



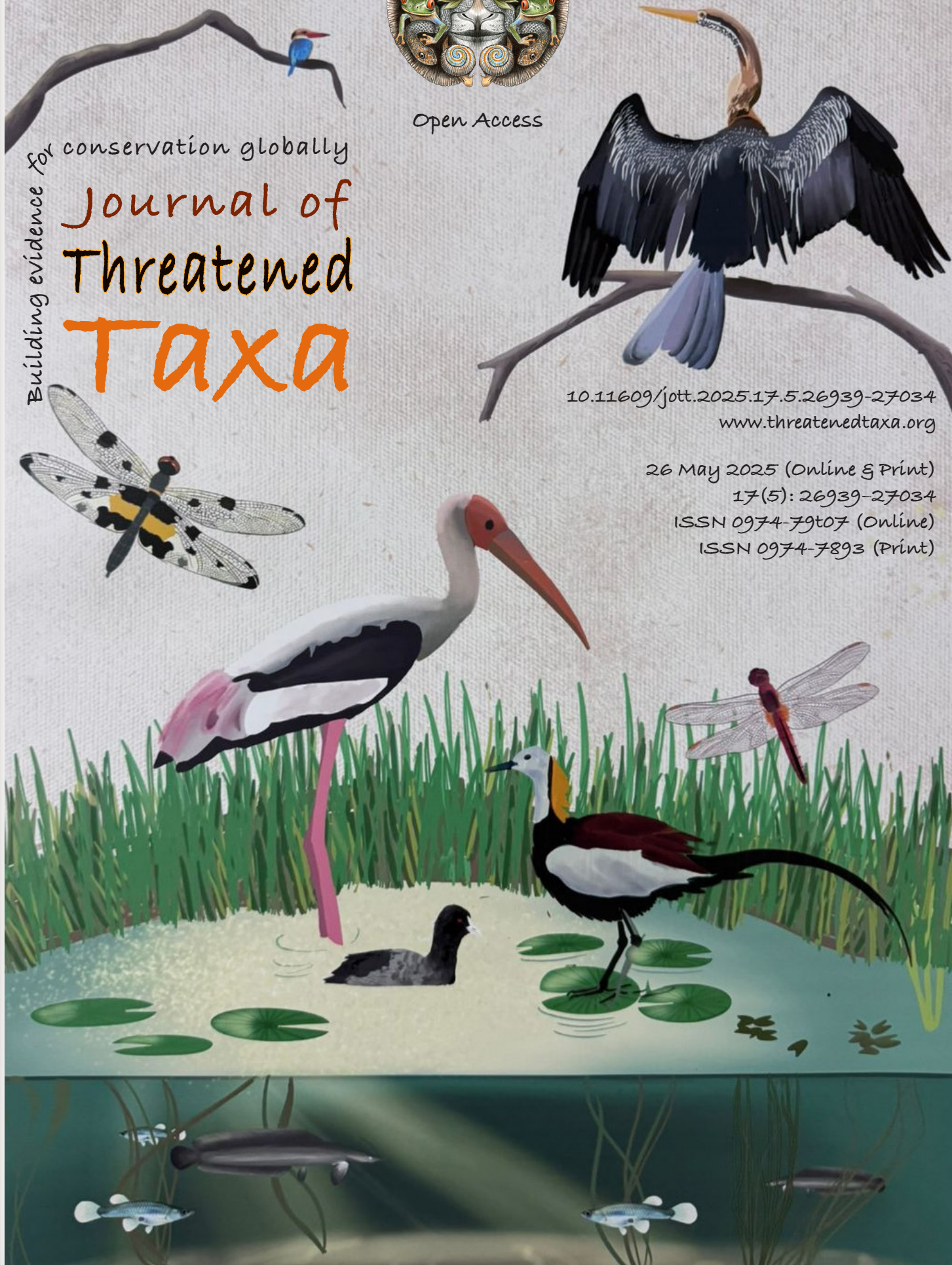
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Cover: A digital art of water birds of Noyyal River and its wetlands in Coimbatore District by Megha A. Kashyap.



Morphological and molecular identifications of sea turtles *Lepidochelys olivacea* and *Eretmochelys imbricata* from the Turtle Bay of Cilacap, Indonesia

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Abstract: DNA barcoding is a powerful tool for accurately identifying marine turtle species, especially when morphological identification is challenging, owing to insufficient clues in the samples available. This study focused on two sea turtle samples of dead adults washed ashore and babies hatching out from nests, which pose a challenge for morphological identification. They represented two species, the Olive Ridley Turtle *Lepidochelys olivacea* and Hawksbill Turtle *Eretmochelys imbricata*, from Turtle Bay, Cilacap, Indonesia. For one sample, morphological identification initially suggested it as a Green Sea Turtle *Chelonia mydas*, based on traits like a pair of prefrontal scales, brown carapace colouration, and the absence of serrations on the posterior carapace. The degraded condition of the specimen and shared juvenile traits between *C. mydas* and *E. imbricata* made conclusive identification challenging. Using mtDNA barcoding with the CO1 gene provided more accurate species identification, revealing the sample to be *E. imbricata* with a perfect genetic match in the BLAST search (0% divergence). This result highlights the advantages of molecular approaches when traditional methods fall short. Phylogenetic analysis of *L. olivacea* and *E. imbricata* sequences revealed close clustering of sampled sequences with published sequences from Ghana, Australia, and China. High bootstrap values of 92% for *L. olivacea* and 98% for *E. imbricata* confirmed the molecular identifications of these samples. The study underscores the value of combining DNA barcoding and phylogenetics for marine turtle identification and evolutionary insights, with implications for conservation and species management.

Keywords: Biodiversity, DNA barcoding, dead specimen, % divergence, extraction, genetics, phylogenetic, scutes, taxonomy, threatened.

Indonesian Abstrak: Barkoding DNA merupakan alat yang ampuh untuk mengidentifikasi spesies penyu laut secara akurat, terutama ketika identifikasi secara morfologi sulit dilakukan, karena petunjuk yang tersedia tidak memadai. Penelitian ini difokuskan pada dua sampel yaitu penyu laut dewasa yang mati terdampar di pantai dan anakan yang menetas dari sarang, sehingga menimbulkan tantangan untuk identifikasi secara morfologi. Sampel tersebut mewakili dua spesies, Penyu Belimbing *Lepidochelys olivacea* dan Penyu Sisik *Eretmochelys imbricata*, yang berasal dari Teluk Penyu, Cilacap, Indonesia. Untuk satu sampel, identifikasi morfologis awalnya menunjukkan bahwa penyu tersebut adalah Penyu Hijau *Chelonia mydas*, berdasarkan ciri-ciri seperti sepasang sisik prefrontal, warna karapas coklat, dan tidak adanya gerigi pada karapas posterior. Kondisi spesimen yang terdegradasi dan kesamaan ciri-ciri juvenil antara *C. mydas* dan *E. imbricata* membuat identifikasi konklusif menjadi sulit. Penggunaan barkoding mtDNA dengan gen CO1 memberikan identifikasi spesies yang lebih akurat, yang menunjukkan bahwa sampel tersebut adalah *E. imbricata* dengan kecocokan genetik yang sempurna dalam pencarian BLAST9 (divergensi 0%). Hasil ini menggambarkan keuntungan pendekatan molekuler ketika metode tradisional tidak berhasil. Analisis filogenetik dari sekuens *L. olivacea* dan *E. imbricata* menunjukkan pengelompokan yang erat dari sekuens sampel dengan sekuens yang dipublikasikan dari Ghana, Australia, dan Tiongkok. Nilai bootstrap yang tinggi sebesar 92% untuk *L. olivacea* dan 98% untuk *E. imbricata* mengonfirmasi identifikasi molekuler dari sampel-sampel ini. Studi ini menggarisbawahi nilai penggabungan barkoding DNA dan filogenetik untuk identifikasi penyu laut dan wawasan evolusi, dengan implikasi untuk upaya konservasi dan pengelolaan spesies.

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INTRODUCTION

Sea turtles are globally recognized for their ecological importance, with seven species documented worldwide (Robinson & Paladino 2013). Of these, six species inhabit the waters of Indonesia, reflecting the region's rich biodiversity (Ario et al. 2016). Olive Ridley Sea Turtle *Lepidochelys olivacea*, Green Sea Turtle *Chelonia mydas*, Hawksbill Sea Turtle *Eretmochelys imbricata*, Leatherback Sea Turtle *Dermochelys coriacea*, Flatback Sea Turtle *Natator depressus*, and Loggerhead Sea Turtle *Caretta caretta* are found in Indonesian Waters (Pardede & Yealta 2013; Suraeda et al. 2018). Despite legal protection and their inclusion in Appendix I of the Convention on International Trade in Endangered Species (CITES), illegal trade in sea turtles remains widespread. Transactions often involve parts such as eggs, meat, and shells, complicating taxonomic identification (Nijman & Nekaris 2014; Foran & Ray 2016). One notable site, Turtle Bay in Cilacap Regency, was once iconic for turtle landings but has since been transformed into an industrial and tourist hub, threatening the local turtle populations (Thahira & Wirasmoyo 2022). Although Pertamina (2019) designates the area as a turtle landing site, the lack of sufficient data on sea turtle activity poses a challenge, leaving classification to rely mostly on morphological identification. This study focuses on two species of particular interest: the Olive Ridley *Lepidochelys olivacea* and the Hawksbill Turtle *Eretmochelys imbricata*, both of which are critically affected by habitat disruption and illegal trade.

Morphological characteristics and tracks have traditionally been the primary methods for identifying sea turtle species, with traits such as carapace shape, colouration, head and limb structure, track width, and egg size serving as key indicators. Studies like Rupilu et al. (2019) have detailed how features such as the number of costal scutes on the carapace, inframarginal scutes on the plastron, and prefrontal & postocular scales provide essential markers for distinguishing species. In Indonesia, as well as globally, these characteristics have been widely used to identify sea turtles, offering a foundational approach to understanding their development, evolution, and ecological role (van Dam & Diez 1998). Despite its historical significance in biology and paleontology, morphological identification is not without limitations. A major challenge is the risk of misidentification when body parts are degraded or worn out, making it difficult to rely on a limited set of morphological features for accurate identification.

Molecular techniques have become vital in

overcoming the limitations of morphology-based species identification, especially in regions like Indonesia, where illegal sea turtle trade complicates classification. Various markers, such as 16S, 12S, CYTB, and ITS, have been used for accurate species identification, with this study focusing specifically on mitochondrial Cytochrome oxidase 1 (CO1). This molecular approach is particularly useful in cases where body parts are traded, as highlighted by Madduppa et al. (2019). Recent studies, including those by Santhosh et al. (2018) and Ollinger et al. (2020), stress the importance of integrating genetic data with morphological analysis to enhance species identification. Molecular methods are not without challenges, such as contamination or incorrect marker usage, which can lead to misidentification. Despite these weaknesses, genetic analysis remains crucial in understanding sea turtle diversity, particularly in areas like Turtle Bay, and Cilacap, where traditional methods fall short.

Sea turtles are threatened wildlife often found dead and decaying on beaches, posing challenges for species identification, critical to conservation intervention. In such cases, DNA barcoding serves as a powerful tool for accurately identifying marine turtle species, particularly when morphological identification is hindered by the lack of sufficient diagnostic traits. This study employed both morphological and genetic analyses to identify sea turtle species in Turtle Bay, Cilacap, Indonesia. Morphological characteristics such as shell structure, curved carapace length, plastron color, and prefrontal scales were assessed alongside genetic data to reduce biases associated with relying solely on physical traits, especially when dealing with deceased & decayed individuals, juveniles, or embryos. Tissue samples from nesting turtles at sites SP1–SP8 were collected for genetic analysis to complement morphological findings. The objective of this research was to accurately identify the species inhabiting the region, integrating both approaches to enhance conservation, and protection efforts.

METHODS

A combination of morphometric data and genetic studies was used to identify the sea turtle species in the study area. For the genetic studies, clutches retrieved from nesting sites were carefully incubated in the sand within the conservation area at Sodong Beach, Cilacap. For the dead and decaying turtle sample, tissue was carefully obtained from the carapace, avoiding

contamination. The collected sample was designated unidentified GT for Green Turtle *Chelonia mydas* and OR1, OR2, and OR3 for Olive Ridley baby turtles and embryo *Lepidochelys olivacea*. Data collection for this study occurred during the new and full moon (July, August, and September 2023) phases to facilitate taxonomic studies. Tissue samples gathered from the field were preserved at -20°C and subsequently subjected to analysis at Genetica Science, Indonesia, employing a DNA barcoding protocol.

Study area

The study was conducted in Turtle Bay, Cilacap, Indonesia, across a designated area spanning SP1 to SP8, with each station covering a distance of 2 km (Image 1). Cilacap Beach, nestled in Cilacap Regency, Central Java, Indonesia, stretches along the Indian Ocean, offering a serene yet vital coastal landscape. Geographically, it sits at about 7.730°S , 108.980°E , surrounded by a mixture of sandy beaches and coastal hills. Positioned roughly 200 km southwestern of Semarang and 300 km southeastern of Jakarta, the area is more than just a beautiful stretch of coastline. Despite the growing industrial activity, the beach holds immense ecological value as a key nesting ground for sea turtles. This makes it a focal point for conservation efforts, where preserving the natural habitat becomes essential for the survival of these endangered species. Global Positioning Systems (GPS) were utilized to ascertain each station's research locations and coordinates. The sampling process involved monitoring during the nesting season at eight designated stations (SP1–SP8) with coordinates

as follows: SP1 (7.691°S , 109.181°E), SP2 (7.691°S , 109.191°E), SP3 (7.696°S , 109.231°E), SP4 (7.692°S , 109.244°E), SP5 (7.698°S , 109.260°E), SP6 (7.698°S , 109.261°E), SP7 (7.700°S , 109.292°E), SP8 (7.700°S , 109.287°E).

Data collection method

During the nesting season, samples were collected from *L. olivacea* and *E. imbricata*. Monitoring of nesting sea turtles occurred during the early morning hours (0200–0600 h.) using rechargeable torchlights. Eggs retrieved from the nesting sites were buried in sandy soils at the conservation area, Sodong Beach, Cilacap, and allowed to hatch. After approximately 45 days (± 15 days), samples were obtained from unhatched eggs and deceased hatchlings (Olive Ridley). Additionally, a sample was obtained from a dead adult Hawksbill Sea Turtle *E. imbricata* carried offshore by ocean currents. They were immersed in a 96% ethanol solution to preserve the samples and stored at -20°C , following established protocols (Roden et al. 2013; Dutton et al. 2014b). The specific methods used to obtain these samples are detailed below.

For the morphological studies, the length and width of the carapace of deceased adult turtles were measured using a measuring tape. Observations were made regarding the number of scutes, carapace colour, shell shape, prefrontal scale, and plastron colour. This information was meticulously recorded in a dedicated research notebook and compared to a reference publication by Pritchard & Mortimer (1999). Genetic studies followed the procedure of using Olive Ridley

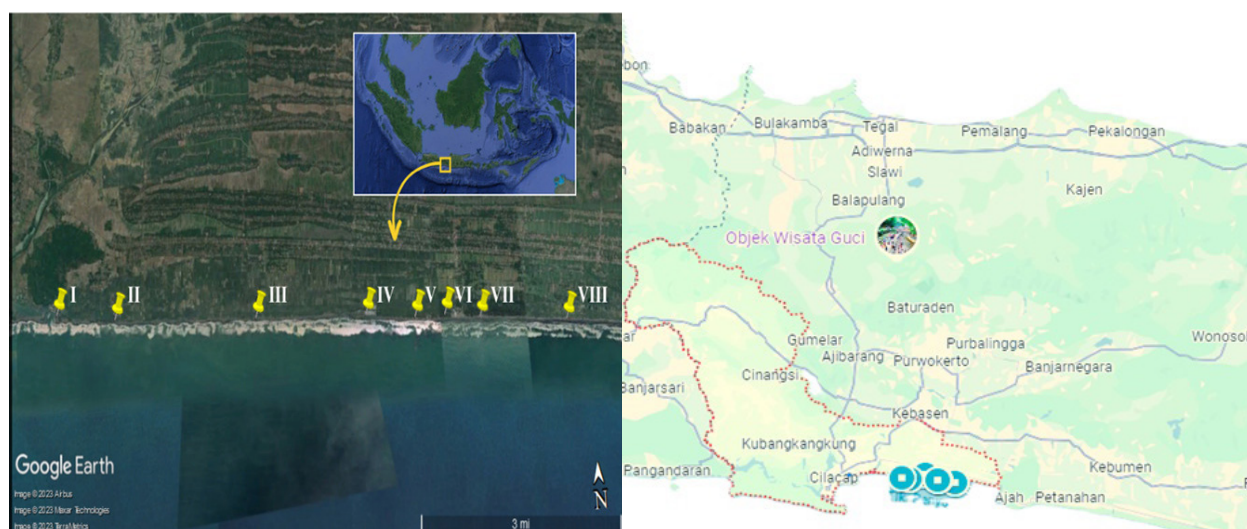


Image 1. Map of study sites in Turtle Bay of Cilacap for data collection.

Lepidochelys olivacea eggs as described below.

Collection tubes filled with 96% ethanol solution were prepared. An unhatched egg was selected and thoroughly rinsed with distilled water. Gloves were worn and changed for each sample collection to prevent contamination. After drying the eggshell with a tissue and placing it in a petri dish, an ethanol swab was used for additional cleaning. Using forceps to grasp a part of the egg, the shell was gently opened with sterile scissors. The embryo was carefully collected, transferred to sample collection tubes, and securely sealed using a wooden skewer. The samples were labelled using a pencil. The containers were wrapped and stored in a cool, dry place until they were later transported to the Animal Taxonomy Laboratory in a cooler with ice. Subsequently, they were stored at -20 °C for the next stage of the process.

Table 2 outlines the protocol optimization based on the methods described by Madduppa et al. (2019), with modifications to the extraction and amplification procedures. In the study involving dead Olive Ridley baby turtles, embryo, and Hawksbill Sea Turtles (initially labelled as GT sample, unverified specimen), gloves were worn and changed for each sample collection. The rear end of the flipper was cleaned with an ethanol swab. A small section of the hind flipper was carefully cut using forceps with sterile scissors. The sample was then transferred into a plastic vial filled with ethanol using a wooden skewer. The samples were labelled using a pencil. The containers were wrapped and stored in a cool, dry place until later transported to the Animal Taxonomy Laboratory at the Faculty of Biology, UNSOED, in a cooler with ice. Subsequently, the samples were stored at -20°C for the next stage of the process as described below.

DNA Barcoding was conducted at Genetica Science, Indonesia, using CO1 gene to identify the sea turtle species. The Genomic DNA Extraction was done using the Quick-DNA™ Tissue/Insect Miniprep kit (ZYMO, D6016) following the manufacturer's protocol. A 50 mg tissue sample was added to a ZR BashingBead™ Lysis Tube (2.0 mm). Then, 750 µl of BashingBead™ buffer was added to the tube and tightly capped. It was secured in a bead beater fitted with a 2 ml tube holder assembly and processed at maximum speed for 10 min. The ZR BashingBead™ Lysis Tube (2.0 mm) was centrifuged in a microcentrifuge at ≥10,000 x g for one min. Subsequently, 400 µl of the supernatant was transferred to a Zymo-Spin™ III-F filter in a collection tube and centrifuged at 8,000 x g for 1 min. Then, 1,200 µl of genomic lysis buffer was added to the filtrate in the

collection tube, and the mixture was thoroughly mixed. Further, 800 µl of the mixture from the previous step was transferred to a Zymo-Spin™ IICR Column one in a collection tube and centrifuged at 10,000 x g for 1 min. The eluate from the collection tube was discarded, and the procedure was reiterated.

Subsequently, 200 µl of DNA pre-wash buffer was introduced to the Zymo-Spin™ IICR column within a fresh collection tube and subjected to centrifugation at 10,000 x g for 1 min. Following this, 500 µl of g-DNA Wash Buffer was applied to the Zymo-Spin™ IICR Column, followed by centrifugation at 10,000 x g for 1 min. Ultimately, the Zymo-Spin™ IICR column was relocated to a sterile 1.5 ml microcentrifuge tube, and 100 µl (with a minimum requirement of 35 µl) of DNA elution buffer was directly administered to the column matrix. The column was centrifuged at 10,000 x g for 30 s to facilitate DNA elution (ZYMO Research Manual 2021).

PCR was performed in a 25 µL reaction volume consisting of 1 µL template DNA, 12.5 µL MyTaq HS Red Mix 2X, and 1.0 µL of each of the four primers (10 µM), 7.5 µL of ddH₂O, and the conditions were set as seen in Table 1. The four primer sequences used were as follows: VF2_t1: TGTAACGACGGCCAGTCAACCAACCACAAAGA CATTGGCAC
FishF2_t1: TGTAACGACGGCCAGTCGACTAATCATAAAG ATATCGGCAC
FishR2_t1: CAGGAAACAGCTATGACACTTCAGGGTGACCG AAGAATCAGAA
FR1d_t1: CAGGAAACAGCTATGACACCTCAGGGTGTCCGA ARAAYCARAA

Data analysis

Species identification followed the reference guidelines provided by Pritchard & Mortimer (1999). Sequences obtained were analyzed using the BOLD system and GenBank databases to confirm species identity, and similarity. Forward and reverse primer sequences were assembled into contigs, creating a single sequence for each sample. These sequences were subjected to a BLAST search on GenBank to identify species. They were also submitted to the NCBI database, where they were assigned the following GenBank accession numbers: OR1 (OR562509.1 and 627 bp), OR2 (OR562510.1, and 648 bp), OR3 (OR562511.1, and 656 bp), and GT (OR562508.1, and 648 bp). The BIN for each closest-matching species was retrieved from BOLD, with OR1, OR2, and OR3 *Lepidochelys olivacea* assigned BIN AAC1248, and GT *Eretmochelys imbricata* assigned BIN ACE8206 (pending compliance with metadata

requirements).

In addition, sequences from 10 individuals of *Lepidochelys olivacea*, 10 individuals of *Eretmochelys imbricata*, and seven individuals of *Lepidochelys kempii* were downloaded from BOLD to construct a phylogenetic tree with the sequences from the study. Sequences were concatenated and edited to remove stop codons. One sequence of *Dermochelys coriacea* was used as the outgroup tree was built using the Neighbor-Joining method in MEGA, illustrating the phylogenetic relationships of species inhabiting Turtle Bay of Cilacap. The results were then discussed for further insights into species positioning.

RESULTS

Of the eight sampling sites surveyed, eggs were found in four areas (Table 3). The number of clutches retrieved from one nest in a sampling site within the study area ranged between 30 (SP8) and 100 (SP3). These data cannot be used to quantify species identity due to irregularities caused by constant poaching prior to egg retrieval. Eggs were recovered from SP1, SP3, SP7, and SP8, with SP8 having the highest number of clutches (Figure 1). At SP7, nesting was also observed twice, but no eggs were found despite visible turtle tracks (noted as NA), indicating poaching. Figure 1 provides a chart representation of egg retrievals across the sampling stations (SP1–SP8), where four sites had no egg records.

A single dead turtle (Image 2) was labelled as “GT” due to certain features, including a brownish plastron colour, absence of strong or prominent serrations, and a pair of prefrontal scales (Table 4). The morphological data for the OR samples, derived from two deceased baby turtles (Image 2), are also presented in Table 4. These data indicated 5–7 costal scutes, grey carapace colouration, slightly overlapping carapace shape, four prefrontal scales, and a white plastron.

Due to the juvenile stage of the specimens, DNA barcoding was employed to confirm the species identity of these two OR samples apart from the decayed GT sample. Similarity and identity results from BOLD

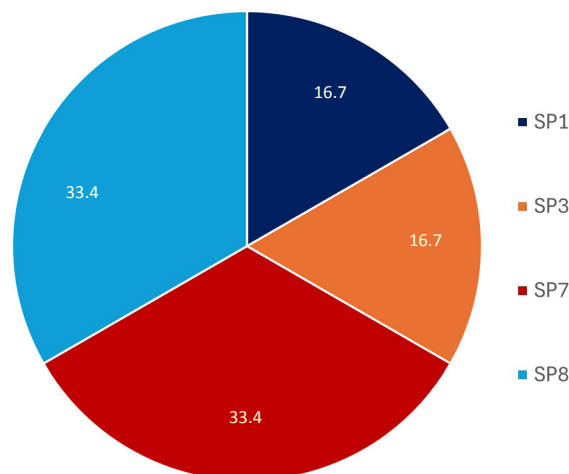


Figure 1. Chart representation of stations with laid eggs of sea turtles in the study area.

Table 1. PCR Conditions details used in the present study.

Steps	Temperature °C	Time	Cycles
Initial denaturation	95	3 min	1
Denaturation	95	15 sec	35
Annealing	50	30 sec	
Extension	72	45 sec	
Final extension	72	3 min	1
Hold	4	∞	1

Systems and GenBank are presented in Table 5. Both *Eretmochelys imbricata* and *Lepidochelys olivacea* showed a 100% similarity (0% divergence) to reference sequences, signifying high confidence in species identification. Taxonomic identities, including taxon assignment, and associated probabilities for the two species identified in Turtle Bay, Cilacap, are summarized in Table 6.

DISCUSSION

This study utilized a combination of fieldwork for data collection and wet lab experiments. The morphological aspect of the study involved a series of body

Table 2. Protocol optimization for tissue sampling following Maduppa et al. (2019).

	Tissue type	Tissue sampling	Preservation	Extraction	Amplification
1	Dead baby turtle	rear-flipper	96% ethanol	ZYMO, D6016	Bioline, BIO-25048
2	Dead GT turtle	Flipper	96% ethanol	ZYMO, D6016	Bioline, BIO-25048
3	Egg Clutches	Embryo	96% ethanol	ZYMO, D6016	Bioline, BIO-25048

Table 3. Number of sea turtle egg clutches retrieved from the study locations.

	Code	Sampling site	Date (found)	Eggs
1	SP1	Sodong	July 2	65
2	SP2	Srandil	-	-
3	SP3	Welahan Wetan	Aug. 21	100
4	Sp4	Widarapayung Kulon	-	-
5	Sp5	Sidayu	-	-
6	Sp6	Widarapayung Wetan	-	-
7	Sp7	Sidaurip	July 9, 11	NA, 96
8	Sp8	Pagubugan	July 7	30, 80

The eggs were retrieved from SP1, SP6, SP7, and SP8, respectively.

Table 4. Morphological identification of samples of a dead turtle (GT sample) and baby turtles (OR) sourced from the study area.

	Features	GT Sample	OR (baby turtles)
1	Costal scutes	4	5–7
2	Carapace color	Brown	Grey
3	Carapace length CCL	66 cm	-
4	Carapace width CCW	57 cm	-
5	Carapace shape	Oval	Slightly overlapping
6	Prefrontal scales	2	4
7	Plastron	White	White



Image 2. Studied samples of two species of sea turtles in Turtle Bay of Cilacap (left): dead and partially decayed sample of *E. imbricata* ("GT") (right): babies of *L. olivacea* (OR). © Mr. Jumawan and Ikegwu, C.M.

measurements and observations. As shown in Table 4, morphometric characters were collected and compared with data from previous studies. Morphological identification involved body measurements and observations based on the guidelines of Pritchard & Mortimer (1999). In Olive ridley, (OR samples), morpho-data were obtained from baby turtles. The recorded costal scutes were 5–7, grey colouration, slightly overlapping carapace, two pairs of prefrontal scales, and white plastron colour. It's morphometrics were consistent with Pritchard & Mortimer (1999), who reported 6–9 costal scutes in *L. olivacea* (with occasional occurrences of five in hatchlings), two pairs of prefrontal scales, and a slightly overlapping carapace in juveniles.

For the GT sample, which was a dead and decayed specimen with degraded body parts, morphological identification proved challenging. Initially, the sample was tentatively identified as *Chelonia mydas* due to

certain features such as the presence of four costal scutes—a trait shared by both Green and Hawksbill Turtles. Additionally, the measured curved carapace length (CCL) was 66 cm, classifying the specimen as juvenile, since *C. mydas* & *E. imbricata* reach sexual maturity at >85 cm, and 78.8 cm CCL, respectively (Liles et al. 2011; Zárate et al. 2013). The juvenile state complicated the classification as either *C. mydas* or *E. imbricata* based solely on CCL data. Most external morphological characteristics aligned with those of *C. mydas*, despite the initial uncertainty. These included the presence of a pair of prefrontal scales, which is a distinguishing feature of Green Turtles compared to Hawksbill Turtles. The observed specimen had a pair of prefrontal scales, supporting its tentative classification as a Green Turtle. Regarding plastron colour, the remaining intact parts were brown, consistent with Pritchard & Mortimer's (1999) description of immature *C. mydas*.

Table 5. Similarity (BOLDsystems) and identity (GenBank) and accession number details.

	Sample Initial	Similarity %	Identity %	Reference Species
1	GT	100	100	<i>Eretmochelys imbricata</i> JX454970
		100	100	<i>E. imbricata</i> GQ152886
		100	100	<i>E. imbricata</i> KU254594
		100	100	<i>E. imbricata</i> KP221806
2	OR1	100	100	<i>Lepidochelys olivacea</i> JX45991
		100	100	<i>L. olivacea</i> NC_028634
		100	100	<i>L. olivacea</i> JX454979
		100	100	<i>L. olivacea</i> JX454987
3	OR2	100	100	<i>L. olivacea</i> JX454991
		100	100	<i>L. olivacea</i> NC_028634
		100	100	<i>L. olivacea</i> JX454979
		100	100	<i>L. olivacea</i> JX454987
4	OR3	100	100	<i>L. olivacea</i> JX454991
		100	100	<i>L. olivacea</i> NC_028634
		100	100	<i>L. olivacea</i> JX454979
		100	100	<i>L. olivacea</i> JX454987

as having brown colouration with radiating streaks. This characteristic varies significantly in adults, ranging from plain to streaked or spotted in shades of brown, buff, or other earth tones, further supporting the identification as *C. mydas*.

In terms of carapace shape, both Green and Hawksbill Turtles have an oval carapace. In contrast, Hawksbill Turtles possess strongly serrated posterior margins, whereas Green Turtles have a broadly oval, non-serrated carapace. The observed carapace lacked prominent serrations, but given the specimen's degraded condition, it is possible that the absence of strong serrations resulted from damage, complicating definitive identification. Overall, while the morphological evidence pointed toward *C. mydas*, the degraded state of the specimen and overlapping characteristics with *E. imbricata* highlighted the challenges of relying solely on morphological identification.

In contrast, molecular analysis provided more definitive results. Tissue samples were collected following the protocols of Dutton (1996) and Carreras et al. (2007) for DNA extraction and amplification (Table 2). The CO1 mitochondrial DNA gene was used for species identification through DNA barcoding, a method praised for its precision, and wide applicability (Madduppa et al. 2019). DNA barcoding allowed for accurate species identification even in the absence of complete morphological data, as demonstrated by Abreu-Grobois et al. (2006). This method has been successfully applied across various taxa, including fish larvae (Nuryanto et al. 2023a), fish from family Sparidae (Nuryanto et al. 2023b), Antarctic Fish (Belchier & Lawson 2013), and sea turtles (Bahri et al. 2017). Furthermore, Hernandez et

Table 6. Identification summary for *Eretmochelys imbricata* and *Lepidochelys olivacea*.

	Linnaean rank	<i>Eretmochelys</i> sp.	<i>Lepidochelys</i> sp.	Probability %
1	Phylum	Chordata	Chordata	100
2	Class	Reptilia	Reptilia	100
3	Order	Testudines	Testudines	100
4	Family	Cheloniidae	Cheloniidae	100
5	Genus	<i>Eretmochelys</i>	<i>Lepidochelys</i>	100
6	Species	<i>Eretmochelys imbricata</i>	<i>Lepidochelys olivacea</i>	100

al. (2013) supported taxonomic sufficiency at the genus level for analyzing assemblage diversity.

For the GT sample, DNA barcoding contradicted the initial morphological identification. While certain morphological characters initially suggested it was *Chelonia mydas*, the genetic analysis revealed it to be *Eretmochelys imbricata*, as confirmed by a 100% (0% divergence) match in the BLAST search, although the BIN (ACE8206) on BOLD is still undergoing metadata validation. This outcome demonstrates the advantages of molecular approaches over traditional methods, particularly when morphological data is incomplete or unreliable. Additionally, studies such as those by Pereira et al. (2013), Lim et al. (2016), Briñoccoli et al. (2020), and Mohammed-Geba et al. (2021) have shown that intraspecific genetic similarities ranging from 92.5% to 99% can serve as species borders in various research contexts (Nuryanto et al. 2017; Winarni et al. 2023). Čandek & Kurtner (2015) further emphasized the importance of geographic locality information for accurate species delineation.

The CO1 target was used in this study to assess the similarity and identity of sea turtles in Turtle Bay, Cilacap. Amplification of two tissues (two baby turtles), an embryo for OR, and tissue from an unidentified GT, from different individuals yielded 100% similarity (0% divergence) to CO1 sequences in BOLD Systems and GenBank for *L. olivacea*, and from one tissue yielded *E. imbricata* (Table 5). Nuryanto et al. (2023) reported that 99% genetic similarity is a reliable species delineation threshold, consistent with findings from other studies (Ratnasingham & Hebert 2013; Nuryanto et al. 2018; Amatya 2019; Kusbiyanto et al. 2020).

In the context of phylogenetic studies, Yang & Rannala (2010) revealed that phylogenetic analysis employs molecular techniques to identify and analyze the connections among closely related species in systematics and taxonomy. The current study provides a phylogenetic

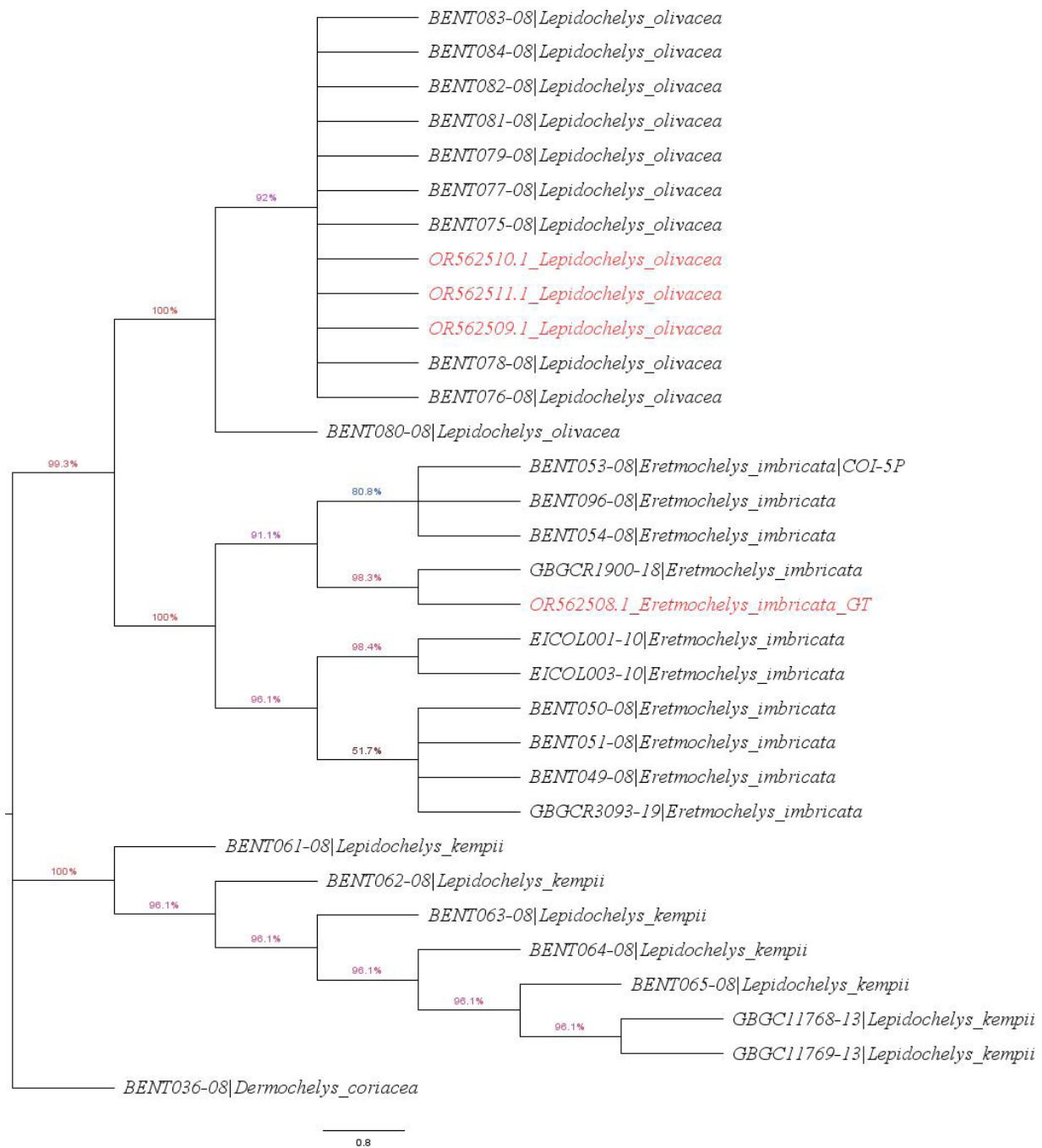


Figure 2. Strongly resolved Neighbor-Joining tree of CO1, showing molecular identifications of *Lepidochelys olivacea* and *Eretmochelys imbricata* samples studied nested within the published sequences of these species.

overview of *Lepidochelys olivacea* and *Eretmochelys imbricata* found in Indonesia. The phylogeny was largely consistent with other marine phylogenies (Bowen et al. 1993; Dutton et al. 1996). Phylogenetic analysis, supported by high bootstrap values, clearly showed that all sequences of *L. olivacea* obtained in Indonesia could be grouped into a single genetic lineage. Specifically, OR1,

OR2, and OR3 identified as *L. olivacea* clustered with sequences BENT083-08, BENT084-08, and BENT082-08 from Ghana, as well as BENT081-08, BENT079-08, BENT077-08, BENT075-08, BENT078-08, and BENT076-08 from Australia, achieving a bootstrap value of 92%. At the larger clade level, the bootstrap support is 100%. Meanwhile, for GT, the sequence in the present study

falls within a well-supported *Eretmochelys imbricata* clade, with a 91.1% bootstrap value for its subclade, indicating strong support, while another subclade within *E. imbricata* from BOLD has 96.1% support. The larger *E. imbricata* clade is highly supported at 100%. Additionally, the sequence in the present study forms a sister species relationship with GBGCR1900-18 from Chinese waters, with a strong bootstrap support of 98.3%, highlighting their close genetic similarity.

For *L. olivacea*, despite being from different geographical locations, the clustering in a single clade implies low genetic divergence, indicating a close evolutionary relationship. This suggests divergence from a common recent ancestor, even though they are separated by distance. The single individual sequenced from Indonesia identified as *E. imbricata* also clustered with a species from China, further implying low genetic divergence.

Despite the findings, there may be limitations in this study due to the restricted number of morpho-characters analyzed and the limited data available to demonstrate the complete relationship of *E. imbricata* with other species, as only one individual was identified. This study contributes to systematic biology by demonstrating the importance of integrating morphological and molecular techniques for species identification, particularly in cases where morphological data alone are insufficient. By using DNA barcoding alongside traditional morphological methods, the research highlights how molecular tools, such as the CO1 gene, can provide more precise identification of species, addressing the limitations of morphology-based approaches. The identification of *Eretmochelys imbricata* through genetic analysis, despite initial misclassification based on morphology, underscores the growing need for molecular methods in systematics.

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