

Genetic of stranded *Dugong dugon* (Müller 1776) in the Java Sea, Indonesia, through COX1-based DNA barcoding

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Abstract. Dewi CSU, Wahyudi S, Tarno H, Wiadnya DGR, Iranawati F, Sukandar, Martinah A, Sani LMI, Subhan B, Herandarudewi SM, Faiqoh E, Ciptadi G. 2025. Genetic of stranded *Dugong dugon* (Müller 1776) in the Java Sea, Indonesia, through COX1-based DNA barcoding. *Biodiversitas* 26: 951-962. The dugong (*Dugong dugon* (Müller 1776)), an endangered herbivorous marine mammal, is listed as the Red List of the IUCN and CITES Appendix I. Considering its protected categorizing, its population is persistently decreasing globally, particularly in Indonesia, accordingly requiring significant conservation measures. This study aimed to assess the conservation status of the species, analyze the morphology, morphometry, and genetic profile of stranded dugongs, and evaluate surrounding seagrass meadows. Fieldwork undertaken in February, March, and September 2023 concentrated on stranded dugongs, gathering morphological, morphometric, and genetic data, in addition to surveying seagrass habitats using satellite photography. Morphological observations encompassed 14 criteria, whilst tissue samples from pectoral fins were utilized for genetic profiling by phylogenetic analysis. Geographic coordinates identified stranded dugongs, whereas seagrass regions were assessed with Sentinel-2 data and QGIS. The results indicated that genetic investigation verified the species' strong affinity with populations in the Java Sea and New Zealand, with more divergence from dugongs in Kerala, India. The seagrass beds adjacent to the dugongs' habitat ranged from 0.21 to 16.68 hectares within a larger network of 55.38 to 85.35 hectares, underscoring their potential as a protection zone. These findings underscore the essential link between dugong conservation and seagrass ecosystem conservation, making it clear that both aspects are interconnected and necessitate combined protection methods.

Keywords: Conservation, morphology morphometry, phylogenetic analysis, seagrass bed, stranded dugong

Abbreviations: BKSDA: Natural Resources Conservation Agency; BPSPL: Coastal and Marine Resources Management Center; BRIN: National Research and Innovation Agency; CITES: Convention on International Trade in Endangered Species of Wild Fauna and Flora; COI: Cytochrome c Oxidase Subunit I; Cyt b: Cytochrome b; D loop: Displacement Loop; DIB: Depository Ihtologiikum Brawijaya; DNA: Deoxyribonucleic Acid; PCR: Polymerase Chain Reaction; FPIK: Faculty of Fisheries and Marine Sciences; IUCN: International Union for Conservation of Nature or the International Union for Nature Conservation; KKP: Ministry of Marine Affairs and Fisheries; KLHK: Ministry of Environment and Forestry; KSDAE: Conservation of Natural Resources and Ecosystems; QGIS: Quantum Geographic Information System; SAJI - DN: Domestic Fish Type Transport Letter; SKIKH: Secretariat of Scientific Authority for Diversity Hayati; UB: Universitas Brawijaya

INTRODUCTION

Dugong dugon (Müller 1776) is a herbivorous marine mammal from the order Sirenia (Domning and Beatty 2012; Marsh and Soltzick 2019). Furthermore, it is the only

remaining species of the Dugongidae family after *Hydrodamalis gigas* (Zimmermann, 1780) was declared extinct (Yamato et al. 2021; Awadh et al. 2024). Dugong is reported to be distributed in more than 40 countries in the world, including Vanuatu and Africa (Cleguer and Marsh

2023; Cleguer et al. 2024). However, its global population, such as in Indonesia, continues to decline due to internal biological factors, including low reproductive ability (Burgess et al. 2012; Heudier et al. 2023). This condition is elevated by the presence of disruptive environmental factors, such as coastal land conversion, sea transportation routes, fishing activities, tourism activities, pollution, or input from land which leads to flooding (Plön et al. 2019; Lin et al. 2022; Gole et al. 2023). Several of these environmental factors have disrupted and damaged the seagrass ecosystem, a habitat of dugong (Cleguer et al. 2020; Kayanne et al. 2022; Seal et al. 2024).

Coastal ecosystem degradation, including seagrass beds, as the only source of food for dugongs, worsens the conservation status (Scott et al. 2018; Budiarsa et al. 2021; Panyawai and Prathep 2022; Cleguer et al. 2024). This mammal has also been reported to thermoregulate and rest in coral reef and mangrove ecosystems (Kayanne et al. 2022; Cleguer and Marsh 2023). The utilization of coastal and marine spaces, alongside associated human activities, is considered a significant factor contributing to the increasing reports of dugong strandings. Consequently, efforts are needed to strengthen conservation efforts with the use of marine space (Heudier et al. 2023).

Cases of dugongs stranded dead on the beach have become increasingly frequent, raising concerns about species conservation efforts, including in Indonesia (Lin et al. 2022; Khamis et al. 2023; Dewi et al. 2024). Stranding events of dugongs have been documented in several areas of Indonesia, including East Java. Recent reports indicate that dugongs have been found both alive and dead along the coasts of these regions (Kurniawati and Hidayat 2018; Husna and Kusumawati 2022; Dewi et al. 2024). A primary factor contributing to the stranding is the destruction of seagrass habitat, which serves as the main food source (Lin et al. 2022; Andriansyah 2023). A study focused on the morphology and morphometry of stranded dugongs for species confirmation remains limited. Existing morphological and morphometric investigations are predominantly conducted using skeletons in museums (Adulyanukosol et al. 2011; Nganvongpanit et al. 2017; Boonsri et al. 2021; Lanyon et al. 2021). In Indonesia, the stranded dugong is typically buried without morphological or morphometric observations, and tissue is not collected for genetic analysis (Kurniawati and Hidayat 2018; Husna and Kusumawati 2022). This is primarily due to the conservation status, which imposes strict restrictions on the utilization of the mammals as study objects.

Genetic information related to dugong remains limited worldwide, while several studies on dugong population have been conducted and reported in Australia, Thailand, and India (Plön et al. 2019; Srinivas et al. 2021; Pommouang et al. 2022). The results present the importance of microsatellite polymorphisms in understanding the genetic structure of the populations and supporting conservation strategies. Previous investigations identified vulnerable populations in the Indian Ocean through phylogeographic analysis, emphasizing the need for conservation efforts in the region (Plön et al. 2019). In Indonesia, genetic information on dugongs is also limited. Several genetic investigations

were conducted using bone tissue collected from various museums in the Netherlands, Norway, and Australia, relying on mitochondrial DNA coding analysis of the D-loop gene (Hunter et al. 2018; Plön et al. 2019; Ooi et al. 2022). This study aimed to (i) trace the conservation status of dugong species, (ii) analyze the morphology and morphometry of stranded dugongs, (iii) analyze the genetic profile of stranded dugongs, and (iv) analyze the area of seagrass beds around stranded mammal.

MATERIALS AND METHODS

Research location

Data collection and sampling were conducted in February, March, and September 2023. The locations for sourcing morphological and morphometric data, coordinates of seagrass beds presence, as well as tissue samples were determined based on community reports of dugong encounters (Dewi et al. 2024). Stranded and dead dugongs were found on Tanjung Beach and Raba Beach in Lebak Village, Bawean Island, Gresik Regency, and Sapulu Beach, Bangkalan Regency, East Java, Indonesia. (Figure 1). The obtained samples were stored at DIB-Marine and Fisheries Resources Utilization Laboratory, FPIK-UB, Malang. Tissue samples for genetics were analyzed at the Oceanogen Laboratory-Bogor. Meanwhile, other data analysis was conducted at the Marine and Fisheries Resources Utilization Laboratory, FPIK-UB, Malang, East Java, Indonesia.

Dugong accidentally stranded

Two dugongs found were discovered stranded on different beaches and dates on Bawean Island, Gresik Regency, East Java, Indonesia (Figures 2.A and 2.B). The island was located 146 km from the center of Gresik City, a remote area with minimal visitors. Due to the isolation, the stranded dugongs were discovered in a worrying condition, possibly having been dead for several days. The incident was reported by Subhan, Head of the Green Leaf Monitoring Group, to the East Java First Response Network, established by the BPSPL Unit in Denpasar, East Java. This was followed by reporting the incident to the East Java KSDA Center, as the discovery was a fully protected species.

An enumerator coordinated the collection of tissue samples from the pectoral fins before storage in 70% alcohol (the most appropriate preservation method in local conditions). These samples, intended for DNA, were transferred to the DIB located at the Exploitation Laboratory, FPIK-UB. Each sample was assigned a DNA access code, and the preservation solution was replaced with saturated alcohol.

The classification system for stranded marine mammals was divided into five categories. These include stranded alive (i), stranded dead but in good condition (ii), stranded and showed early signs of decomposition (iii), experienced advanced decomposition (iv), and discovered in a mummified state or only in the form of a skeleton, respectively (v) (Herandarudewi 2019; Husna and Kusumawati 2022).

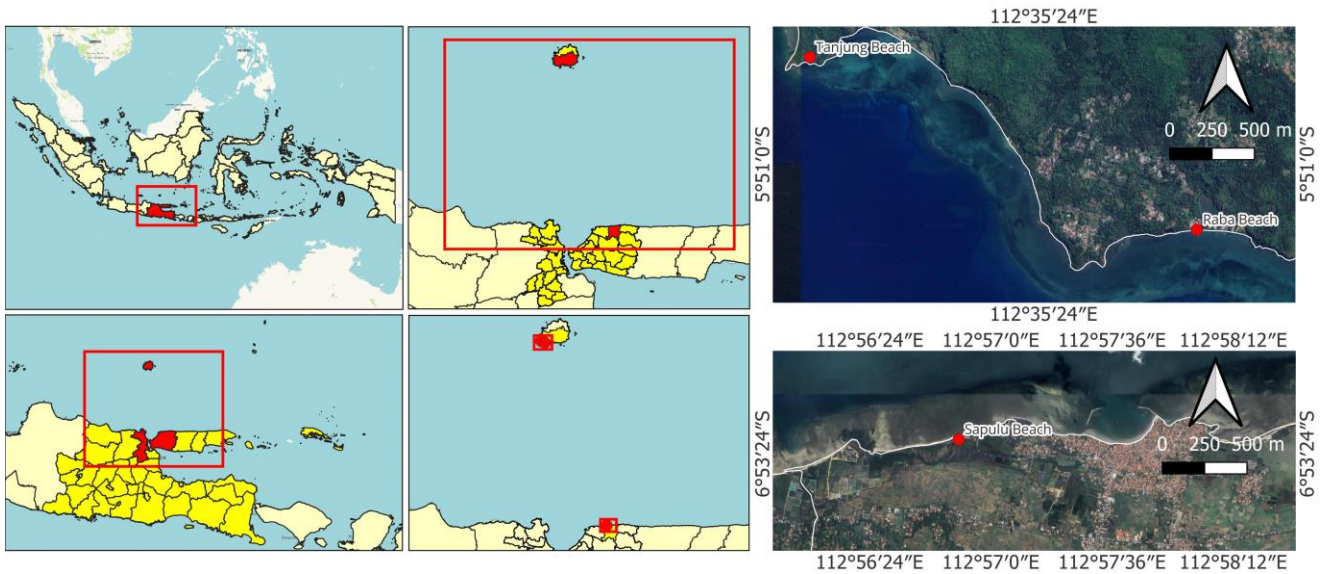


Figure 1. Locations where stranded and dead dugongs were found: Tanjung Beach and Raba Beach in Lebak Village, Bawean Island, Gresik Regency, and Sapulu Beach, Bangkalan Regency, East Java, Indonesia

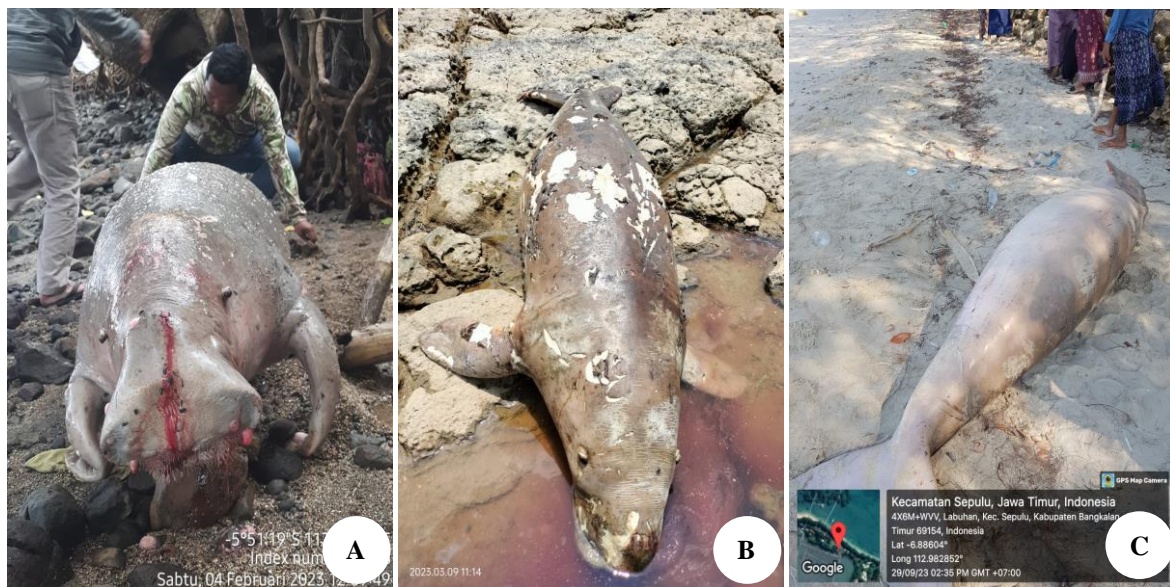


Figure 2. Stranded and dead dugongs were found at the research location, with tissue samples collected for identification purposes. Three dugongs were stranded at: A. Tanjung Beach, Lebak Village, Bawean Island, Gresik ($-5^{\circ}51'10''$ S, $112^{\circ}36'54''$ E); B. Raba Beach, Lebak Village, Bawean Island, Gresik ($-5^{\circ}85'08''$ S, $112^{\circ}58'87''$ E); and C. Tengket - Sapulu Beach, Bangkalan ($-6^{\circ}88'85''$ S, $112^{\circ}90'83''$ E), where biological tissue samples were collected for genetic analysis

The first stranded dugong was identified on 4 February 2023 and classified under Category 3 because it was dead, bloated, and smelled, as shown in Figure 2.A. The second, which was discovered on 9 March 2023, also on Bawean Island - Gresik, fell under Category 4, signifying severe decomposition with peeling skin and a pungent odor, as shown in Figure 2.B. Meanwhile, the last dugong was identified on 28 September 2023 at Sapulu Beach, Bangkalan Regency. It was classified under Category 2 because it had recently died, remained in good condition, and showed no signs of decay and odor, as presented in Figure 2.C.

Regulatory framework and authorization procedures for the use of protected organisms

The research protocol for studying dugong (*Dugong dugon*) genetics involved multiple administrative and regulatory compliance steps. The conservation status assessment began with reviewing the IUCN Red List criteria and CITES listings, while the Indonesian protection status was determined through comprehensive legal document analysis from governmental sources. The research authorization process followed a structured pathway that commenced with obtaining faculty approval from FPIK-

UB, followed by securing an ethics permit (Certificate No. 166-Kep UB-2023) from Brawijaya University's Bioscience Laboratory. Subsequently, the research team acquired a Domestic Fish Transport Letter (SAJI-DN No. B.2057/BPSPL.4/PRL.430/VI/2024) from the Ministry of Maritime Affairs and Fisheries through BPSPL 4-Denpasar. The process continued with obtaining a genetic resource access recommendation (No. B-4860/IV/KS.00/6/2024) from SKIKH-BRIN. The final authorization was granted through Decree No. 191 of 2024 from KSDAE-KLHK, permitting genetic resource utilization in accordance with Indonesian Laws No. 5/1990 and No. 32/2024 on Biological Natural Resources and Ecosystem Conservation. This comprehensive permitting process ensured ethical research conduct while maintaining compliance with national and international conservation regulations.

Morphological observation and morphometric measurement of Dugong

Morphological observations and morphometric measurements were performed on stranded and dead dugongs. At the same time, the process of obtaining a permit for the analysis was initiated. During the permit application process,

another stranded dugong was discovered on Sapulu Beach, Bangkalan Regency, East Java. This information, provided by the Bangkalan Regency fisheries extension officer, Arief Rachman, prompted an amendment of the permit. The condition of this dugong was relatively fresh, as the location was only 34 km from the city center, enabling the enumerators to conduct morphological observations and morphometric measurements. These observations and measurements aimed to identify distinguishing characteristics and morphological variations that contribute to the taxonomy of the species. This process includes the analysis of 14 traits, as shown in Figure 3 and described in Table 1. Furthermore, the weight of the stranded dugong was estimated using the following equation: $y = 19.108 \cdot x^{2.8103}$ ($R^2 = 0.945$). Where y represents the weight of the dugong (kg) and x represents the length of the dugong (m) (Adulyanukosol et al. 2011).

A total of 14 specific characteristics of dugongs were observed and measured (Table 1). These observations and measurements were modified from the previous analysis (Adulyanukosol et al. 2011; Nganvongpanit et al. 2017; Boonsri et al. 2021; Lanyon et al. 2021).

Table 1. Codes and descriptions for morphological observations and morphometric assessments of Dugongs

Code	Measurement	Assessment description
a	Total length	Distance from the anterior tip of the dugong's mouth to the tail (cm)
b	Maximum body circumference	Maximum abdominal circumference of the dugong (cm)
c	Pectoral fin width	Maximum width from the left to the right side of the pectoral fin (cm)
d	Pectoral fin length	Maximum length from the base to the tip of the pectoral fin (cm)
e	Tail shape and width	Shape of the caudal fluke in a horizontal position and the distance from the right to the left tip of the tail (cm)
f	Nose diameter	Diameter of the nostrils (cm)
g	Mouth width	Distance from the right tip to the left tip of the dugong's mouth (cm)
h	Pad width	Distance to the widest part of the pad located at the base of the dugong's head (cm)
j	Umbilicus	Presence in the middle of the body (present/absent)
k	Genital slit	Presence between the umbilicus and the anus (present/absent)
l	Anus	Presence near the tail (present/absent)
m	Distance from umbilicus to genital slit	$m > n =$ Female
n	Distance from genital slit to anus	$m < n =$ Male

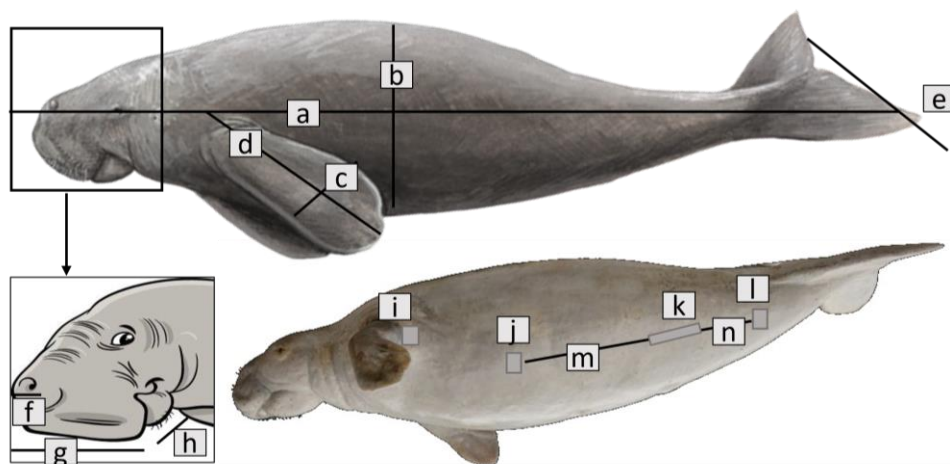


Figure 3. Dugong body parts function as differentiating factors in morphological observations and morphometric measurements. For more detailed information, see Table 1

DNA extraction

DNA extraction was performed using Qiagen DNeasy Blood and Tissue Kits to obtain genomic DNA from collected tissue samples (Jeunen et al. 2022). Approximately 10 g of the samples were cut into small pieces, followed by the addition of 180 μ L Buffer ATL and 20 μ L Proteinase K. The mixture was vortexed and incubated at 56°C until the tissue was completely dissolved (~100 minutes). Following this, 200 μ L of Buffer AL was added, vortexed, and incubated at 56°C for 10 minutes. DNA binding was facilitated by adding 200 μ L of absolute ethanol to the sample, which was then vortexed and centrifuged at 8,000 rpm on a spin column for 1 minute. DNA washing was performed by adding 500 μ L of AW1 solution to the spin column, followed by centrifugation at 8,000 rpm for 1 minute. A second wash includes adding 500 μ L of AW2 solution and centrifuging at 14,000 rpm for 3 minutes to ensure the removal of the residual solution. DNA elution was completed by adding AE solution to the spin-column membrane, followed by centrifugation at 8,000 rpm for 1 minute. The extracted DNA, preserved in AE solution, was stored at -20°C until further analysis.

PCR, electrophoresis, and DNA sequencing

The PCR process used primers LCO 1490 and HCO 2198, with a target of 700 bp mitochondrial DNA, appropriate to the CO1 region commonly adopted for the identification of metazoan taxa (Folmer et al. 1994). In this analysis, DNA amplification was performed using 13 μ L MyFi 2x hotstart polymerase (bioline), 1 μ L forward primer, 1 μ L reward primer, 9 μ L ddH₂O, and 3 μ L of DNA isolate. The amplification process comprised 35 cycles, starting with initial denaturation at 95°C for 480 seconds, followed by denaturation at 95°C for 45 seconds, annealing at 45°C for 45 seconds, and extension at 72°C for 300 seconds. Additionally, the PCR products were visualized by electrophoresis on a 2% agarose gel, and sequencing was performed using the Sanger method at Apical Scientific, Malaysia.

Sequencing analysis

The forward and reverse sequences of each specimen were checked and edited using Chromas (www.technelysium.com.au) and EuGene (www.eugene.toulouse.inra.fr) to ensure precise matching between the sequences. Mesquite software (www.mesquitproject.org) was applied to detect possible stop codons. The edited sequences were then compared with those uploaded to GenBank (www.ncbi.nlm.nih.gov) through BLAST (Basic Local Alignment Search Tool) and integrated with the Barcode of Life Data (BOLD) system (www.v4.boldsystems.org). Species identification was performed by searching with high similarity (>99% identity) in the nucleotide database of GenBank. The indicators considered during the BLAST analysis were query cover, identity, and e-value. Query

cover signified the validity of the analysis performed, with low values suggesting that high identity results may be invalid due to inadequate coverage (Gaffar and Sumarlin 2020). An identity close to 100% represents a strong similarity between the query and the reference sequence, which is typically expected in BLAST analysis. However, high identity without adequate query cover could raise doubts about the accuracy of the match (Gaffar and Sumarlin 2020). A low e-value, especially close to 0, represents the high significance of the search results, implying minimal occurrence probability by chance. In many analyses, an e-value below 0.01 is considered a strong indicator of the result's validity (Anwar et al. 2022).

Phylogenetic analysis in this study included 4 dugong sequences retrieved in NCBI, as shown in Table 2, with accession numbers KF430645 (Kerala, India), NC003314 (Australia), AJ421723 (Australia), and AY075116 (New Zealand). The phylogenetic tree was constructed using 2 manatee sequences, *Trichechus manatus* (Linnaeus, 1758) as outgroups. The sequences were sourced from French Guinea (HQ918423) and Mexico (KX451316). Several base substitutions per site were calculated based on the average of all sequence pairs among the groups. This analysis was performed using the Maximum Probability model (Kimura 2-parameter, 1000 bootstrap replicates) (Tamura et al. 2021). The dataset includes three sequences from dead-stranded dugongs, four dugong sequences from NCBI, and two manatee sequences from NCBI. The matching process was performed by smoothing each base pair of sequences (with the pairwise deletion option). Additionally, the final dataset consisted of 666 positions, and all genetic analyses were executed using MEGA11 (Tamura et al. 2021).

Confirmation of seagrass area

Confirmation of the seagrass beds' existence and extent at each location was essential, as dugongs are associated with the ecosystems. Therefore, the extent of seagrass beds in each area was mapped. The analysis includes several stages, such as location determination, image processing, and accuracy testing. Advanced technology, such as Sentinel-2A imagery, was utilized due to its geometric and radiometric correction processes, making it suitable for observing changes in seagrass habitats. In the initial stage, the image was cropped to focus on the desired location, followed by the separation of land and sea using a specific algorithm. Depth correction was applied using the Lyzenga Equation or Depth Invariant Index to overcome the effects of the water column, which produced more accurate seabed data (Rosalina et al. 2023). Furthermore, supervised classification was performed using the Maximum Probability method to group image pixels based on spectral characteristics. Accuracy was assessed by comparing the results of image classification with field data, adopting matrices to evaluate classification accuracy.

Table 2. Genetic sequencing of *Dugong dugon* at NCBI

Accession number	Gene	Gen release time NCBI	Location
KF430645	COX1, 658 bp	23 November 2013	Kerala, India
NC003314	Complete genome, 16850 bp	3 April 2023	Australia
AJ421723	Complete genome, 16850 bp	26 July 2016	Australia
AY075116	Complete genome, 16847 bp	26 July 2016	New Zealand

RESULTS AND DISCUSSION

Regulations and authorization procedures for protected organisms

The last evaluation of *Dugong dugon* by the IUCN Red List in 2015 classified the species as vulnerable based on the A2bcd+4bcd criteria. Meanwhile, the utilization status - trade of dugongs worldwide is recorded in Appendix 1 of CITES (Marsh and Soltzick 2019). In Indonesia, this marine mammal has been officially protected by the state under Law Number 5 of 1990 in conjunction with Law Number 32 of 2024 concerning the Conservation of Natural Resources and Ecosystems.

Morphology and morphometry of Dugong

Morphological observations and morphometric measurements were performed on 3 dugongs that were stranded and dead, covering 14 specific characteristics, as shown in Table 3. The lengths recorded were 220 cm, 200 cm, and 280 cm, while the body circumferences measured 160 cm, 152 cm, and 177 cm, respectively, as shown in Table 3. The corresponding weights calculated using the

previously mentioned formula were 174.20 kg, 134.03 kg, and 345.04 kg. Furthermore, the three-stranded dugongs were observed to have an umbilicus, genital opening, anus, and a horizontal caudal fin shape. However, further measurements were only conducted on the specimens stranded at Sapulu Beach in Bangkalan, as shown in Figure 4.

Morphological observations and morphometric measurements of individuals found on Tanjung Beach, Bawean (Dugong A), Raba Beach, Bawean (Dugong B), and Sapulu Beach, Bangkalan (Dugong C) correspond to the 14 specific characteristics described in Table 3.

Phylogenetic analysis

BLAST analysis performed on 3 sequences obtained from stranded specimens showed a query cover of 99%, with an identity percentage exceeding 99% and an e-value of 0 when compared to the AY075116.1 sequence in NCBI. This confirms the presence of *Dugong dugon* species, as shown in Table 4.



Figure 4. Dugong body parts serve as distinguishing factors in morphological observations and morphometric measurements. These morphological adaptations enable dugongs to function as marine mammals capable of diving while still requiring oxygen from the air, with: G. Their nostrils automatically closing during submersion. Additionally, D. The unique shape of their flippers supports movement and balance underwater, while F. Their distinctively shaped mouths reflect a grazing-bulldozer feeding pattern, allowing them to efficiently forage on seagrass meadows and leaving characteristic feeding trails in their habitats. See Table 3 for more detailed information

Table 3. Results of morphological observations and morphometric measurements on stranded *Dugongs*

Character code	Description Genebank accession number	Dugong A PQ039761	Dugong B PQ039762	Dugong C PQ039763
A	Total length (cm)	220	200	280
B	Body circumference (cm)	160	152	177
C	Pectoral fin width (cm)	Not measured	Not measured	39.5
D	Pectoral fin length (cm)	Not measured	Not measured	25
E	Tail shape and tail width (cm)	Caudal fluke horizontal	Caudal fluke horizontal	Caudal fluke horizontal - 80
F	Nostril diameter (cm)	Not measured	Not measured	2.5
G	Mouth width (cm)	Not measured	Not measured	24.5
H	Bottom pad width (cm)	Not measured	Not measured	10
I	Nipples/Mammary glands (present/absent)	Not measured	Not measured	Yes
J	Navel (present/absent)	Yes	Yes	Yes
K	Genital opening (present/absent)	Yes	Yes	Yes
L	Anus (present/absent)	Yes	Yes	Yes
M	Navel distance - Genital opening	Not measured	Not measured	m > n = Female
N	Genital opening distance - Anus	Not measured	Not measured	

Table 4. BLAST analysis results based on NCBI for species identification

Sample	Query cover (%)	Per. ident (%)	E value	Genbank accession number of the comparative species	Species identified
PQ039761	99%	99.85%	0	AY075116.1	<i>Dugong dugon</i>
PQ039762	99%	100%	0	AY075116.1	<i>Dugong dugon</i>
PQ039763	99%	99.39%	0	AY075116.1	<i>Dugong dugon</i>

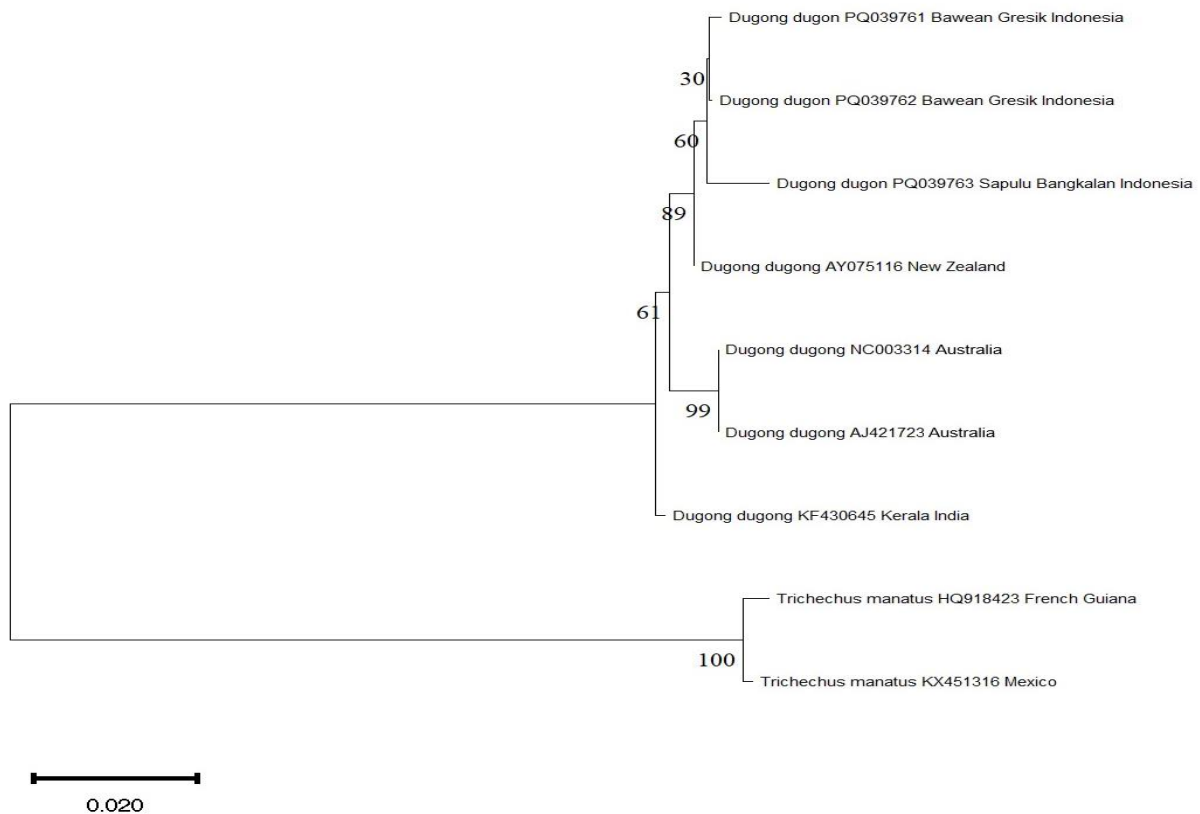


Figure 5. The *Dugong dugon* phylogenetic tree was designed using the Maximum Probability method with the Kimura 2 parameter evolution model, and the accuracy was tested with 1000 bootstrapping times

The phylogenetic tree obtained from the analysis shows that the 2 sequencing data from dugong specimens stranded on Bawean Island in Gresik had a closer relationship compared to sequencing data from dugongs in Sapulu Beach, Bangkalan (Figure 5). These sequences collectively formed a unique group, referred to as Java Sea Dugongs, distinct from their Australian and New Zealand counterparts. This group had a closer affinity to the New Zealand Dugong population than the Australian counterparts, with the furthest genetic distance observed in comparison to Kerala Dugongs, India; a total of two sequences clustered separately, representing *T. manatus* as the outgroup.

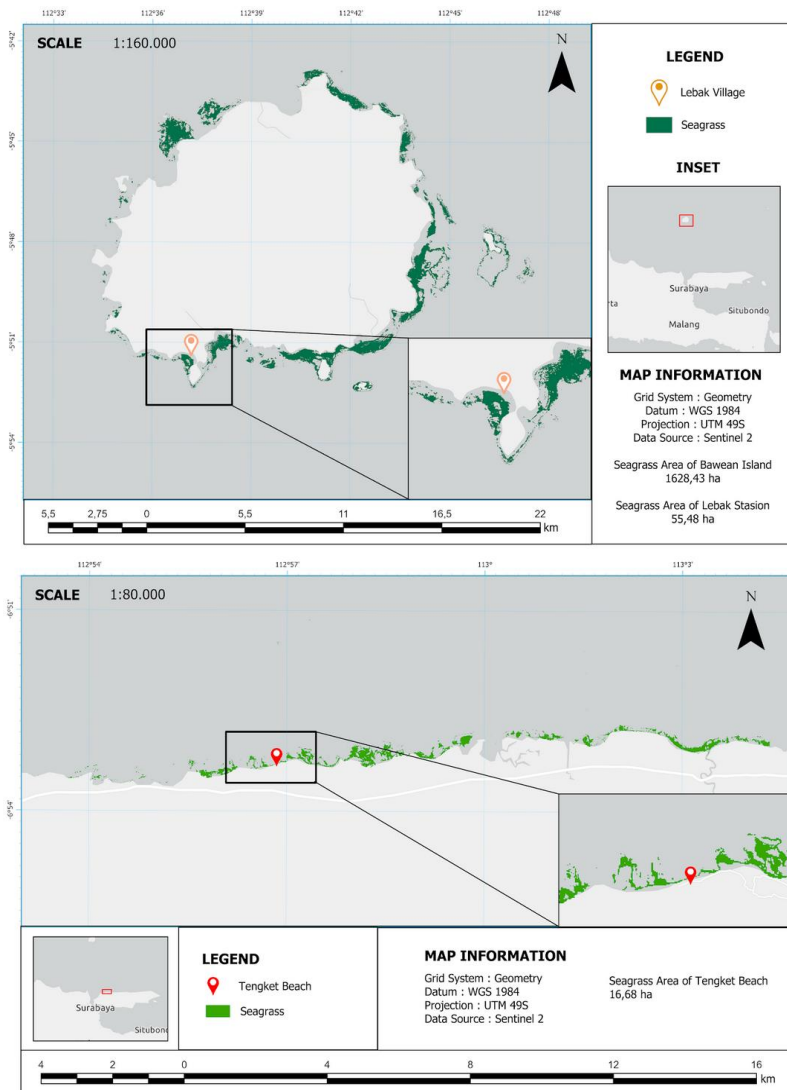
Seagrass bed area

The results of Lyzenga analysis using Sentinel-2 Imagery conducted on the QGIS application showed the presence of seagrass beds on Bawean Island in Gresik and the North Coast of Sapulu District in Bangkalan, with an area of 1,628.43 Ha and 85.35 Ha respectively, as presented in Figure 6. The beds around the stranded dugong on Tanjung and Raba Beach, Lebak Village, Bawean Island in Gresik

had an area of 0.26 Ha and 0.21 Ha, while the total area in Lebak Village was 55.38 Ha. In Tengket Beach, Sapulu District in Bangkalan, an area of 16.68 Ha was recorded. Additionally, the total area of seagrass beds in the Sapulu District of Bangkalan was 85.35 Ha, as shown in Table 5.

Table 5. The extent of the seagrass beds around the location where the dugong was stranded

Location name	Seagrass field area (Ha)
Bawean Island	1,628.43
Lebak Village, Bawean Island	55.38
Tanjung Beach, Bawean Island (Location 1)	0.26
Raba Beach, Bawean Island (Location 2)	0.21
Sapulu, Bangkalan	85.35
Tengket Beach, Sapulu, Bangkalan (Location 3)	16.68



A

B

Figure 6. The area of seagrass beds on: A. Bawean Island; B. The north coast of Bangkalan Regency

Discussion

Dugong is included in the IUCN Red List as vulnerable, reflecting the population decline due to limitations in reproduction and anthropogenic pressures that directly or indirectly threaten its habitat (Ooi et al. 2022; Andriansyah 2023; Awadh et al. 2024). IUCN assigns the full conservation status of this marine mammal with the code A2bcd+4bcd (Marsh and Soltzick 2019), which reflects factors such as population size, geographic range, rate of population decline, and number of specimens capable of reproducing. Specifically, the code signified that the population has been declining for some time, and the ability to recover remains uncertain. Dugongs are also reported to have a fragmented, isolated distribution pattern, leaving only a small number of adults in the population.

The dugong is not only protected but also exploited and traded in the world, leading to its inclusion in Appendix 1 of CITES (Marsh and Soltzick 2019). The status has been in effect since 1975 in over 40 countries, except Australia. In the same year, the dugong population in Australia was high, leading to the inclusion in Appendix 2 of CITES. The population has continued to decline, prompting the reclassification into the CITES Appendix 1 list on 19 July 2020. Wildlife trade, including dugong, persists, especially in high-tourist locations such as Bali, where parts of the body are exploited (Lee and Nijman 2015; Chavez et al. 2024).

The protection of endangered species in Indonesia has received special attention since the colonial era, in 1931 through the Wildlife Protection Ordinance (Dierenbeschermingsordonnantie). This ordinance was revised in 1941 to become the Nature Protection Ordinance (Natuur Beschermings Ordonnantie), aimed at protecting areas and types of wildlife in the Dutch East Indies. The first regulation related to wildlife protection after Indonesian independence was Presidential Decree No. 43 of 1978, which is the legal basis for wildlife protection, including marine mammals. This regulation was later developed into Law No. 5 of 1990 and Law No. 32 of 2024, concerning the Conservation of Natural Resources and Ecosystems, which provides official protection for wildlife. More specifically, regulations for the protection of special types of fisheries and marine life are stipulated in Law No. 31 of 2004. In its development, the law extended to include habitat protection, as stated in Law No. 32 of 2009 concerning Environmental Management, which provided a legal framework for the protection of marine ecosystems, crucial habitats for marine mammals.

A more detailed explanation of Law No. 5 of 1990 concerning the protection of wildlife, including dugongs, is regulated in Government Regulation No. 7 of 1999. This law is further strengthened by the Regulation of the Minister of Marine Affairs and Fisheries No. 6 of 2016, which explains the protection, management, and study of marine mammals to ensure sustainability. In 2018, the Ministry of Marine Affairs and Fisheries issued the Decree of the Minister of Marine Affairs and Fisheries No. 79 of 2018 in the form of a National Action Plan for Marine Mammal Conservation. In the same year, the Ministry of Environment and Forestry issued the Regulation of the Minister of Environment and Forestry No. 20 of 2018

concerning the conservation of plant and animal species. Professionals in the field were crucial in enforcing these regulations. Based on the technical implementation, the parties responsible for licensing the trade of protected organisms are divided into two parts. This included scientific (BRIN) and management authority holders (KLHK and KKP) as stated in Hanif (2021).

The process of managing the permit for the utilization of the dugong network started in August 2023, with all administrative documents finalized by September 2024, with a completion time of 13 months. A notable challenge during this process was the limited availability of clear information related to the technical stages of the administration process. Available electronic resources are very limited and tend to be sectoral, requiring a direct approach to each targeted sector. The development of a comprehensive standard operating procedure (SOP), including BRIN, KLHK, and KKP, accompanied by extensive socialization, could resolve these challenges and streamline the management of permits related to the dugong network.

Dugong is a marine mammal with unique morphological adaptations, including the tail and pectoral fins. The tail is known to be flat, resembling an inverted W, enabling efficient propulsion during swimming (Figure 3.E). It is not only crucial for movement maneuvers but also for maintaining balance when grazing seagrass beds (Nakaoka et al. 2002). The pectoral fins were 39.5 cm long and 25 cm wide, as shown in Figures 3.C and 3.D. These fins have a strong bone structure, facilitating locomotion and enhancing the ability to uproot seagrass at the bottom of the waters (Marshall et al. 2003; Lanyon et al. 2021).

The structure of the dugong skull, specifically the rostrum, is a fascinating example of evolution. It shows a long and flat shape, with a mouth width of 24.5 cm (Figure 3.F), a nostril diameter of 2.5 cm located at the top of the rostrum (Figure 3.G), and a 10 cm in diameter round perioral pad (Figure 3.H). These adaptations allow the dugong to graze in seagrass beds, with strong cranial bones to withstand mechanical pressure due to hard substrates (Boonsri et al. 2021). The nostrils, strategically positioned at the top of the rostrum, play a crucial role in the dugong's life, facilitating efficient breathing during feeding activities at the bottom of the waters and allowing efficient breathing when diving (Domning and Beatty 2007, 2012). Meanwhile, the presence of the perioral pad had fine books that are useful for dugongs in estimating the presence of seagrass (Sun et al. 2017).

Their body structure and reproductive organs can distinguish the male and female dugongs. The females are known to have mammary glands located in the anterior part, near the pectoral fins, generally visible when the dugong surfaces to breathe or rest (Burgess et al. 2012; Lanyon et al. 2021). This was confirmed in the study, where mammary glands were observed in the right and left anterior parts near the pectoral fins, as shown in Figure 3.I. The morphology, characteristics, and functions of the mammary glands are similar to mammals in general. A previous study reported that mammary glands in dugongs had an important function in parenting, namely providing nutrition to children (Rodrigues et al. 2014; Lanyon et al.

2021; Thibault et al. 2024). This emphasizes the nurturing aspect of these marine mammals. A key distinction between the male and the female lies in the distance between the genital opening and the anus, compared to the distance from the genital opening to the navel (Adulyanukosol et al. 2011; Nganvongpanit et al. 2017). In this study, it was discovered that the distance from the genital opening to the anus was shorter compared to the navel. Hence, the stranded dugong was female, as shown in Table 3. Another physical characteristic that stands out in males is the appearance of tusks during puberty, which can be used as an indicator of sexual maturity (Marshall et al. 2003).

Phylogenetic analysis conducted in the study showed that the two stranded dugongs discovered in Bawean were more closely related than those stranded in Sapulu, Bangkalan, as presented in Figure 4. This condition is in line with previous publications that specimens in geographical proximity tend to have close genetic distances due to high opportunities for interaction and gene exchange (Plön et al. 2019; Cleguer et al. 2024). In this study, the three sequences are known to be clustered, forming a group referred to as the Java Sea dugong.

The Java Sea dugong population had a closer genetic distance to the New Zealand and Australian population, than to the Indian counterpart, as shown in Figure 4. According to a previous report, dugongs in the Indo-Pacific region had the same ancestors, while the Indian Ocean possessed unique and different genetic ancestry (Plön et al. 2019; Furness et al. 2024). This is closely related to the fragmentation scenario of the supercontinent Gondwana, which gave rise to the east-west separation. The split between Australia and New Zealand occurred approximately 80 million years ago. In comparison, Madagascar separated 160-121 million years ago, and South America parted from Gondwana around 165-140 million years ago, during the formation of the Indian and Pacific Ocean basins (Toon et al. 2010).

The genetic differences that occurred in dugongs in this study may have occurred due to environmental factors and habitat conditions faced. When the environmental pressures faced are the same, it will produce a fairly close genetic composition (Srinivas et al. 2021). History shows that sea level fluctuations in the Indo-Pacific facilitate the movement and mixing of marine biota populations, including dugongs (Blair et al. 2014; McGowan et al. 2023). The habitats and seagrass beds in the Indo-Pacific, including Australia, New Zealand, and Indonesia have relatively similar distribution patterns and types (Collier and Waycott 2014). Halophila and Halodule seagrasses are the favorite foods of dugongs. Hence, population stability is maintained (Satyaningtijas et al. 2020; Khamis et al. 2023). This also contributes to the genetic connectivity of dugongs. Furthermore, the existence of seagrass beds at the location of dead dugongs facilitates the migration of mammals, which is vital for maintaining genetic diversity and population connectivity (Cleguer et al. 2020; Srinivas et al. 2021).

The distribution of seagrass beds at the study location showed a random distribution pattern, as shown in Figure 5. This condition presents that dugongs at the study location are thought to perform their daily activities by moving

from one seagrass bed to another. According to a previous study, the dugong home range varies, in Indonesia 4.1 km², Torres Strait 942.6 km², Shoalwater Bay 60.6 km², Harvey Bay 24 km², and Cleveland Bay 63.6 km² (De Iongh et al. 2007; Gredzens et al. 2014). These variations in home range size are largely influenced by habitat conditions and the availability of seagrass, a crucial food source (Awadh et al. 2024). While dugongs are not long-distance migratory mammals, they can swim up to 560-1,000 km² in search of new seagrass beds and varied food sources (Kayanne et al. 2021, 2022). However, the movements are limited by water depth, as dugongs tend to avoid predators and have a limited ability to dive for extended periods (Poommouang et al. 2021). Considering the reliance of dugongs on seagrass beds, establishing a network of Marine Protected Areas (MPAs) for seagrass ecosystems is essential for conservation. These efforts should prioritize areas that are at risk of environmental threats (Seal et al. 2024).

This research carries substantial significance for Indonesia's marine conservation efforts, particularly as one of the last remaining strongholds for dugong populations in Southeast Asia. The country's extensive coastal and seagrass ecosystems serve as critical habitats for this marine mammal, making population genetics research essential for evidence-based conservation strategies. Genetic data provides crucial insights into population structure, genetic diversity, and potential inbreeding depression - key indicators of population viability. Moreover, this genetic information reveals both historical and contemporary population connectivity patterns, facilitating the identification of critical habitat corridors and isolated populations requiring specialized conservation attention. The study not only strengthens Indonesia's capacity for marine mammal research and protection but also supports its commitments to international conservation agreements. Furthermore, the genetic findings can inform management strategies by identifying distinct genetic lineages, guiding potential translocation efforts, and enabling the development of targeted conservation measures to maintain genetic diversity within dugong populations.

The study highlights the critical need for robust conservation strategies to protect *Dugong dugon* populations and their habitats. Morphological and genetic analyses reveal distinct local adaptations and connectivity among Java Sea dugongs, emphasizing the ecological importance of preserving seagrass ecosystems. The genetic proximity of Java Sea dugongs to New Zealand populations suggests historical connectivity facilitated by shared environmental conditions and seagrass distribution. However, the observed fragmentation and habitat degradation underscore the vulnerability of these marine mammals to anthropogenic pressures. Comprehensive legal frameworks, as demonstrated by the multi-level authorization process, provide a foundation for regulating research and conservation activities. This underscores the need for streamlined administrative procedures to enhance collaboration across sectors. Establishing networks of Marine Protected Areas targeting critical seagrass habitats, coupled with sustained public awareness and habitat restoration initiatives, is vital for ensuring the long-term survival of dugongs in Indonesia

and beyond. Future research should further explore population genetics and ecological dynamics to strengthen conservation efforts.

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