 2. Morphological comparison of the collected specimen; (a) Lateral view of sample K5; (b) Reference image of *Aethaloperca rogaa* from Fishider database for taxonomic comparison.

Molecular Identification

The molecular analysis provided a significant correction to the initial field identification. Gel electrophoresis results showed clear, specific DNA bands at approximately 650–700 base pairs (bp) for sample K5, consistent with the expected CO1 barcode fragment length for fish (Hebert *et al*., 2003; Abdullah *et al*., 2019). The amplified bands were highly intense and free of nonspecific contamination or primer-dimer artifacts, indicating high PCR specificity and optimal amplification efficiency (Halisah *et al*., 2024). The quality of the electrophoresis result confirmed suitability for downstream sequencing (Anggreni *et al*., 2022). Among all seven specimens, sample K5 showed the most

consistent and stable band intensity, justifying its selection for sequencing and phylogenetic analysis.

BLAST analysis of the K5 consensus sequence against the NCBI GenBank database yielded a 100% identity match with *Aethaloperca rogaa* (Accession: MH707290.1), obtained from an Egyptian specimen. This result fundamentally contradicted the initial morphological identification of sample K5 as *Epinephelus* sp. The 100% sequence similarity leaves no ambiguity regarding species assignment, as intraspecific CO1 divergence for fish typically falls below 2%, while interspecific divergence generally exceeds this threshold (Hebert *et al*., 2003). This result confirms the critical utility of DNA barcoding in overcoming the limitations of morphological identification, particularly for cryptic grouper species in the Indo-Pacific (Marfuah *et al*., 2021). The occurrence of *A. rogaa* in the waters of Labuhan Lombok is also supported by previous reports from Sulawesi Sea fisheries (Achmad *et al*., 2023).

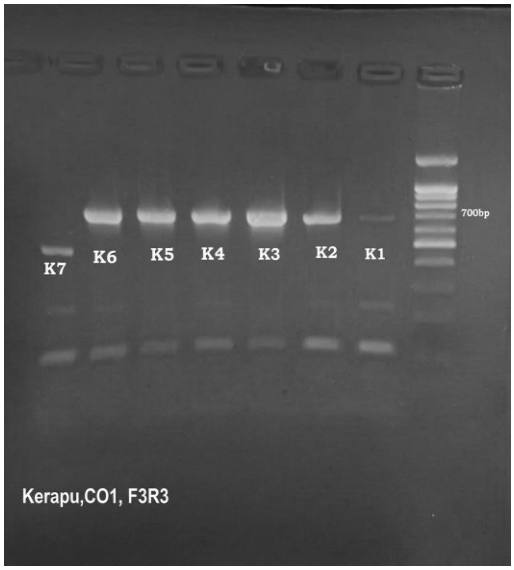

Figure 3. Agarose gel electrophoresis (1%) of the PCR-amplified CO1 gene. Lane M: 100 bp DNA ladder; Lane K5: Successfully amplified product at approximately 700 bp.

While sample K5 was visually identified as a member of the genus *Epinephelus*, the DNA barcoding results confirmed it as *Aethaloperca rogaa* with 100% similarity to the GenBank database (Accession: MH707290.1). The Neighbor-Joining phylogenetic tree constructed from 31 CO1 sequences (604 bp) demonstrates clear topological groupings consistent with established Serranidae taxonomy (Blackburne & Whelan, 2013). Sample K5 formed a strongly supported monophyletic cluster with *Aethaloperca rogaa* sequences from geographically distant populations, including isolate G_12 (PV998116.1) from Sri Lanka and Ez2_1 (MH707290.1) from Egypt, indicating high inter-population genetic similarity across the Indo-Pacific. The bootstrap support value for this clade was high, reflecting strong statistical confidence in the grouping (Tamura *et al*., 2021).

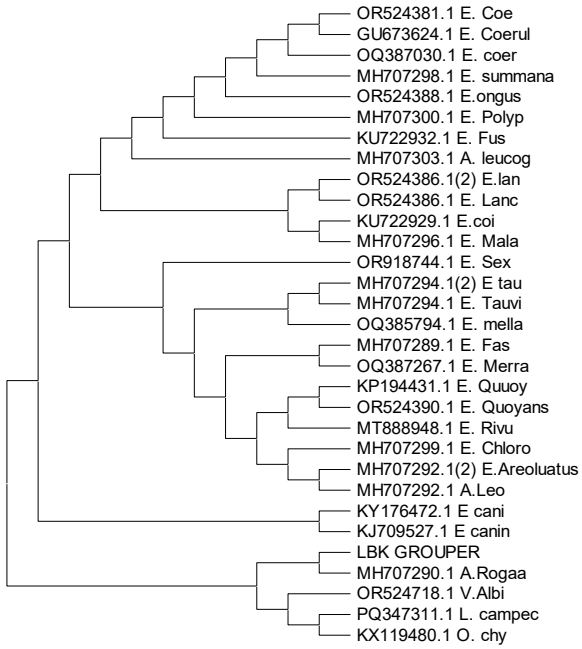

Figure 4. Evolutionary tree constructed using the Neighbor-Joining approach based on CO1 gene data with the Kimura 2-Parameter substitution model. Bootstrap support values from 10,000 iterations are displayed at branch points. Sample K5 is emphasized, demonstrating its monophyletic grouping with *Aethaloperca rogaa*.

The NJ tree also clearly separated the genus *Aethaloperca* from *Epinephelus*, *Variola*, and *Anyperodon* clades, consistent with phylogenomic findings of Sachithanandam *et al*. (2022), who validated *Aethaloperca* as a genetically distinct monophyletic group within Epinephelinae. The out-group taxa (*Lutjanus campechanus*, *Lates calcarifer*, *Ocyurus chrysurus*) were positioned on a basal branch with large genetic distances (0.20–0.24) from in-group members, appropriately anchoring the

phylogenetic reconstruction. These inter-generic distance values are within the range previously documented for marine fish phylogenetics (Darmawan *et al*., 2024; Fietri *et al*., 2021).

The genetic distance between K5 and *A. rogaa* reference sequences was 0.00 (K2P model), confirming conspecific status. Comparisons with *Epinephelus* species yielded intergeneric distances ranging from 0.16 to 0.19, well above the standard 2% species delineation threshold for CO1 barcoding (Hebert *et al*., 2003). This genetic differentiation at the genus level is consistent with the molecular divergence reported by Basith *et al*. (2021) for *Epinephelus* spp. from Madura Island and with the findings of Rueck *et al*. (2020) on the role of oceanographic barriers in shaping genetic differentiation among marine fish populations.

Nucleotide Composition and Phylogeny

The nucleotide composition of the K5 CO1 fragment (604 bp) was: T = 29.8%, C = 27.3%, A = 25.5%, and G = 17.4%, yielding a G+C content of 44.7%. This composition was identical across all positions to the *Aethaloperca rogaa* reference sequence (MH707290.1), providing further independent evidence of species-level identity (Altschul *et al*., 1990). The observed low guanine content relative to other bases reflects the well-documented anti-G bias in the CO1 gene of teleost fishes (Johns & Avise, 1998). In teleost mitochondrial DNA, the third codon position shows particularly elevated C content (~41.3%) and drastically reduced G (~5.5%), a pattern attributable to asymmetric mutational pressure during mtDNA replication (Peng *et al*., 2018). These thermodynamic properties of the G+C content (~44.7%) are consistent with the stability characteristics reported across Serranidae sequences analyzed in comparable studies (Santosa *et al*., 2021).

The average nucleotide composition across all 31 sequences analyzed was: T = 29.4%, C = 27.6%, A = 24.7%, G = 18.2%. The consistently low G content among all analyzed sequences, including those of *Epinephelus*, *Variola*, *Anyperodon*, and outgroups, supports the conclusion that anti-G bias is a conserved feature of CO1 across teleost fishes, validating the marker's utility for comparative analysis and barcoding (Otsuka & Matsui, 2023).

Conclusion

This study successfully analyzed the phylogenetic relationships of grouper (*Aethaloperca rogaa*) landed at the Pondok Sampan Fish Landing Site in Labuhan Lombok, based on CO1 gene sequences. The phylogenetic analysis revealed that the grouper specimens collected from Labuhan Lombok share a very close genetic relationship with *Aethaloperca rogaa* specimens recorded from Egypt, suggesting a high degree of genetic similarity across geographically distant populations. The use of CO1 markers proved to be an effective molecular tool for determining interspecific relationships within the family Serranidae, providing essential genetic data that supports the sustainable management and conservation of grouper species in Indonesia.

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