Jurnal Teknologi Perikanan dan Kelautan
Vol. 17 No. 1, February 2026: 126-138
ISSN: 2087-4871
EISSN: 2549-3841
DOI: https://doi.org/10.24319/jtpk.17.126-138

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INTEGRATIVE MORPHOLOGICAL AND DNA BARCODING CONFIRMATION OF THE DATA-DEFICIENT INDIAN HALIBUT IN CILACAP WATERS, INDONESIA

KONFIRMASI INTEGRATIF MORFOLOGI DAN DNA BARCODING IKAN SEBELAH YANG KEKURANGAN DATA DI PERAIRAN CILACAP, INDONESIA

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(Received December 22, 2025; Revised February 28, 2026; Accepted March 2, 2026)

ABSTRACT

The Indian halibut (*Psettodes erumei*) is classified as data deficient on the IUCN Red List. Accurate species identification is a fundamental prerequisite for sustainable fisheries management and biodiversity conservation. This study aims to identify the Indian halibut landed from Cilacap waters, Indonesia, using an integrative morphological and molecular approach. Four specimens collected in March and June 2025 were analyzed using an integrated morphological and molecular approach. Morphological identification was based on detailed morphometric and meristic analyses, cross-referenced with standard taxonomic guides. Molecular identification involved DNA extraction, PCR amplification of the mitochondrial Cytochrome C Oxidase Subunit I (COI) gene, electrophoresis, sequencing, and comparison with global genetic databases using BLAST. Morphological characteristics aligned with the description of *P. erumei*, though significant morphometric differences from a historical reference suggest potential local phenotypic variation. DNA barcoding confirmed the identity with 100% sequence similarity to conspecifics from other Indo-Pacific regions (GenBank accession: PX599017.1). Phylogenetic analysis further placed the Cilacap samples within a genetically homogeneous cluster of *P. erumei* from across Indonesian waters. This research successfully contributes to Indonesia's biodiversity database and DNA barcode library for a commercially exploited yet understudied species. It also provides essential baseline data to inform stock assessments and conservation strategies in the region.

Keywords: DNA barcoding, Indian halibut, species identification, fisheries management

ABSTRAK

Ikan sebelah (*Psettodes erumei*) merupakan spesies yang diklasifikasikan sebagai kekurangan data (*data deficient*) dalam IUCN Red List. Identifikasi spesies yang akurat merupakan prasyarat penting bagi pengelolaan perikanan yang berkelanjutan serta konservasi keanekaragaman hayati. Penelitian ini bertujuan untuk mengidentifikasi spesies ikan sebelah yang berasal dari perairan Cilacap, Indonesia, melalui pendekatan integratif morfologi dan molekuler. Empat spesimen dikumpulkan pada Maret dan Juni 2025 untuk dianalisis menggunakan pendekatan terpadu morfologi dan molekuler. Identifikasi morfologi didasarkan pada analisis morfometrik dan meristik terperinci, yang diverifikasi ulang dengan panduan taksonomi standar. Identifikasi molekuler melibatkan ekstraksi DNA, amplifikasi PCR gen Cytochrome C Oxidase Subunit I (COI) mitokondria, elektroforesis, sekuensing, dan perbandingan dengan basis data genetik global menggunakan BLAST. Karakteristik morfologi selaras dengan deskripsi *P. erumei*, meskipun perbedaan morfometrik yang signifikan dari referensi historis mengindikasikan potensi variasi fenotipik lokal. Barcoding DNA mengonfirmasi identitas dengan kemiripan sekuens 100% terhadap konspesifik dari wilayah Indo-Pasifik lainnya (aksesi GenBank: PX599017.1). Analisis filogenetik lebih lanjut menempatkan sampel dari Cilacap ke dalam kluster genetik yang homogen dari *P. erumei* di seluruh perairan Indonesia. Hasil penelitian ini memberikan kontribusi pada basis data keanekaragaman hayati dan pustaka *DNA barcoding* Indonesia untuk spesies yang dieksploitasi secara komersial namun kurang dipelajari. Penelitian ini juga menyediakan data dasar esensial untuk menginformasikan kajian stok dan strategi konservasi di wilayahnya.

Kata kunci: *DNA barcoding*, identifikasi spesies, ikan sebelah, pengelolaan perikanan

INTRODUCTION

The Indian halibut (flatfish) species occurring in Cilacap waters is suspected to be *Psettodes erumei* (Bloch 1801) and is utilized in the commercial fishery (Froese and Pauly 2024). The fish is easily caught by bottom-sweeping fishing gear, such as trawls, beach seines, bottom gillnets, and trammel nets, because these species inhabit habitats exposed to the operation of the fishing gear (1-1-100 m in depth) (Nelson 1994; Ryer 2008). In 2020, the annual production reached 700 kg and decreased in 2021 to only 260 kg. Its value increased to 450 kg for two consecutive years in 2022 and 2023 before it decreased again in 2024 to 240 kg (Cilacap Ocean Fishing Port 2025). The fluctuating production values indicated various fisheries-related issues with the resources.

Munroe (2014) stated that the Indian halibut resources in tropical waters, such as Cilacap waters, are under pressure from overfishing as well as exposure to rapidly increasing anthropogenic disturbances, habitat degradation, and climate change (Gama and Agustina 2022; Struebig *et al.* 2022). The management approach in Indonesia reveals other issues. The management of Indian halibut in Indonesia has not yet been carried out specifically at the local level. The determination of fishery management areas remains at the provincial and national levels (Regent Regulation of Tulang Bawang Regency Number 13 of 2012; Regent Regulation of Sleman Regency Number 44 of 2012). The maximum sustainable yield and total allowable catch (TAC) are also still estimated at the national level. Their values are not estimated per species but rather for broader fishery commodity groups (demersal fish) (Decree of the Minister of Marine Affairs and Fisheries Number 19 of 2022).

Non-specific management leads to inaccurate fisheries management decisions. In this case, the flatfish in Cilacap waters cannot be assessed in detail. Species-based quota management cannot be determined, as stakeholders do not identify the commodity that is landed from their waters. There are no published studies or scientific reports that explicitly confirm the species as *P. erumei.* Therefore, identification of the species is required to be conducted as the first step of fisheries management (Hutubessy and Mosse 2015; United Nations 2024).

There are two methods of fish identification for the study of flatfish in Cilacap waters, namely morphological and molecular identification (Santanumurti *et al.* 2024a). Morphological studies aim to identify the fish based on its morphology, morphometrics, and meristics, while molecular studies aim to record and evaluate its genetic information (e.g., DNA sequence). The combination of these studies can definitively identify the species, allowing for accurate fisheries management (Bone and Moore 2008). However, these studies do not just benefit fisheries management; they are also expected to contribute to biodiversity and conservation aspects.

Fish species identification, both morphologically and molecularly, can become a cornerstone of effective biodiversity conservation (Scribner *et al.* 2016). However, it remains fragmented in Indonesia, hindering conservation efforts (Hubert *et al.* 2015). The suspected *P. erumei* is one of many species classified as Data Deficient on the IUCN Red List due to unavailable population data. The lack of knowledge regarding the species is particularly critical (Munroe *et al.* 2020). Establishing comprehensive DNA barcode libraries is therefore vital, as genetic analysis allows researchers to trace evolutionary lineages, resolve ambiguous relationships, and accurately classify species (Gostel and Kress 2022; Brown 2024; Tan *et al.* 2025). Ultimately, these genetic insights can assess fisheries conditions by revealing molecular-level impacts from threats like overfishing or degradation, directly informing targeted conservation strategies (Ramzan *et al.* 2025). The study aims to identify the Indian halibut landed from Cilacap waters morphologically and molecularly, and evaluate the results for producing scientific insights related to its fisheries management and conservation.

METHODS

Time and location

All research was conducted in March–August 2025. The fish sample was from Penyu Bay, and its collection was conducted at the fish auction site in Cilacap Regency (Figure 1) in March 2025. A morphological study was conducted in the Research and Community Service Laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University (March–May 2025). Meanwhile, a molecular study was conducted in Bionesia Laboratory, Bali, Indonesia (June–August 2025).

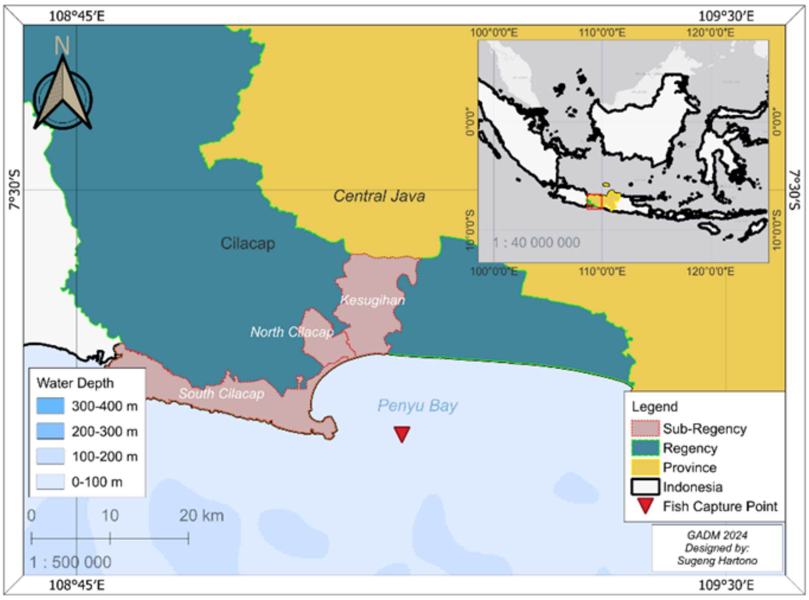

Figure 1. Location of fish sample collection in Cilacap waters, Central Java, Indonesia.

Data collection

Samples were transported to the laboratory in a cooler box for measurement, dissection, and morphological study. Fish dimensions were measured to record length (cm) and weight (g), with each measurement conducted using a measuring board (with 1 mm precision) and a digital scale (with 0.1 g precision), respectively. Moreover, samples were observed to determine their characteristics morphologically, morphometrically, and meristically (Yedier *et al.* 2023). Some tissues of fish organs were preserved in a container with ethanol 95% for molecular study (Pramono *et al.* 2019; Santanumurti *et al.* 2024b).

Morphological identification

Fish morphology was studied, tabulated, and documented according to its shape, colour, and general properties. Furthermore, some body parts were measured and counted for morphometry and meristic study (Santanumurti *et al.* 2024b). The results were guided and confirmed by using a fish identification book with the title "*The Living Marine Resources of the Western Central Pacific. FAO Species Identification Field Guide for Fishery Purposes*" (Carpenter and Niem 2001). A t-test was performed to compare groups of data in a Python environment.

Molecular identification

DNA testing was conducted once on a specimen labeled as UNSOED02.25.SH through several sequential steps, including (1) preparation of tissue samples at Jenderal Soedirman University (approximately 10 grams of tissue); (2) DNA extraction using the 10% Chelex protocol in BIONESIA Laboratory (Bali, Indonesia); (3) DNA amplification using the Polymerase Chain Reaction (PCR) technique by following BIONESIA laboratory protocol (annealing at 50°C for 30 seconds); (4) visualization of PCR results using 1% agarose gel electrophoresis stained with GelRed® Nucleic Acid Gel Stain; and (5) DNA sequencing at Genetika Science Company, Jakarta, using the Sanger dideoxy method (Ward *et al.* 2005; Apriliyanti *et al.* 2018; Patra *et al.* 2020; Baruna *et al.* 2024; Pramono *et al.* 2025). The target gene used in the study was the Cytochrome C Oxidase Subunit I (COI) gene. The primers used were FISH F1 (5'-TCA ACC AAC CAC AAA GAC ATT GGC AC-3') and FISH R1 (5'-TAG ACT TCT GGG TGG CCA AAG AAT CA-3') as described by Ward *et al.* (2005).

The sequence data were edited and aligned using the ClustalW method in MEGA XII software (Kumar *et al.* 2024). The sequence results were compared to genetic information available in the National Center for Biotechnology Information (NCBI) GenBank database using the Basic Local Alignment Search Tool (BLAST) on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi). The degree of similarity and data accuracy were recorded for each sequence (Landi *et al.* 2014).

Genetic data, such as nucleotide sequences, were analyzed to produce a phylogenetic tree. The aim was to review its genetic relationship with other samples and to evaluate the evolutionary relationship among nucleotide sequence data in the genetic database. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with 1,000 bootstrap replications in MEGA XII software (Kumar *et al.* 2024).

RESULTS AND DISCUSSION

Morphological study of flatfish in Cilacap waters

The results of morphological observations and other physical characteristics can be seen in Table 1. The results identified the flatfish caught in Cilacap waters as *Psettodes erumei*. The coloration, body part characteristics, eye position, and fin characteristics align with the features of *P. erumei* described in the identification guide and reference (Heemstra 1986; Carpenter and Niem 2001). Figure 2 also displayed the appearance of the fish sample collected from Cilacap waters.

The results of morphology observation on suspected *P. erumei* from Cilacap waters become the first study on this species in Indonesia. There are no other reports regarding this topic, even though its resources are actively exploited commercially (White *et al.* 2013). Some authors in Indonesia only discussed its biology (Tresnati and Tuwo 1997), vulnerability (Yonvitner *et al.* 2020), and stock assessment (Tresnati *et al.* 2024).

Studies from other localities, however, showed similar results compared to the current morphological study. Aung (2018) described the characteristics of *P. erumei* from Mon State waters as identical to the illustrations by Carpenter and Niem (2001). Khan *et al.* (2023) also stated that the species characteristics were aligned with the fish identification book (Carpenter and Niem 2001).

Morphological study was followed by morphometric and meristic studies. The results of the morphometric study were provided in Tables 2 and 3. Meanwhile, the meristics were displayed in Table 4. All results were compared to available previous studies from different localities. There is only one reference available for morphometric comparison, conducted by Nielsen (1984).

Table 1. Morphological characteristics of Psettodes erumei from Cilacap waters, Indonesia.

Characteristics	Observation
Colour	The eyed side of the body appeared brownish; the dorsal, anus, and posterior part of the caudal fin were darker; and the blind side of the body was pale grey.
Body parts	The body was oval-shaped, flat but fairly thick; the supra maxilla bone was well developed; the anterior parts of the dorsal and anal fins consisted of spines; the caudal peduncle was deeper than its long; the scales were small (ctenoid); the lateral line was present on both sides of the body, slightly curved above the pectoral fin; there were no broad, dark crossbars.
Eye position	Both eyes were on the right side of the head; the upper eye was on the dorsal surface of the head.
Fins	The origin of the dorsal fin was well posterior to the upper eye; the caudal fin was separated from the dorsal and anal fins; the caudal fin was free from the dorsal and anal fins, with a doubly truncated posterior margin (double truncated); dorsal, anal, and caudal fin tips were black.

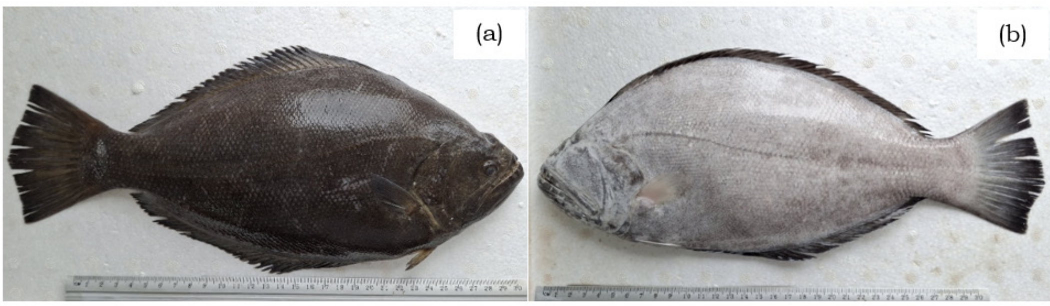

Figure 2. External morphology of the fish sample (*Psettodes erumei*) collected from Cilacap waters, Indonesia: (a) eyed side and (b) blind side.

Table 2. Morphometric measurements of *Psettodes erumei* specimens collected from Cilacap waters, Indonesia.

Characteristics	Sample 1	Sample 2	Sample 3	Sample 4	Nielsen (1984)
Total weight (g)	660	650	748	714	-
Gutted weight (g)	617	612	682	660	-
Total length (cm)	34.5	34.5	35.7	35.9	21.39
Standard length (cm)	29	30.3	31.1	31.4	18.57
Fork length (cm)	34.5	34.5	35.7	35.9	21.39
Pre-orbital length (cm)	1.9	1.8	2.0	2.0	1.03
Pre-dorsal fin length (cm)	4.5	4.5	6.2	5.2	5.9
Pre-pectoral fin length (cm)	7.0	7.0	7.6	7.2	4.45
Pre-pelvic fin length (cm)	5.5	6.5	6.1	5.8	3.47
Pre-anal length (cm)	8.5	7.8	11.1	10.3	5.58
Head length (cm)	5.5	5.4	5.8	5.6	4.77
Head width (cm)	2.0	2.0	1.9	2.6	-
Head depth (cm)	5.5	6	6.2	6.2	-
Eye diameter (cm)	0.9	0.8	0.9	0.9	0.71
Snout length (cm)	3.0	3.0	3.4	2.8	-
Distance between eyes (cm)	1.2	1.2	1.3	1.2	-
Body depth (cm)	13.4	13.3	14.2	13.5	8.21
Body width (cm)	2.9	2.9	3.0	2.9	-
Longest dorsal fin spine length (cm)	2.9	2.8	3.2	2.9	-
Dorsal fin length (cm)	22.5	22.8	23.4	24	-
Dorsal fin base length (cm)	18.6	18.8	22.4	23.2	-
Pectoral fin length (cm)	4.2	4.0	4.3	4.2	-
Pelvic fin length (cm)	2.6	2.6	2.6	2.6	-
Anal fin base length (cm)	16.7	17	16.4	15.9	-
Caudal peduncle length (cm)	2.1	2.0	2.2	2.2	-
Caudal peduncle height (cm)	3.84	3.8	4.1	4.0	-
Caudal fin length (cm)	6.0	5.9	6.2	6.2	-
Caudal fin height (cm)	9.5	9.2	9.6	9.5	-
Top caudal fin length (cm)	5.2	4.8	5.7	5.4	-
Bottom caudal fin length (cm)	5.5	5.0	5.9	5.6	-

Eleven of the morphometric traits in Table 3 were used to analyze the results statistically. Six traits showed a significant difference from the reference. The first four traits with p–value < 0.001 were pre-pectoral fin length, pre-orbital length, head length, and body depth. The other two were pre-pelvic fin length and pre-anal length, with p–value < 0.05. Meanwhile, two traits showed insignificant differences, namely eye diameter and pre-dorsal fin length, with p–value > 0.05.

The morphological differences between contemporary specimens and the historical reference are substantial and statistically significant. While sample size limitations constrain definitive conclusions, the patterns suggest meaningful biological divergence that warrants further investigation through integrated morphological, genetic, and ecological approaches. These specimens also likely represent a distinct morphological variant of *P. erumei*, possibly reflecting population-level adaptation or environmental responses (Gwilliam *et al.* 2018). On the other hand, meristic results showed otherwise.

The current study on *P. erumei* from Indonesia revealed several meristic characteristics that are largely consistent with previous regional studies. Current samples exhibited dorsal fin spines (IX-X) and anal fin spines (I) that fall within the ranges reported by Nielsen (1984) for the Western Indian Ocean sample (IX-XI) and all other studies. Dorsal fin ray counts (40–44) align closely with the ranges from Nielsen (38–45) and Heemstra (1986) for Mozambique (38–45), as well as Shen (1993) for Taiwan (38–45), but are notably lower than the counts reported by Amaoka (1971) for the South China Sea (52–56) and Liew (1986) for Australia (49–56).

Anal fin rays (37–38) fall within the broader ranges of most studies (33–44) and are similar to those of Amaoka (37–43) and Shen (37–43). Pectoral fin rays (14–16) match exactly with Heemstra (14–16) and Shen (14–16), and overlap with Nielsen (13–16), but are relevant to Amaoka's single value of 14. Pelvic fin spines (I) and rays (5) agree with Nielsen's reports where available. Several characteristics, including vertebrae, lateral line scales, and scales around the caudal peduncle, were not recorded in our study but show interesting variation across regions in other studies, with lateral line scales ranging from 61 to 77 and caudal peduncle scales from 32 to 38.

The current study produced a lower value of dorsal fin rays than others, which may indicate regional subpopulations or environmental factors. As an organism that is widely distributed in tropical and subtropical waters (Coulson and Poad 2021), *P. erumei* has created a large population group. However, a distinct group in a specific region, such as Indonesian waters, shares specific characteristics and has limited exchange (genetic or demographic) with other groups. This group might have a genotype or phenotype difference due to local adaptation, genetic drift, or natural selection. According to Cadrin *et al.* (2014), the differences in dorsal and anal ray counts were also observed on winter flounder from Georges Bank, north of Cape Cod, and south of Cape Cod. The distinction can be explained by another factor, such as the aquatic environment.

Variations in meristic traits can occur when early development takes place in different locations or under differing environmental conditions. These countable characteristics are influenced by a combination of genetic factors and the environment experienced during the egg and larval stages (Swain *et al.* 2005). Factors such as temperature, salinity, dissolved oxygen and carbon dioxide levels, and light exposure have been shown to affect meristic count traits (Lindsey 1988). According to Jordan's rule, the number of vertebrae, fin rays, and scales generally increases as water temperature decreases. In addition to environmental conditions, several other factors in fish development and ecology may also affect countable traits. These include the fish's body size, evolutionary lineage, overall shape, and style of swimming (McDowall 2008).

The meristic analysis reveals that certain traits, such as the anal spines and pelvic rays, remain invariant, indicating a strong degree of evolutionary conservation. As an evolutionarily primitive species, no major divergence in its physical characteristics was detected. Consequently, these findings do not support the need for a taxonomic revision. The meristic profile of *P. erumei* demonstrates stability in fundamental traits while also exhibiting notable geographical variations in others. This pattern suggests a species that retains a conserved core morphology while displaying evidence of regional adaptation. However, the study still strongly suggests increasing or maintaining the quality of the aquatic environment for the sustainability of these fisheries resources.

Table 3. Comparison of selected morphometric ratios of *Psettodes erumei* between the present study and Nielsen (1984).

Characteristics	Sample 1	Sample 2	Sample 3	Sample 4	Nielsen (1984)
Total length (TL) (cm)	34.5	34.5	35.7	35.9	21.39
Standard length in TL	84.06%	87.83%	87.11%	87.47%	86.82%
Fork length in TL	100%	100%	100%	100%	100%
Pre-orbital length in HL**	35%	33%	34%	36%	22%
Pre-dorsal fin length in TLNS	13%	13%	17%	14%	17%
Pre-fin length in TL**	20%	20%	21%	20%	13%
Pre-pelvic fin length in TL*	16%	19%	17%	16%	10%
Pre-anal length in TL*	25%	23%	31%	29%	16%
Head length in TL**	16%	16%	16%	16%	14%
Eye diameter in HLNS	16%	15%	16%	16%	15%
Body depth in TL**	39%	39%	40%	38%	24%

Note: **$p < 0.001$, *$p < 0.05$, NS = Not Significant

Table 4. Comparison of meristic characters of *Psettodes erumei* from the present study and previous studies.

Characteristics	Current Study (2025) in Indonesia (n=4)	Amaoka (1971) in the South China Sea	Nielsen (1984) in the Western Indian Ocean	Heemstra (1986) in Mozambique	Liew (1986) in Australia	Shen (1993) in Taiwan
Vertebrae	-	24	23–25	24	24	23–24
Dorsal fin spines	IX–X	-	IX–XI	IX–XI	-	IX–XI
Dorsal fin rays	40–44	52–56	38–45	38–45	49–56	38–45
Caudal fin rays	-	-	-	-	-	-
Anal fin spines	I	-	I	I	-	I
Anal fin rays	37–38	37–43	33–43	33–42	34–44	37–43
Pectoral fin spines	-	-	-	-	-	-
Pectoral fin rays	14–16	14	13–16	14-16	-	14–16
Pelvic fin spines	I	-	I	-	-	-
Pelvic fin rays	5	-	5	-	-	-
Lateral line scales	-	65–72	61–77	68–75	-	-
Scales around the caudal peduncle	-	-	33–38	32–38	-	-

Molecular study of Indian halibut in Cilacap waters

Results from agarose gel electrophoresis confirmed the successful amplification of the target DNA fragment, producing a single, prominent band at approximately 655 base pairs (bp) (Figure 3) in the experimental sample lane (1PE). The positive control lane yielded an identical band, validating the efficacy of the PCR reagents and thermal cycling conditions. Crucially, the negative control lane showed no detectable amplification, confirming the absence of DNA contamination in the reaction components. These results also allowed the subsequent analysis, namely, DNA sequencing.

The sequencing results were provided in Table 5. The results could provide the molecular information used for identification, classification, and evolutionary analysis. In the current study, it was compared against a public database to find matching sequences and identify the organism.

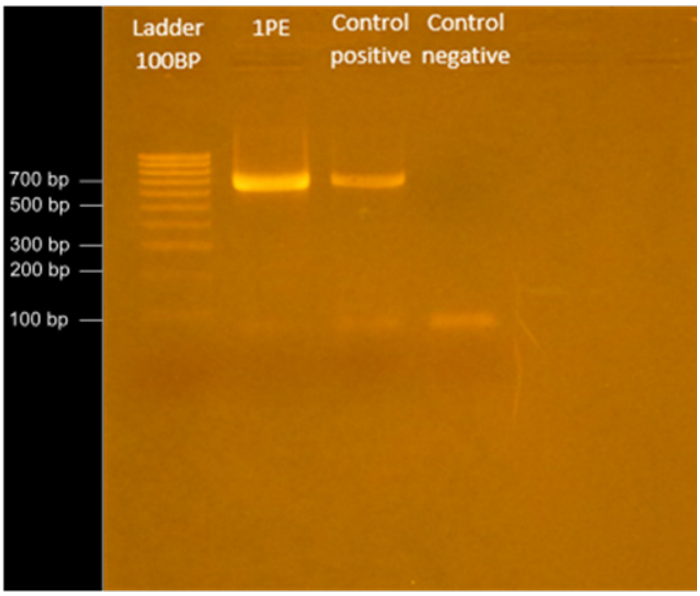

Figure 3. Agarose gel electrophoresis of PCR-amplified DNA of *Psettodes erumei* from Cilacap waters, Indonesia.

Table 5. Sequence results from the current molecular study on the fish sample, *Psettodes erumei*.

Specimen Voucher	Sequence Results
UNSOED02.25.SH	CCTTTATCTAGTATTTGGTGCTTGAGCCGGCATAGTAGGCACAGCCCT-GAGCCTGCTAATTCGGGCAGAACTTAGCCAGCCCGGAACCCTCCTGGGAGA-TGACCAAATCTACAATGTCATCGTAACAGCACACGCCTTCGTTATAATTTTCTT-TATAGTAATGCCTATCATGATCGGAGGCTTCGGAAACTGACTTATCCCCCTA-ATAATCGGCGCCCCAGACATAGCATTCCCCCGTATGAATAACATGAGTTTCT-GACTCCTTCCCCCTTCTTTCTTACTGCTACTTGCCTCTTCAGGAGTTGAGGCTG-GTGCGGGTACTGGATGAACCGTTTATCCGCCTCTGGCCGGCAACCTAGCCCACG-CAGGAGCATCCGTTGACCTGGCTATCTTTTCCCTCCACCTGGCCGGAATCTCCT-CAATTCTTGGGGCCATTAATTTTATCACTACGATCATCAACATAAAACCCCCGACC-GTCTCTATGTACCAAATCCCCCTCTTTGTCTGAGCCGTGCTCATTACAGCTGTCCT-GCTTCTCCTCTCTCTTCCCGTCCTAGCTGCTGGCATTACAATGCTCCTAACAGATC-GCAACCTTAACACCACATTCTTCGACCCTGCAGGGGGAGGAGACCCCATTCTATAT-CAACACCTATTC

The sequence of the current study was matched with one species, namely *Psettodes erumei*, when compared to the nucleotide collection database on NCBI. The similarity reached 100% with a 0.00 E value (statistically significant). The record was stored under accession number PX599017.1 on NCBI. The comparison of the current study sequence with other records from different localities is provided in Table 6.

There were at least eleven records of DNA sequences on the DNA database (NCBI and BOLD) for *P. erumei* from Indonesian waters. The list can be seen in Table 7. Analysis of the COI (cytochrome c oxidase subunit I) gene sequences from *P. erumei* specimens sampled across Indonesia shows a very high level of genetic similarity, reflecting minimal genetic variation within the species. The data indicate sequence similarities ranging from 97.25% to 100%, with most samples (8 out of 11) showing 99.69% or higher similarity. Specifically, four sequences (KP856821.1, KP856822.1, BIFL2211-18/BIF8359, and MN257527.1) perfectly match the reference sequence. This high degree of genetic consistency is evident across different collection years (2009–2025) and diverse locations in Indonesia, such as Sukabumi, Cilacap, Banjarbaru, and Aceh.

The very low genetic divergence, with most differences below 1%, supports the interpretation that all sampled individuals belong to a single, genetically uniform population. The lack of clear genetic differentiation across geographic regions suggests ongoing gene flow among populations (Slatkin 1987). Although one sequence shows a slightly lower similarity (97.25%), this value falls within the normal range of within-species variation for marine fish and does not suggest the presence of a hidden or cryptic species (Lins *et al.* 2021). These molecular findings align with the morphological results, reinforcing that *P. erumei* in Indonesian waters is a stable species with conserved genetics and no major evolutionary divisions. The observed genetic uniformity has important implications for fisheries management and conservation, as it indicates that the species likely constitutes a single stock throughout the Indonesian archipelago.

Table 6. Sequence comparison for query and similarity retrieved from the NCBI website.

No	Accession	Species	Location	Description	Query	Similarity
1	OR592667.1	*Psettodes erumei*	India	COI	100%	100.00%
2	EF609580.1	*P. erumei*	India	COI	100%	100.00%
3	NC_020032.1	*P. erumei*	China	Complete genome	100%	100.00%
4	ON248018.1	*P. erumei*	India	COI	100%	100.00%
5	PV714486.1	*P. erumei*	Singapore	COI	100%	100.00%
6	MN257527.1	*P. erumei*	Indonesia	COI	96%	100.00%
7	KP856821.1	*P. erumei*	Indonesia	COI	94%	100.00%
8	ON384442.1	*P. erumei*	India	COI	90%	100.00%
9	KU945083.1	*P. erumei*	Taiwan	COI	84%	100.00%
10	PV828941.1	*P. erumei*	Taiwan	COI	84%	100.00%
11	MT076806.1	*P. erumei*	UAE	COI	100%	99.85%

Table 7. Record list of *Psettodes erumei* collected from Indonesian waters.

No	Accession/ Process ID/ Sample ID	Primer Name	Collector (Collection year)	Location	Related Reference	Similarity
1	GU674026.1	BCL	William White (2009)	Sukabumi, Indonesia (ID)	NCBI (2021a)	99.85
2	GU674038.1	BCL	William White (2009)		NCBI (2021b)	99.69
3	HQ564328.1	VF2_t1; FishF2_t1	William White (2010)	Cilacap, ID	NCBI (2019)	99.00
4	KP856821.1	RH1	Asadatun Abdullah (2012)	ID	Abdullah and Rehbein (2017)	100
5	KP856822.1	RH1	Asadatun Abdullah (2012)			100
6	KP856823.1	RH1	Asadatun Abdullah (2012)			97.25
7	BIFL2211-18, BIF8358	VF2_t1; FishF2_t1	Hubert *et al.* (2017)	Banjarbaru, ID	Hubert *et al.* (2018)	99.85
8	BIFL2211-18, BIF8359	VF2_t1; FishF2_t1	Hubert *et al.* (2017)			100
9	BIFL2211-18, BIF8360	VF2_t1; FishF2_t1	Hubert *et al.* (2017)			99.69
10	MN257527.1	BCL	Andriyono S and Damora A (2019)	Aceh, ID	Andriyono *et al.* (2022)	100
11	PX599017.1	Fish F1	Hartono *et al.* (2025)	Cilacap, ID	NCBI (2025)	100

The production of a phylogenetic tree confirmed the relationship between *P. erumei* in Cilacap waters and other samples from various regions in Indonesia molecularly. It also confirmed the relationship with other species from Psettodidae, namely *Psettodes belcheri* and *Psettodes bennettii* (Nelson 1994). The tree clearly classified *P. erumei* from Indonesian waters in one group and other species in another distinct group (Figure 4).

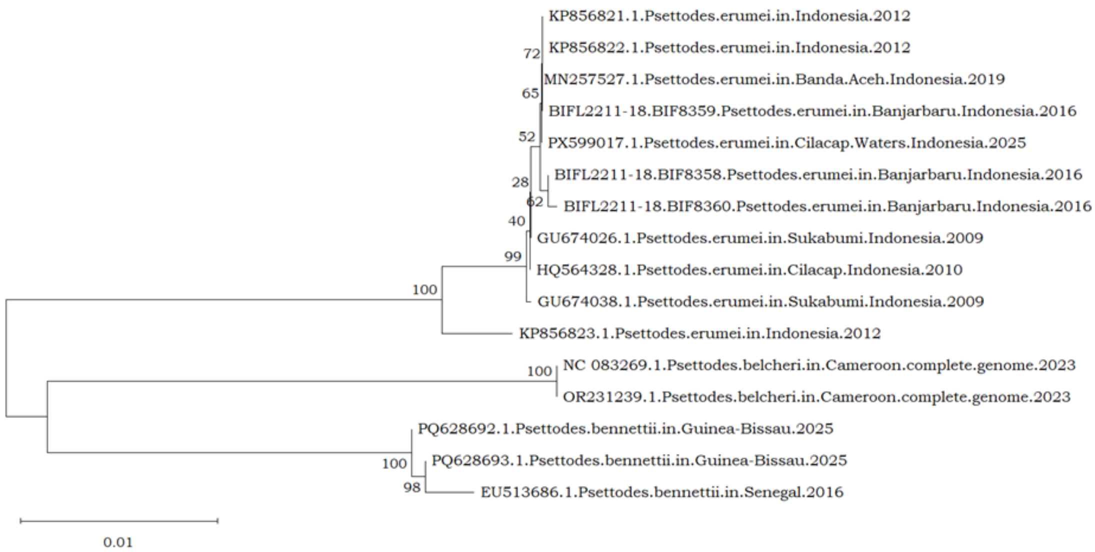

Figure 4. Phylogenetic tree of *Psettodes erumei* from Indonesian waters combined with other species from Psettodidae (with bootstrap value in each node).

This study has successfully identified a data-deficient species from Cilacap waters as *P. erumei* through integrated morphological and molecular approaches. The results revealed minimal intraspecific genetic divergence compared to conspecifics from other regions, suggesting a stable population without significant signs of environmental degradation at the genetic level (Andres *et al.* 2023). These findings contribute valuable data to Indonesia's fisheries biodiversity records and DNA barcoding libraries, enriching the scientific basis for species management.

Despite this, concerted conservation efforts remain imperative, as scientific literature on *P. erumei* remains limited. Munroe (2014) notes that tropical flatfish fisheries constitute a minor component of global fisheries landings, and a substantial portion of the catch is often discarded due to low commercial value, exacerbating pressures from overfishing and habitat destruction. In Indonesia, Tresnati *et al.* (2024) have already urged fisheries stakeholders in South Sulawesi to adopt management measures due to high exploitation rates. Furthermore, the species might face heightened vulnerability due to the limited suitable muddy bottom as its habitat in Cilacap waters, since it is on the southern coast of Java Island (Hartono *et al.* 2020). Therefore, implementing stock assessments and quota-based fisheries management is urgently needed to ensure sustainability amid ongoing exploitation pressures in the region (Azizah *et al.* 2023).

CONCLUSION

This study successfully confirms the presence of the Indian halibut (*Psettodes erumei*) in Cilacap waters through morphological and molecular evidence. The morphological analysis confirmed key diagnostic traits of the species, while revealing statistically significant differences in several morphometric measurements compared to a historical benchmark. Crucially, molecular analysis provided definitive confirmation, with DNA barcoding showing 100% sequence similarity to *P. erumei* records from across the Indo-Pacific. The phylogenetic assessment, along with a review of existing genetic data, indicates high genetic homogeneity among Indonesian populations. The confirmation of this Data Deficient species in Cilacap represents a vital contribution to Indonesia's ichthyofaunal records and public DNA barcode libraries. These findings provide the foundational taxonomic clarity required for effective fisheries management. However, this scientific clarity emphasizes an urgent need for action.

ACKNOWLEDGEMENT

We would like to thank Jenderal Soedirman University and its Institute for Research and Community Service for providing a research grant with number 14.550/UN23.34/PT.01/V/2025.

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