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FIRST CONFIRMED RECORD OF THE NEAR-THREATENED BOMBAY DUCK (*Harpadon nehereus*) IN CILACAP WATERS, INDONESIA

REKAMAN TERKONFIRMASI PERTAMA IKAN KADAL (*Harpadon nehereus*) YANG HAMPIR TERANCAM PUNAH DI PERAIRAN CILACAP, INDONESIA

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ABSTRACT

Accurate species identification is an essential foundation for effective fisheries management and conservation in Indonesia, particularly for near threatened species. This study aims to identify Bombay duck (*Harpadon nehereus*) from Cilacap Waters through morphological and molecular analyses, document its first record in Cilacap Waters, and provide a scientific basis for population studies, fisheries management, and conservation. Morphological identification was performed using physical characteristics such as shape, colour, and general features for morphometric and meristic analyses, guided by identification keys. Molecular confirmation was carried out through DNA barcoding of the mitochondrial COI gene. Primer Fish F1 and Fish R1 were used, along with the Sanger dideoxy sequencing method. The subfamily was confirmed by the presence of inner rays of the pelvic fin that are subequal or slightly longer than the outer rays. The genus was identified by the lateral line scales being enlarged and extending as a median lobe of the caudal fin. The primary rays of the caudal fin were without scales, and the body was compressed. The species was identified as *H. nehereus* because the pectoral fins reach beyond the origin of the dorsal fin. The obtained sequence (accession PV688437.1) showed approximately 96.928–99.495% identity with conspecific references in global databases and formed the same clade with other *H. nehereus* sequences in phylogenetic analysis, confirming its genetic identity. As the first confirmed record of *H. nehereus* in Cilacap, these findings provide essential baseline data for stock assessment, fisheries management, and conservation efforts in Indonesian waters.

Keywords: Bombay duck, DNA barcoding, fisheries management, species identification

ABSTRAK

Identifikasi spesies yang akurat merupakan dasar penting untuk pengelolaan dan konservasi perikanan yang efektif di Indonesia, khususnya untuk spesies yang hampir terancam punah. Penelitian ini bertujuan untuk mengidentifikasi ikan kadal (*Harpadon nehereus*) dari perairan Cilacap melalui analisis morfologi dan molekuler, mencatat temuan pertamanya di perairan Cilacap, dan menyediakan dasar ilmiah untuk kajian populasi, pengelolaan perikanan, dan konservasi. Identifikasi morfologi dilakukan berdasarkan karakteristik fisik, seperti bentuk, warna, dan ciri-ciri umum untuk kajian morfometri dan meristik, dengan berpanduan pada buku identifikasi. Konfirmasi molekuler dilakukan melalui *DNA barcoding* pada gen mitokondria COI. *Primer Fish F1* dan *Fish R1* digunakan, bersama dengan metode sekuensing dideoksi Sanger. Subfamili dikonfirmasi dengan adanya jari-jari bagian dalam sirip perut yang hampir sama panjang atau sedikit lebih panjang daripada jari-jari bagian luar. Genus diidentifikasi berdasarkan sisik garis lateral yang membesar dan memanjang sebagai lobus median sirip ekor. Jari-jari utama sirip ekor tidak bersisik, dan tubuhnya pipih. Spesies diidentifikasi sebagai *H. nehereus* karena sirip dada melampaui pangkal sirip punggung. Urutan genetik yang diperoleh (aksesi PV688437.1) menunjukkan identitas sekitar 96,928–99,495% dengan referensi sejenis dalam basis data global dan membentuk klad yang sama dengan urutan *H. nehereus* lainnya dalam analisis filogenetik, yang mengonfirmasi identitas genetiknya. Sebagai laporan pertama tentang *H. nehereus* yang dikonfirmasi di Cilacap, temuan ini memberikan data dasar yang penting untuk pengkajian stok, pengelolaan perikanan, dan upaya konservasi di perairan Indonesia.

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

INTRODUCTION

Thirty species of Synodontidae inhabit Indonesian waters. One of the species is *Harpadon nehereus*, commonly known as the Bombay duck. The species has been classified as near-threatened (NT) on the IUCN Red List of Threatened Species due to its declining trend in its population (Carpenter and Niem 1999; Russell *et al.* 2019).

The Bombay duck population has been impacted by the degradation of fisheries and their environments due to human activities. For the last decade, the species has been heavily exploited by trawl and other fishing gear across regions. It is fully exploited or declining in at least parts of Indonesia (Nugroho *et al.* 2015a; Prasetyo *et al.* 2014). It is also considered overfished in Pakistan, India, Bangladesh, and China (Kalhoro *et al.* 2013; Koya *et al.* 2017; Liang and Pauly 2017; Sarker *et al.* 2017). Furthermore, the species inhabits soft-bottomed nearshore waters and can be very common and abundant in estuaries. However, estuarine degradation keeps occurring throughout much of its range due to pollution and high human population in coastal areas, including Indonesia (Macusi *et al.* 2011; Hasin 2016).

One of the Indonesian regions that exploits the Bombay duck is Cilacap, Central Java. The report from Cilacap Ocean Fishing Port (2024) showed that the fish production was high in number, but the trend fluctuated considerably over the period of 2014–2018. The production reached 63.84 tons in 2014 and increased to 142.77 tons in 2015. However, it decreased to 49.60 tons in 2016, before it was increased again in 2017 (80.45 tons). The production reached 44.66 tons in 2018 and decreased significantly in the following period. In 2019 and 2020, the value was only recorded at 1.11 tons and 0.27 tons, respectively. Furthermore, it was 1.55 tons (in 2021), 6.49 tons (2022), and 10.08 tons (2023).

Bombay duck from Cilacap Waters is overlooked for its viable values and conservation urgency. It is considered a minor commercial fishery in the area. As a small demersal fish, it is priced only USD 0.41–1.23 per kg according to the report by Cilacap Ocean Fishing Port (2024), despite its advantage for culinary (Koya *et al.* 2017). Additionally, it is possibly an irrelevant object for fisheries management in the area since there is no scientific report regarding its resources. Azizah *et al.* (2023) reported that the small demersal fish in the southern Java Sea, where the Cilacap waters are located, have been exploited. The cumulative conditions on the exploited fish have increased the vulnerability of its resources.

The fisheries management must be conducted on Bombay duck in Cilacap Waters, procedurally. However, a crucial first step in fisheries management has not been executed yet, which is fish identification at the species level of taxonomy. There is no report that confirms the fish as *H. nehereus* specifically.

Hutubessy and Mosse (2015) stated that fisheries management in Indonesia has regulated and developed area-based and species-based fisheries management plans. The management must be conducted precisely at the species level to produce reliable and precise management. However, the identification of this matter still relies on non-scientific and traditional methods, which depend on morphological trait assessment or even naked eye observation. These methods consume much time and effort but do not guarantee the accuracy of identification (Ward *et al.* 2009). Some fish species that were identified traditionally have also been proven to represent two taxa in some areas (Zemlak *et al.* 2009). Furthermore, physical characteristics might alter throughout a life cycle (Tillett *et al.* 2012). It is also resistant to deterioration and environmental changes (Moran *et al.* 2016). Some species with identical physical traits, such as cryptic species, are also difficult to notice (Melo *et al.* 2016). These circumstances can lead to species misidentification and errors in fisheries analysis, particularly for management and conservation purposes (Garcia-Vazquez *et al.* 2012). Thus, a proper investigation must be conducted to validate the suspected *H. nehereus* precisely for further fisheries assessment needs.

The research aims (1) to confirm the taxonomic identity of suspected *H. nehereus* specimens collected from Cilacap waters through an integrated morphological examination and molecular DNA barcoding of the mitochondrial COI gene; (2) to document the first verified record of this near-threatened species in Cilacap waters, thereby filling a critical gap in its known distribution range; and (3) to provide a scientifically validated foundation for future population assessments, fisheries management strategies, and conservation actions. Morphological identification was conducted by examining diagnostic physical features, including morphometric and meristic characteristics (Winans 1985; Carpenter and Niem 1999). Molecular identification was carried out by analyzing fish DNA (Hebert *et al.* 2003a; Steinke and Hanner 2011). These studies could contribute to the richness of

reference regarding ichthyofauna biodiversity in Indonesian waters (Hubert *et al.* 2015).

METHODS

Time and location

The present study was conducted between February and May 2025. It started by collecting samples in February 2025 using a fisherman's trammel net. Sample collection was conducted once in Cilacap Waters at 7°46'49.7"S, 109°07'32.1"E (Figure 1). Furthermore, the morphological identification (February 2025) and DNA test (April–May 2025) were conducted simultaneously in the laboratory of the Faculty of Fisheries and Marine Sciences, Jenderal Soedirman University (Purwokerto), and Bionesia Laboratory (Bali), Indonesia.

Materials and sample collection

A sample was collected and taken to the laboratory using a cooler box to be measured, dissected, and analyzed. 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 (Mostarda *et al.* 2016). It was then examined to record its morphological characteristics. Fin and muscle tissues of the fish were preserved in the container with 95% ethanol as a preliminary step for testing the DNA (Vences *et al.* 2022; Santanumurti *et al.* 2024a).

Morphological identification

Fish morphology was studied, tabulated, and documented according to its shape, colour, and general properties. Some physical properties were measured and counted for morphometric and meristic analysis (Nugroho *et al.* 2015b; Santanumurti *et al.* 2024b). The results were guided and confirmed by using the fish identification book by Carpenter and Niem (1999).

Molecular identification

The specimen was sent to the Bionesia Laboratory (Bali, Indonesia). It was labelled with the specimen code UNSOED01.25.SH and tested once. DNA testing was conducted through several sequential steps, including (1) preparation of tissue samples, (2) DNA extraction using the 10% Chelex protocol, (3) DNA amplification using the Polymerase Chain Reaction (PCR) technique, (4) visualization of PCR results, and (5) DNA sequencing (Apriliyanti *et al.* 2018).

DNA amplification via PCR followed the Bionesia Laboratory protocol. The target gene used in the study was the Cytochrome C Oxidase Subunit I (COI) gene (Hebert *et al.* 2003b). 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).

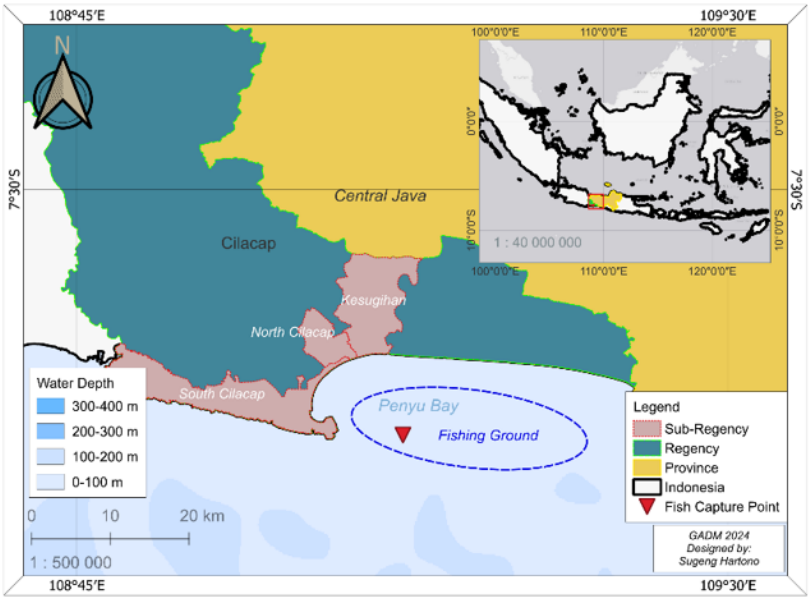

Figure 1. Samples collection site at Cilacap waters, Indonesia.

PCR product visualization was performed using 1% agarose gel electrophoresis stained with GelRed® Nucleic Acid Gel Stain. Positive samples showed visible DNA bands under UV light (Meliyana *et al.* 2024). DNA sequencing was carried out using the Sanger dideoxy method at Genetika Science Company, Jakarta. The sequencing output was in the form of sequence files (Ab1 format), which were further analyzed computationally. The sequence data were edited and aligned using the ClustalW method in MEGA XII software. Each base arrangement was manually checked to ensure the quality of the data used (Newman *et al.* 2016).

Further analysis was conducted by matching the sequences with genetic information available in the 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 percentage identity were recorded for each sequence (Landi *et al.* 2014). Genetic data were analyzed to produce a phylogenetic tree. The aim was to review its genetic relationship with other samples (Zhang *et al.* 2000). A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with 1,000 bootstrap replications on MEGA XII software (Kumar *et al.* 2024).

RESULTS AND DISCUSSION

Morphology of *Harpadon nehereus* in Cilacap Waters

Fish samples from Cilacap waters were identified as *H. nehereus* morphologically. The observation results showed that the fish matched the characteristics of the species based on Russell's key identification (Russell 1999). The sub-family was confirmed by the existence of inner rays of the pelvic fin, which are subequal to or slightly longer than outer rays (Figure 2). The genus was determined by some conditions, specifically the enlarged lateral-line scales, enlarged and extended as a median lobe of the caudal fin. Furthermore, the primary rays of the caudal fin were without scales, and the body was compressed (Figure 3). It was then identified as *H. nehereus* due to pectoral fins reaching beyond the origin of the dorsal fin (Figure 4). The detailed results of the morphology observation are shown in Table 1.

Bombay duck has been identified in several Indonesian Waters by previous authors. Andriyono *et al.* (2020) identified the fish molecularly in Gresik (East Java). Nugroho *et al.* (2017) and Setiawan *et al.* (2020) reported it on Tarakan Island (North Kalimantan). Meanwhile, Hariyadi *et al.* (2021) only reported the fish occurrence in Natuna Waters, and Kholis *et al.* (2024) supposedly caught it in Bengkalis Strait Waters. There were two authors who have conducted morphological studies on *H. nehereus* in other Indonesian waters, namely Nugroho *et al.* (2015b) and Setiawan *et al.* (2020), and their findings are consistent with those of the present study.

The snout length size difference with samples in Kumar *et al.* (2014) indicates an extremely truncated snout. This strongly suggests a difference in measurement protocol, such as the use of alternative anatomical landmarks, such as the snout tip to the anterior vs. posterior nostril or to the eye. A re-evaluation of the measurement method is essential. There were no other studies that could be compared for fish snout length, suggesting that morphometric data on the species are deficient.

The smaller eye diameter relative to other studies could be explained by two non-exclusive factors, namely allometric growth, where eye size increases at a slower rate than overall body size in larger fish, or genuine taxonomic variation (von Bertalanffy 1957; Kinne 1960; Brett 1979). However, the specimen's eye size falls within the broad population range (0.14–0.57 cm) provided by Setiawan *et al.* (2020), indicating it is a plausible value.

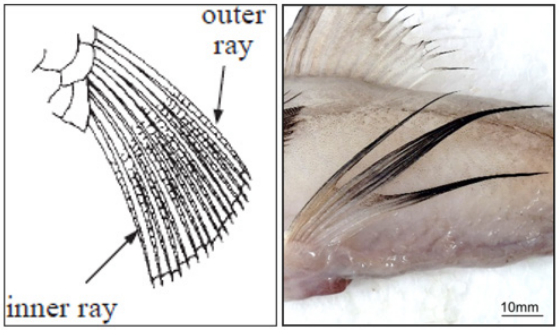

Figure 2. The condition of the inner rays of the pelvic fin of *Harpadon nehereus* in Cilacap Waters.

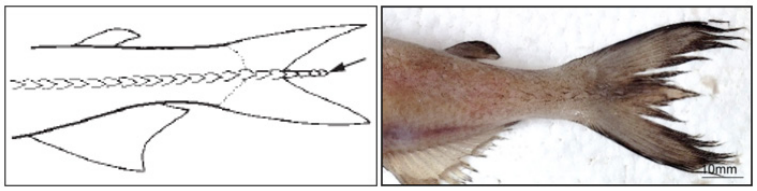

Figure 3. The scaleless primary rays of the caudal fin of *Harpadon nehereus* in Cilacap waters.

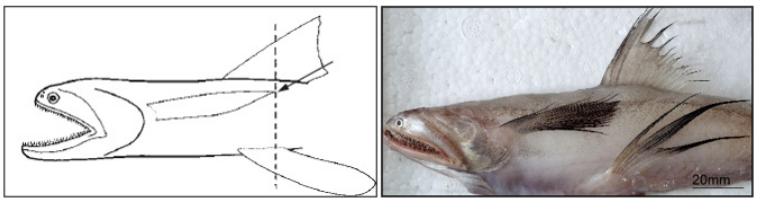

Figure 4. The pectoral fins of *Harpadon nehereus* in Cilacap waters reach beyond the origin of the dorsal fin.

Table 1. Morphological observations of *Harpadon nehereus* collected from Cilacap waters.

Details	Observation Results
Appearance	Aulopiform fishes, slender, small to moderate-sized (24.92 cm in total length). Colour is variable, light reddish, brown, or silvery, with red or yellow markings. Peritoneum colour is pale with many small black spots on each side of the midventral line. Anus located just anterior to the anal-fin origin.
Gill	Extending far forward into the mouth, well in advance of the angle of gape. Gill opening is large. Gill membranes are free from isthmus. Opercular flap with free edge formed by both opercle and subopercle.
Fins	Fins with pronounced soft rays except a few anterior secondary caudal-fin rays, none greatly prolonged; no spines. Dorsal fin about midway on back, over pelvic-fin insertion; first 2 rays unbranched, all others branched, with last ray branched to its base. Dorsal adipose fin is over the base of the anal fin. Pectoral fins extend beyond the origin of anterior to dorsal fins; first and last rays unbranched, all other rays branched. Pelvic fins close together and inserted abdominally, posterior to pectoral-fin origin; first and last rays unbranched, all other rays branched; inner rays of pelvic fins subequal or slightly longer than outer rays. Anal fin posterior to end of dorsal fin; first 2 rays always unbranched, all other rays unbranched, and last ray branched to its base.
Body shape lateral	Elongated.
Cross section	Body compressed.
Type of eyes	Eye size was small to moderate, positioned laterally.
Dorsal head and nape profile	Head clearly convex, depressed to compressed.
Position of mouth/type of mouth/snout	Terminal. Large, gape tending to be slanting. Upper jaw not protractile, bordered its entire length by premaxilla, extending well past the posterior margin of orbit in adult specimens; maxilla reduced or vestigial; supramaxilla size was small. Lower jaw with a fleshy knob at its tip. Teeth of moderate size, cardiform and depressible, unbarbed; no distinct canines; teeth on palatines (present in 2 bands) and on tongue. Vomer present.
Type of scales	Scales are absent on the procurrent and principal caudal fin rays and are restricted to the posterior half of the body. The head and body are covered with naked skin, except for a series of scales present along the lateral line and on the caudal peduncle.
Caudal shape	Forked.

Morphometry and meristics of *H. nehereus* in Cilacap waters

The present study measured approximately 37 external shapes of *H. nehereus* collected from Cilacap Waters to analyze their morphometry (Table 2). The study produced numerous measurements not commonly reported in previous literature, including body width (2 cm), detailed adipose fin dimensions (height: 0.28 cm; base: 1.25 cm), and various pre- and post-fin lengths.

Table 2. Morphometric measurements of *Harpadon nehereus* collected from Cilacap waters compared with previous studies.

Characteristics	Present Study (n = 1)	Nakabo (2002) (n = 1)	Kumar *et al.* (2014) (n = 373)		Setiawan *et al.* (2020) (n = 234)
			Male	Female	
Weight (g)	100	-	200	198	-
Total length (cm)	24.92	13.49	30	30.5	18.6–29
Fork length (cm)	22.31	12.33	-	-	17–27
Standard length (cm)	21.57	11.56	25.11	25.29	15.5–25.5
Body depth (in % FL)	16.86	15.65	-	-	10–27.5
Body width (cm)	2	-	-	-	-
Head length (cm)	4.73	2.17	4.37	4.49	3–6.4
Snout length (in % HL)	9.51	-	20.59	19.27	-
Upper jaw length (% HL)	59.83	-	-	-	-
Lower jaw length (% HL)	61.46	-	-	-	-
Head width (cm)	2	-	-	-	-
Head depth (cm)	3.38	1.71	-	-	-
Eye diameter (cm)	0.36	0.15	0.54	0.55	0.14–0.57
Distance between eyes (cm)	1.4	-	-	-	
Pre-orbital length (mm)	3.30	1.30	3.47	3.57	2.5–5.1
Pre-dorsal length (cm)	9.61	4.73	9.49	9.60	4.11–11
Pre-pectoral length (cm)	4.91	2.12	-	-	2–8
Pre-pelvic length (cm)	10.32	4.84	-	-	6.4–14.2
Pre-anal length (cm)	16.74	8.75	-	-	10–20.4
Dorsal fin base length (cm)	3.17	1.85	3.21	3.14	-
Longest dorsal fin length (cm)	3.89	1.99	4.14	3.40	-
Post dorsal and pre fat fin length (cm)	5.16	2.67	-	-	-
Fat fin height (cm)	0.28	-	-	-	-
Fat fin base length (cm)	1.25	-	-	-	-
Post fat fin and pre caudal length (cm)	1.75	1.03	-	-	-
Pectoral fin base length (cm)	0.89	-	-	-	-
Pectoral fin length (cm)	5.48	-	6.09	5.90	-
Pelvic fin base length (cm)	1.44	0.55	-	-	-
Longest pelvic fin length (cm)	6.45	3.15	6.40	6.18	-
Post pelvic and pre anal fin length (cm)	5.42	3.28	-	-	-
Anal fin base length (cm)	2.94	1.47	3.58	3.45	-
Longest anal fin length (cm)	2.54	1.39	-	-	-
Post-anal and pre-caudal length (cm)	2.12	-	-	-	-
Caudal height (cm)	4.57	1.92	-	-	-
Caudal peduncle (cm)	1.20	0.60	1.18	1.18	-
Top caudal fin length (cm)	4.14	2.42	-	-	-
Bottom caudal fin length (cm)	3.78	2.34	-	-	-

Some parts of the fish were compared to previous studies to see the difference among samples from various regions (Nakabo 2002; Kumar *et al.* 2014; Setiawan *et al.* 2020). The specimen's total and standard lengths place it at the upper end of the size range reported by Setiawan *et al.* (2020) and are comparable to the mean values for both sexes reported by Kumar *et al.* (2014). However, its recorded weight of 100 g was approximately half that of similarly

sized specimens in Kumar *et al.* (2014) (200 g).

Several key morphological comparisons were also noted, namely, the specimen's head length (4.73 cm) fell within the reported range (3–6.4 cm). A major discrepancy was found in snout length, measured at 9.51% of head length (HL), which is less than half the value reported by Kumar *et al.* (2014) (20% HL). The eye diameter (0.36 cm) was smaller than the means from Kumar *et al.* (2014) (0.54–0.55 cm) and at the lower end of the range reported by Setiawan *et al.* (2020). For fin dimensions, measurements for the longest pelvic fin length (6.45 cm), pectoral fin length (5.48 cm), and dorsal fin base length (3.17 cm) were closely aligned with the sex-specific means from Kumar *et al.* (2014) (e.g., pelvic fin: 6.18–6.40 cm).

The study also found that the current fish sample was at a lower body weight despite similar linear dimensions, pointing to differences in body condition. It could be influenced by seasonal nutritional status, preservation history, or population-specific growth patterns relating to habitat quality (Gregory 2011). Since the samples in the comparison were collected from different localities, the possibility of finding a difference between the samples is reasonable. It is recommended that the stakeholders of fisheries in Indonesia maintain the environmental quality of the waters, as pollution and overfishing may lead to a low growth rate and affect other physical development of the fish (Lawrence and Hemingway 2008).

The results of meristic observation on countable parts of the fish fin are displayed in Table 3. The specimen exhibited a total of 41 vertebrae and possessed one supramaxilla in the upper jaw. The fin spine and ray counts were similar to previous studies (Russell 1999; Nakabo 2002; Kumar *et al.* 2014; Setiawan *et al.* 2020). However, its dorsal fin ray count (10) was lower than the lower bounds reported by Russell (1999) (12–13), Nakabo (2002) (11–13), and Setiawan *et al.* (2020) (12–14). Although it fell within the broader range given by Kumar *et al.* (2014) (10–13). The pectoral fin ray count (11) was within the reported range of 10–12.

The meristic analysis reveals both strong consistency and one notable discrepancy, which is crucial for evaluating the specimen's taxonomic alignment. The complete absence of spines in all fins and the fixed count of 9 pelvic fin rays are highly conserved traits that perfectly match all literature sources, solidifying these as stable diagnostic characters for the species.

The most significant finding is the lower count of dorsal fin rays in the present specimen. In teleost taxonomy, dorsal fin ray counts are often key diagnostic characters with low intraspecific variation (Zafar *et al.* 2025). It also could represent (1) a methodological difference in counting (e.g., whether a minute anterior ray was counted) or (2) a potential taxonomic signal indicating population-level differentiation or misidentification (Andres *et al.* 2023). Therefore, this deviation warrants careful consideration. We suggest re-examining the specimen with magnification or staining and comparing meristic data from other Indonesian populations of *H. nehereus*.

Table 3. Meristic counts of *Harpadon nehereus* collected from Cilacap waters compared with previous studies.

Characteristics	Present Study (n = 1)	Russell (1999) (n = 1)	Nakabo (2002) (n = 1)	Kumar *et al.* (2014) (n = 373)	Setiawan *et al.* (2020) (n = 234)
Dorsal fin spines	0	0	0	0	0
Dorsal fin rays	10	12–13	11–13	10–13	12–14
Pectoral fin spines	0	0	0	0	0
Pectoral fin rays	11	-	10–12	10–12	10–12
Pelvic fin spines	0	0	0	0	0
Pelvic fin rays	9	9	9	9	9
Anal fin spines	0	0	0	0	0
Anal fin rays	13	-	13–15	13–5	13–15
Caudal principal fin rays	19	19	-	-	-
Caudal branched fin rays	17	17	-	-	-
Upper jaw supramaxilla	1	1	-	-	-
Gill arches	-	4	-	-	-
Vertebrae	41	-	-	-	-

The perfect match with Russell (1999) on more specialized osteological characters provides strong positive evidence for correct generic or familial placement. These traits are often phylogenetically informative. The agreement on pectoral fin rays (11 within 10–12) is also supportive.

Molecular study of the Bombay duck in Cilacap waters

The current study has successfully amplified the COI gene of the fish sample from Cilacap Waters. The gel electrophoresis results are shown in Figure 5, where DNA fragments are separated and visualized based on their molecular size (Wittmeier and Hummel 2022). PCR amplification of the target region produced a clear and distinct band in the sample, with an estimated size of 600–700 bp based on migration relative to the 100 bp DNA ladder. The positive control showed a band of identical size, while the negative control displayed no amplification, confirming the absence of contamination and validating the PCR conditions (Bacich *et al.* 2011). Sequencing of the current sample produced a high-quality nucleotide fragment with a total length of 655 bp. The chromatogram showed no ambiguous bases. The fragment was displayed in Table 4.

The nucleotide sequence obtained from the current sample was recorded with accession number PV688437.1 onto the NCBI database as the first record in the study area. It generated dominant similarity that matched *H. nehereus* (Table 5). Together, the gel electrophoresis and BLAST results confirm successful amplification and accurate species-level identification of the sample as *H. nehereus*.

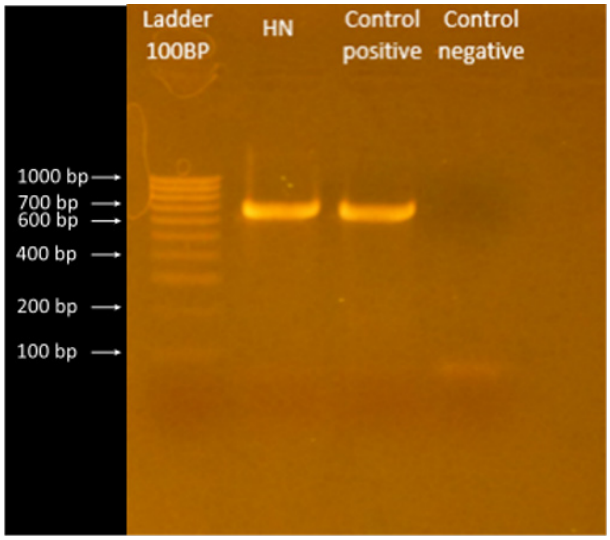

Figure 5. Gel electrophoresis of the COI gene amplified from *Harpadon nehereus* collected from Cilacap waters.

Table 4. COI nucleotide sequence of *Harpadon nehereus* collected from Cilacap waters.

Sample Code	Nucleotide Fragment
UNSOED01.25.SH	CCTCTACCTTGTATTTGGTGCATGAGCTGGAATAGTGGGAACCGCCCT-GAGCCTCTTGATCCGCGCCGAGCTAAGCCAGCCGGGGGCCCTGCTCGGTGAC-GATCAAATTTATAACGTAATCGTTACTGCCCACGCCTTCGTAATAATTTTCTT-TATAGTAATGCCAATTATGATCGGGGGCTTTGGAAATTGACTCATTCCCCT-GATGATCGGTGCCCCCGATATAGCGTTCCCCCGAATGAACAACATAAGCTTTT-GACTCCTCCCACCCTCTTTCCTTCTTCTCTTGGCATCATCGGGAGTC-GAAGCAGGGGCTGGAACCGGCTGAACAGTCTACCCCCCGTTAGCGGGAAACCTT-GCTCACGCTGGGGCCTCTGTAGATCTAACCATCTTCTCGCTACACTTGGCTG-GAATTTCCTCTATTTTGGGAGCCATTAATTTTATTACGACAATTATCAATATA-AAACCTCCCGCCATTTCACAATACCAGACACCCCTCTTTGTCTGAGCTGTACTAAT-TACGGCTGTCCTCCTCCTCCTCTCCTTACCCGTTCTTGCAGCCGGAATCACAAT-GCTCTTAACCGATCGAAATCTTAATACCACCTTCTTTGACCCTGCAGGAGGCGGC-GATCCTATCCTCTATCAGCACTTATTC

Table 5. BLAST comparison of the COI sequence obtained in the present study with NCBI reference sequences.

No	Accessions	Species	Locations	Descriptions	Similarities (%)
1	PV872660.1	*Harpadon nehereus*	Singapore	COI	99.495
2	MT621034.1	*Harpadon nehereus*	Tarakan, Indonesia	COI	97.086
3	MW402952.1	*Harpadon nehereus*	East China Sea	COI	96.947
4	KP112261.1	*Harpadon nehereus*	Wuhan, China	COI	96.947
5	MT734532.1	*Harpadon nehereus*	Wenzhou, China	COI	96.947
6	KY315382.1	*Harpadon nehereus*	Fujian, China	COI	96.947
7	JX534239.1	*Harpadon nehereus*	Zhejiang, China	Genome	96.947
8	JN242630.1	*Harpadon nehereus*	Shanghai, China	COI	96.933
9	KP260457.1	*Harpadon nehereus*	Fujian, China	COI	96.933
10	OL494348.1	*Harpadon nehereus*	Guizhou, China	COI	96.928

The phylogenetic tree is shown in Figure 6. The results of the current study indicated that the fish sample was certainly included in the same clade as *H. nehereus*. There are four species in the subfamily Harpadontinae found in Indonesian waters, namely *H. nehereus*, *H. microchir*, *H. translucens*, and *H. mortenseni* (Carpenter and Niem 1999; Froese and Pauly 2025). Only *H. nehereus* was included in the diagram because reference sequence data for other species were not available. It suggests the limitation of sequence data from other subfamily species. However, the data from other waters still reveal clear phylogenetic relationships and definitively confirm the species identification despite the limitation of sequence data for the subfamily species.

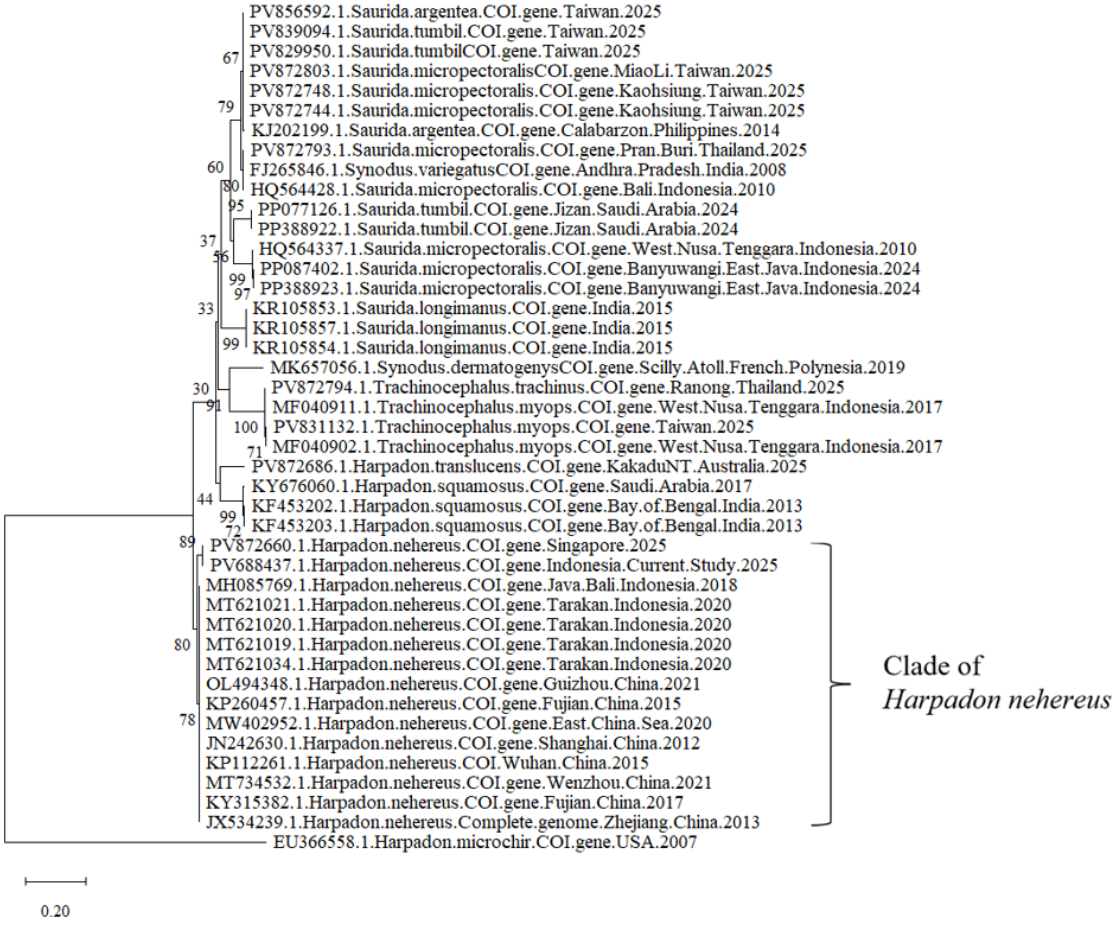

Figure 6. Phylogenetic tree of *Harpadon nehereus* based on COI gene sequences.

There were only about six records of *H. nehereus* in Indonesia itself that have been entered into NCBI, including the one from the current study. The other records were from two different waters, namely Tarakan (North Kalimantan) (MT621021.1; MT621034.1; MT621020.1; MT621019.1) and Bali (MH085769.1). The results of the study contribute to the biodiversity data of *H. nehereus* in Indonesian waters.

The sequence similarity shows exceptionally high similarity (>96.928%) with other *H. nehereus* sequences, indicating normal intraspecific variation due to slightly lower identities (less than 100%) (Yang *et al*. 2022; Dong *et al*. 2023). The phylogenetic tree also formed a distinct cluster from another congener. This strong molecular boundary validates the established taxonomic hierarchy, as all species from the same genera (*Harpadon*, *Saurida*, *Synodus*, and *Trachinocephalus*) cluster together, reflecting deeper evolutionary divergence (Choi *et al*. 2024). Fundamentally, the genetic result provides independent and conclusive validation of the prior morphological identification. The DNA evidence confirms that specimens are genetically *H. nehereus*, perfectly aligning morphology with genetics. As it is confirmed as *H. nehereus*, which has been near-threatened, specific management should be implemented to conserve the resource. Some subsequent actions should include restoring its population biomass, assessing fishing pressure, and implementing measures to maintain habitat quality in Cilacap waters.

CONCLUSION

The current study successfully confirms the first record of the near-threatened species *H. nehereus* (Bombay duck) in Cilacap waters, Indonesia, through an integrated approach combining morphological and molecular analyses. Morphological examination of diagnostic characters aligned precisely with established taxonomic keys for the species. DNA barcoding of the COI mitochondrial gene provided definitive molecular confirmation, with the generated sequence (accession PV688437.1) showing high similarity (96.928–99.495%) to conspecific sequences and forming a robust phylogenetic clade with other *H. nehereus* individuals. The confirmation provides a crucial scientific foundation for accurate population assessment, the development of targeted fisheries management plans, and informed conservation strategies. Subsequent actions should include restoring its population biomass, assessing fishing pressure, and implementing measures to maintain habitat quality in Cilacap waters.

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