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# Assessing Applicability of eDNA-Based Sampling for Population Monitoring of Leatherback Turtles in the Northeast Indian Ocean

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## ABSTRACT

Current population genetics studies of sea turtles primarily rely on invasive tissue sampling or blood draws, which involve the capture and handling of the animals that require specific permits and resources. Moreover, this approach is limited by the sporadic visibility of turtles. In this study, we assessed the applicability of eDNA-based sampling to obtain mitochondrial haplotype data for leatherback turtle (*Dermochelys coriacea*) populations at three beaches in Sumatra, Indonesia (Northeast Indian Ocean). We collected seawater samples at two time points: immediately after a female left the beach (night samples) and 12 h later (morning samples) to reflect the common practice of conducting beach monitoring surveys at dawn. Our findings revealed that the eDNA samples captured identical haplotypes to those obtained from tissue samples. The haplotypes persisted in the eDNA from seawater samples up to 12 h after the females left the beach. We identified five haplotypes that correspond to those previously recorded in the Pacific, Atlantic, and Indian Oceans, showing the broad phylogeographic links between the Sumatra population and other global populations. Our results provide further evidence that noninvasive eDNA techniques could supplement traditional tissue sampling for studying sea turtle population genetics. This applies particularly to understudied populations or remote rookeries where traditional methods are difficult to implement and opens the possibility of using eDNA for population structure studies that could complement traditional monitoring programs.

## 1 | Introduction

Sea turtle populations worldwide have shown divergent trends over recent decades (Hays et al. 2024). While some, such as the Pacific leatherback (*Dermochelys coriacea*), have experienced severe declines over recent years (Benson et al. 2020; Tapilatu et al. 2013), others are recovering. For example, green turtle (*Chelonia mydas*) nesting has increased in Florida (Lasala et al. 2023), loggerhead (*Caretta caretta*) in Cape Verde (Hays et al. 2022), and olive ridley (*Lepidochelys olivacea*) in northern Mexico (Sosa-Cornejo et al. 2021). These contrasting trends reflect both ongoing threats and the impact of sustained conservation efforts (Cheng et al. 2018; Reavis et al. 2022; Roe et al. 2013; Wallace and Saba 2009). They have complex life stages and spend the majority of their lives in widely dispersed oceanic areas, while only females occupy coastal areas during

the nesting season (Hamann et al. 2010). It is difficult to capture the shifting dynamics of sea turtle populations; thus, information from the rookeries becomes crucial as they provide data on population structure through the differentiation of DNA among rookeries, which can be used to define genetic stocks or management units (Komoroske et al. 2017). The management units are primarily established based on significant differences in mitochondrial variants (haplotypes) that are maternally inherited (Komoroske et al. 2017). Therefore, to have effective conservation management of sea turtles, it is important to define whether the rookeries are part of a broader breeding population or represent individual populations. Currently, more than 150 management units have been determined for all species of sea turtles; however, some areas are data deficient, such as Indonesia for leatherback turtles (Wallace et al. 2023).

Leatherback turtles (*Dermochelys coriacea*) occupy extensive oceanic habitats throughout their lives, ranging across entire ocean basins and using both pelagic and coastal areas during different life stages (James et al. 2005; Kaewmong et al. 2022; Kamel and Mrosovsky 2004; Piboon et al. 2025; Roden et al. 2017). While nesting occurs on tropical and subtropical beaches, adults migrate across vast oceanic regions, often spanning thousands of kilometers between nesting and foraging grounds (Benson et al. 2007, 2011). Despite their extensive dispersal, regional fidelity to nesting beaches has led to the formation of discrete genetic stocks (Bowen and Karl 2007; Dutton et al. 1999, 2013). Genetic studies have revealed structured populations across ocean basins: in the Atlantic, at least six distinct management units have been identified; in the Pacific, two distinct management units have been recognized; and in the Indian Ocean, genetic data remain sparse but suggest differentiation between eastern and western rookeries (Dutton et al. 2013; Vargas et al. 2019; Wallace et al. 2010, 2023; Wongfu et al. 2022).

To date, over 1000 leatherback turtle rookeries have been identified globally, yet only 26 have undergone genetic sampling (Wallace et al. 2023). These include 15 from the Atlantic region—Brazil (1), US Florida (1), Costa Rica (2), Dominican Republic (1), Trinidad (2), French Guiana (2), French West Indies (2), Suriname (1), US Virgin Islands (1), Ghana (1), and Gabon (1); nine from the Indo-Pacific—South Africa (2), Malaysia (1), Indonesia (Sumatra (2) and Papua Barat (2)), Papua New Guinea (1), and Solomon Islands (1); and two from the East Pacific—Mexico (1) and Costa Rica (1) (Wallace et al. 2023). Most of these sampled sites represent well-established nesting areas. Recent genetic studies have also revealed population structure in previously unsampled regions such as the Andaman Sea of Thailand and Great Nicobar Island, India, both of which appear to form part of the broader Indo-Pacific genetic group (Piboon et al. 2025; Shanker et al. 2011; Wongfu et al. 2022). These studies to date indicate that leatherback turtles have relatively low mitochondrial diversity (Komoroske et al. 2017); however, in order to detect potential bias, more extensive sampling is needed, particularly from newly identified rookeries in the Indo and western Pacific. In Indonesia, genetic and demographic data are primarily derived from the well-monitored rookeries at Jamursba Medi and Wermon located on the northern coast of the Tambrauw District in Papua Barat Province (now Papua Barat Daya Province), eastern Indonesia (Dutton et al. 2007; Hitipeuw et al. 2007). Four haplotypes have been identified at these sites, one (DcA/Dc1.1) with global distribution, while the other three originated from the Pacific region (Dutton et al. 2007); however, expanded studies with larger sample sizes have identified additional unique haplotypes (Toha et al. 2025). Additional Indonesian rookeries remain unstudied, with a notable knowledge gap in Sumatra, situated in western Indonesia along the Indian Ocean coast. This region has been categorized as part of the Northeast Indian Ocean subpopulation with a "data deficient" status according to the IUCN Red List (Wallace et al. 2013). Recently, six rookeries were discovered in Aceh and West Sumatra, Sumatra, Indonesia, where monitoring programs were initiated to protect the populations (Maslim and Farajallah 2016). However, these rookeries have not yet been analyzed for mitochondrial (mtDNA) haplotypes.

Current sea turtle population genetics studies obtain DNA by collecting skin or blood, which could result in injury and stress for the turtles (Andrews et al. 2021; Bjorndal et al. 2010; Sherrill-Mix and James 2008; Stasiak 2024). Given the critically endangered status of some of the world's sea turtle populations, it is desirable to employ noninvasive methods that minimize direct interaction when possible. Environmental DNA (eDNA) is genetic material shed by organisms into their surrounding environment (e.g., water, soil, or sediment) and can be collected noninvasively, offering potential for population genetic studies that currently require the use of tissue/blood to perform (Andres, Lodge, Sethi, and Andrés 2023; Couton et al. 2023; Farrell et al. 2021; Sigsgaard et al. 2020). Environmental DNA from green turtles (*Chelonia mydas*) was detected and quantified in a marine estuary area in San Diego Bay, California, using qPCR with the markers LCMint2/H950g and LTCM2/HDCM2 (Harper et al. 2020). Farrell et al. (2022) observed a strong positive correlation between the quantity of green turtle and loggerhead turtle eDNA (from water and sand) based on 16S rRNA analysis and the amplification ratio of technical replicates ($R = 0.765$) with the greatest quantity of eDNA obtained coming from a nest surface sand sample that was taken after hatchling emergence. These studies demonstrated that trace DNA of sea turtles can be recovered noninvasively from sand and water samples. In addition, eDNA has also been used to identify mitochondrial haplogroups at the group level for green and loggerhead turtles using shotgun sequencing, enabling population-level inferences about genetic diversity and natal origins without direct handling (Farrell et al. 2022). However, it remains unconfirmed whether eDNA can also be informative for the haplotype identification of a particular individual. Thus, further testing of eDNA from field samples in other species, such as leatherback turtles, is needed to assess its applicability for population genetic analysis and to determine whether it can yield haplotype data from specific individuals within rookeries.

Sea turtle conservation in many countries relies on traditional monitoring programs, which are generally conducted during the nesting season from sunset until sunrise, as sea turtles lay their eggs during that time. However, due to limited resources such as funding, technical knowledge, and personnel, as well as safety, monitoring is often conducted at dusk or in the morning to count the tracks (leatherback tracks are visually distinctive from other sea turtle species) and nests as well as relocate nests requiring protection from the previous night (Hitipeuw et al. 2007;

Swaminathan et al. 2017). The morning survey is typically done within 12 h after the females have laid their eggs, and while the female was not sighted, her track is still clearly visible (Farrell et al. 2022). Integrating eDNA sampling with standard surveys could enhance monitoring by providing real-time genetic information on nesting females, including species identity, haplotype composition, and potentially individual identification, without requiring direct observation. Unlike tagging or tissue collection, which requires the presence of the animal, eDNA can be obtained from environmental traces shortly after nesting, enabling genetic assessment (population structure and genetic diversity) even when females are missed. This is particularly valuable in low-resource or high-risk settings and offers new opportunities to assess genetic diversity and connectivity across nesting populations. In order to determine whether eDNA sampling should form a standard part of sea turtle nesting surveys, we assessed the reliability of eDNA for detecting haplotypes from samples collected during routine morning patrols—approximately 12 h after nesting—focusing on a novel sea turtle species for eDNA studies (*Dermochelys coriacea*) and a geographically and genetically understudied population in Sumatra.

To address the data gaps in the leatherback turtle population in Indonesia, and to improve the current standard monitoring and sampling of turtle eDNA globally, we evaluate three newly monitored rookeries (Along, Buggeisiata, and Selaut Besar) using eDNA samples from water in order to validate methodologies and characterize the mtDNA diversity. These rookeries, located on the west coast of Sumatra, host low-density nesting populations with nesting seasons from October to March. Alongside leatherbacks, olive ridley (*Lepidochelys olivacea*) and green turtles (*Chelonia mydas*) are also known to nest on these beaches, with some degree of temporal overlap, particularly at the beginning and end of the leatherback nesting season. This study aimed to (1) evaluate whether eDNA extracted from water samples provides the same haplotype as the tissue from nesting leatherback turtles (*Dermochelys coriacea*), (2) assess whether eDNA can be used reliably from morning monitoring by testing the persistence time of eDNA and the associated haplotypes in seawater (in situ) 12 h after the leatherback female has laid her eggs, and (3) relate the haplotypes of leatherback turtles from new rookeries on the west coast of Sumatra to those previously identified for leatherback populations globally.

## 2 | Materials and Methods

### 2.1 | Sample Collection and DNA Extraction

Two liters of seawater samples were collected from the surface water within 1 m of the shoreline in the area where the turtle passed, within 10 min after the turtle had returned to the sea. Water samples were collected in 2 L sterile Nalgene HDPE bottles (catalogue number 2120-0005) and filtered through Millipore cellulose nitrate sterile membrane filters (47 mm diameter; 0.45 μm pore size, catalogue number HAWG047S6) using a portable vacuum pump (Joanlab, catalogue number VP-10 L) and Nalgene Reusable Filter Units (catalogue number 300-4100). All membrane filters were preserved in 400 μL Zymo DNA/RNA Shield, formulated to preserve DNA at room temperature, thoroughly shaken, stored at room temperature for up to 1 week, and subsequently transferred to −20°C upon return from the field (Takahashi et al. 2023). Prior to sampling, all reusable eDNA sampling and filtration equipment was cleaned with 1% chlorine solution and then rinsed thoroughly with deionized (DI) water to remove any traces of chlorine.

For each female, three replicates and one negative control using only DI water were collected on two occasions: (1) within 10 min after the turtle returned to the sea during nighttime monitoring (night samples), and (2) 12 h later during the following morning (morning samples), coinciding with standard patrol schedules (Figure 1). Tissue samples from the females were also collected to confirm haplotypes identified through eDNA. Tissue samples were taken using a sterile 2 mm biopsy punch at the trailing edge of the front flipper and stored in 95% ethanol (EtOH)

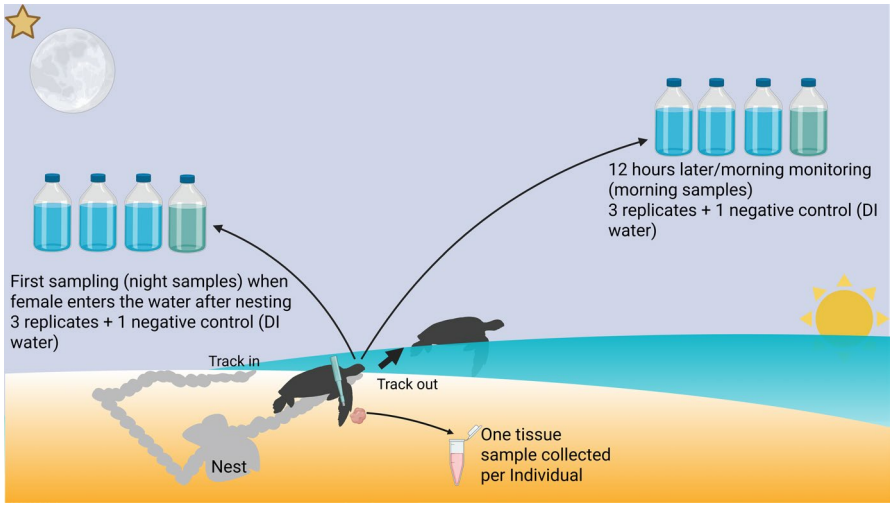


**FIGURE 1** | Overview of sampling strategy for eDNA and tissue samples (created with BioRender.com/s57i107). Each eDNA sample was accompanied by a tissue sample from the nesting female. Tissue samples were collected after nesting activity, while seawater night samples were obtained within 10 min after the female left the beach. Seawater morning samples were collected 12 h later, reflecting the common monitoring practice typically conducted at dawn.

(Dutton and Stewart 2013). In total, 108 seawater samples (including negative controls) and 11 tissue samples were collected from 15 individual turtles across three nesting beaches—Along, Buggeisiata, and Selaut Besar (Table 1). Of the 15 turtles, 11 were observed during night patrols, allowing for the collection of both tissue and water samples. These 11 individuals were confirmed to be different females based on flipper tag identification. Three turtles were not observed directly, with their nesting inferred from fresh tracks and nests; for these, only morning seawater samples were collected. One individual was observed returning to the sea, and both night and morning seawater samples were collected, but no tissue sample was taken. The discrepancy in sample numbers is due to logistical constraints and the opportunistic nature of field-based sampling in remote locations.

DNA extraction for eDNA samples was done using a DNeasy Blood and Tissue extraction kit (Qiagen, catalogue number 69504) with a modified protocol by Nijland (2020). Proteinase K was added to the filter and incubated at 56°C for 10–30 min, followed by the standard protocol for blood samples, with the volumes of buffer AL and ethanol doubled to 400 μL. For tissue samples, DNA extraction followed the standard DNeasy Blood and Tissue Kit protocol. To prevent contamination, the working area was decontaminated using 70% isopropyl alcohol and UV light prior to extraction. All laboratory work was conducted at the Wageningen University Marine Animal Ecology (MAE) laboratory, which does not house any other sea turtle research, ensuring that no residual sea turtle DNA from previous studies was present. The extraction process was carried out in a dedicated UV cabinet, separate from the one used for PCR. eDNA samples were extracted first, followed by amplification, after which extraction and amplification for tissue samples were performed.

## 2.2 | DNA Amplification

To investigate the turtle haplotypes from eDNA and tissue samples, we initially amplified 763 bp of the mtDNA control region using the common primers for leatherback turtles: LCM15382 (5′-GCTTAACCCTAAAGCATTGG-3′), and H950g (5′-GTCTCGGATTTAGGGGTTTG-3′) (Abreu-Grobois et al. 2006). However, these primers did not successfully amplify the eDNA samples. Therefore, we designed three new pairs of primers targeting shorter fragments of the control region (100–300 bp) for eDNA samples (Table 2). The new primers were designed *in silico* using Geneious Prime 2024.0.7 (https://www.geneious.com) based on conserved regions identified across haplotype sequences from Dutton et al. (2013). Default settings were used to target nucleotide differences in regions commonly used for haplotype identification in leatherback population genetics, enabling comparisons between eDNA and tissue sample data (Figure 2). Each primer pair was tested for specificity against the NCBI nucleotide (nt) database

**TABLE 1** | Total samples (eDNA and tissue) per location.

| Location | | Number of samples | | |
|---|---|---|---|---|
| Beach | Province | Night sample (eDNA), including replicates and negative control | Morning sample (eDNA), including replicates and negative control | Tissue samples |
| Selaut Besar, Simeulue | Aceh | 12 | 12 | 3 |
| Along, Simeuleu | Aceh | 12 | 12 | 3 |
| Buggesisiata, Sipora | West Sumatra | 24 | 36[b] | 5[a] |
| Total | | 48 | 60 | 11 |

[a]Tissue samples were not collected from three turtles that were not observed during night monitoring, with their presence inferred from fresh tracks and nests; for these individuals.
[b]Only morning water samples were obtained. Additionally, one turtle observed when leaving the beach had both night and morning eDNA water samples collected, but no tissue sample.

**TABLE 2** | Primers were designed and used for eDNA sample analyses in the mitochondrial control region of leatherback turtles in this study.

| Name | Direction | Sequence (5′–3′) | Target region | Fragment size (bp) | Annealing temperature (°C) | Primer designation |
|---|---|---|---|---|---|---|
| DL06S | Reverse | GGGGGTTTAAGTTTAAGATAAATGAGT | Control region mtDNA | 146 | 59 | CR3 |
| DL05S | Forward | CTTTGTGTCTTCAGGCCCAC | | | | |
| DL04S | Reverse | AATGTATAGAACTCATTAACCAGAGGC | Control region mtDNA | 133 | 64 | CR2 |
| DL03S | Forward | TTATTAATCACGAGAAATAAGCAACCC | | | | |
| DL01aS | Reverse | ACTTCAATAGTGTTATGTGCTTGAA | Control region mtDNA | 292 | 58 | CR1 |
| DL02aS | Forward | ACCCTATGTATTATCGTGCATTCA | | | | |

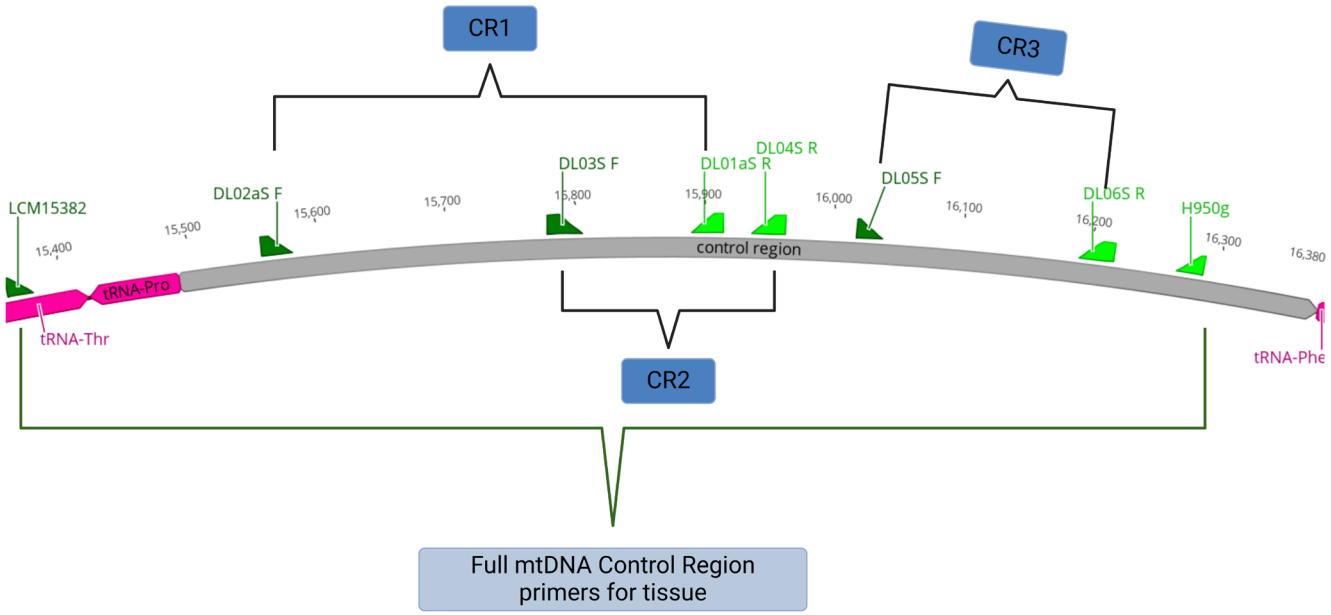


**FIGURE 2** | Locations of six newly designed primers (CR1, CR2, and CR3) for eDNA samples and common primers for tissue sample analyses of the leatherback turtle mitochondrial control region used in this study.

using Primer-BLAST with default settings (Ye et al. 2012, Data S1) to ensure no crossamplification with other sea turtle species. Importantly, no green or olive ridley turtles were observed nesting or in the immediate sampling areas during the study period, reducing the risk of interspecific DNA contamination. Additionally, all amplified products were verified using Sanger sequencing, which provided clear sequence data for species confirmation, thereby supporting the specificity and reliability of the primers for leatherback turtles.

The PCR reactions were carried out in a total volume of 10 μL, containing 5 μL Phire Tissue Direct PCR Master Mix (Thermo Scientific, catalog number F170S), 3.6 μL nuclease-free water (Thermo Scientific, catalog number 10977015), 0.2 μL of forward and reverse primers, and 1.2 μL of DNA template. Thermal cycling conditions varied by primer pair: 98°C for 5 min, followed by 40 cycles of 98°C for 10 s, annealing temperature (Table 2) for 10 s, and 72°C for 10 s. The final extension was performed at 72°C for 10 s, with a final hold at 4°C. DNA amplifications were performed using a T100 PCR thermocycler (Bio-Rad, catalogue number 1861096). The PCR product was run on a 1.3% agarose gel to assess PCR success.

### 2.3 | DNA Sequencing and Analysis

PCR amplification of tissue and eDNA samples, including negative controls, was sent to Eurofins Genomics for Sanger sequencing using the Mix2Seq Kit NXP. Obtained sequences were edited and aligned using Geneious Prime 2024.0.7. (https://www.geneious.com) while haplotype identification was done using DnaSP6 version 6.12.3 (Rozas et al. 2017) and subsequently verified through BLAST (Camacho et al. 2023) searches against the NCBI nucleotide (nt) database to determine whether the identified haplotypes matched published ones or represented novel haplotypes. Haplotype comparisons between tissue and seawater (eDNA) samples were calculated and visualized in R using the ggplot2 package (Wickham 2009), followed by a chi-squared test to assess significant differences between fresh (night) and 12-h (morning) samples. A Bayesian inference phylogenetic tree was constructed for the haplotypes identified in this study, and the standard 763-bp global haplotypes published by Dutton et al. (2007, 2013, Table S1). The analysis was performed using the MrBayes 3.2.6 plugin in Geneious Prime, with 10,000,000 MCMC iterations for each tree and the first 100,000 iterations discarded as burn-in (Ronquist et al. 2012).

## 3 | Results

### 3.1 | Haplotype Identification From Water Samples

A total of 108 seawater samples, including negative controls and replicates, yielded 158 sequences through Sanger sequencing of three mitochondrial DNA Control Region fragments (CR1, CR2, and CR3) using three different primer pairs (Table S2). The three mtDNA fragments demonstrated high amplification success, with overall detection success rates of 83.33% for night samples and 80% for morning samples across all fragments. This outcome highlights the efficacy of these primers in specifically amplifying leatherback turtle DNA from seawater samples. Furthermore, none of the 27 negative control samples produced visible PCR amplification bands, and no readable sequences were obtained following Sanger sequencing, confirming the absence of leatherback DNA and indicating that no contamination occurred during sample collection or laboratory procedures.

Four haplotypes were identified from seawater samples accompanied by tissue samples, based on consensus sequences from all three fragments (Figure 3). All four haplotypes (Dc1.1, Dc1.4, Dc5.1, and Dc9.1) matched those found in tissue samples collected from three locations. Additionally, three haplotypes (Dc1.4, Dc4.1, and Dc5.1) were also amplified from seawater samples not

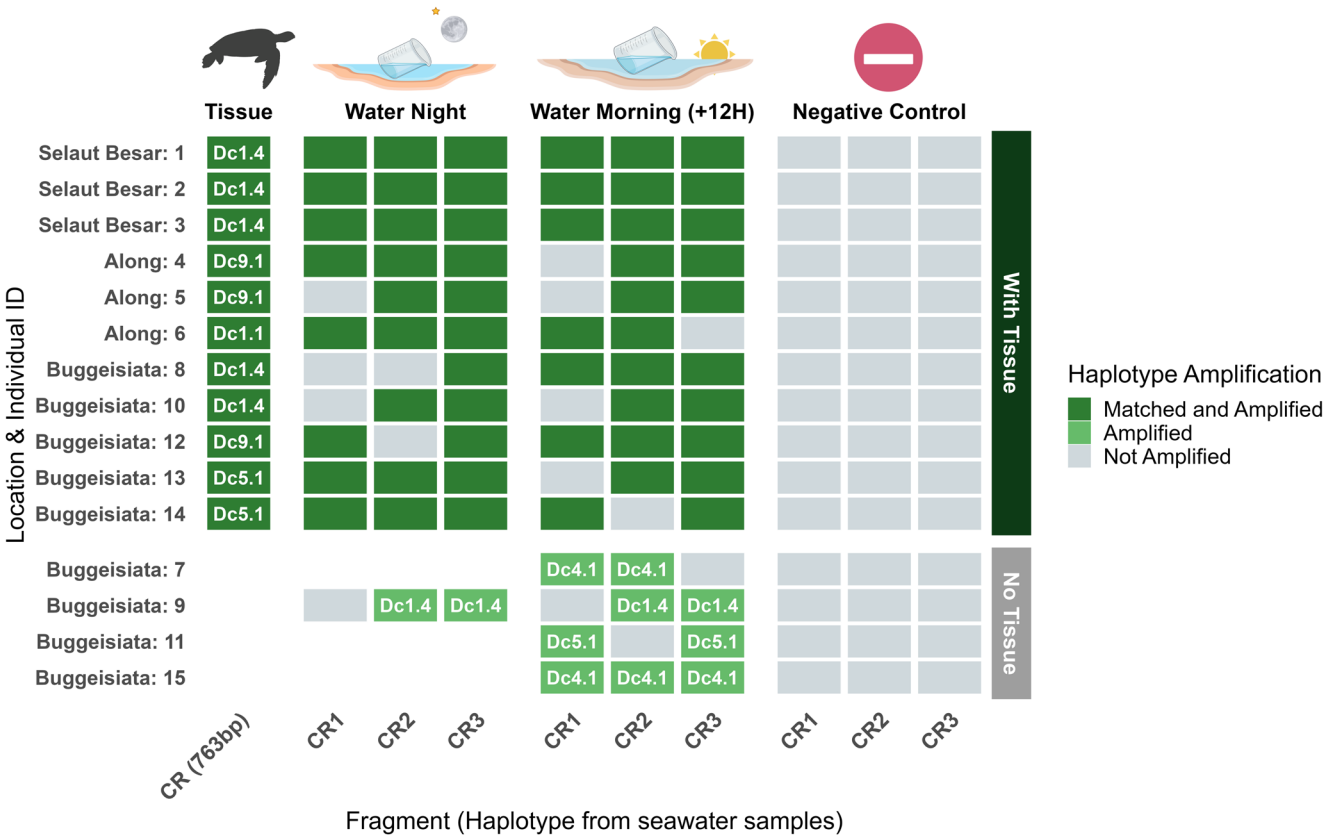


**FIGURE 3** | Haplotype matching between leatherback turtle tissue and seawater samples based on the longer Control Region mtDNA (763 bp) for tissue samples and three shorter Control Region mtDNA fragments (CR1 [292 bp], CR2 [133 bp], and CR3 [146 bp]) for seawater samples (see Figure 2 and Table 2). Seawater samples were collected at the time of nesting (night) and 12 h postnesting (morning). Dark-green boxes indicate seawater samples that matched the haplotypes from corresponding tissue samples (where available), while light-green boxes represent seawater samples that successfully amplified haplotypes despite the absence of corresponding tissue samples.

accompanied by tissue samples (no tissue samples). Two of these haplotypes (Dc1.4 and Dc5.1) matched tissue samples from the same location (Buggeisiata), suggesting that they likely originated from the same individuals, whereas the third haplotype (Dc4.1) did not match any tissue samples. Furthermore, both morning and night seawater samples consistently detected haplotypes identical to those in tissue samples from the same individuals, demonstrating reliable detection across different time periods.

### 3.2 | Haplotypes From Environmental DNA: Comparison Between Night (Fresh) and Morning (12 h Later) Samples

The successful water sampling during both night (immediately after the turtle left the beach) and morning (12 h later) demonstrated a nearly equal capture of the same haplotypes in replicate samples across all locations (Figure 4), indicating consistent haplotype detection between the two sampling periods. The similarity in haplotype detection between night and morning samples is further supported by chi-squared test results ($p$ values: Along and Selaut Besar = 1; Buggeisiata = 0.969), showing no significant differences in haplotype distributions across the two time periods. These findings suggest that eDNA sampling conducted either at

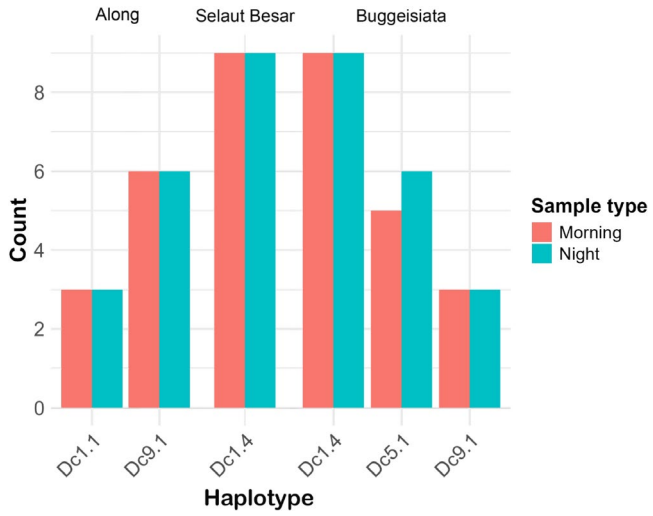


**FIGURE 4** | Haplotype distribution across three nesting sites in Sumatra (Along, Selaut Besar, Buggeisiata) based on eDNA replicate samples collected at night and the following morning. Each bar represents the number of replicate samples (maximum of three per timepoint per individual) in which a given haplotype was detected. The figure illustrates detection consistency across time points rather than the number of unique individuals.

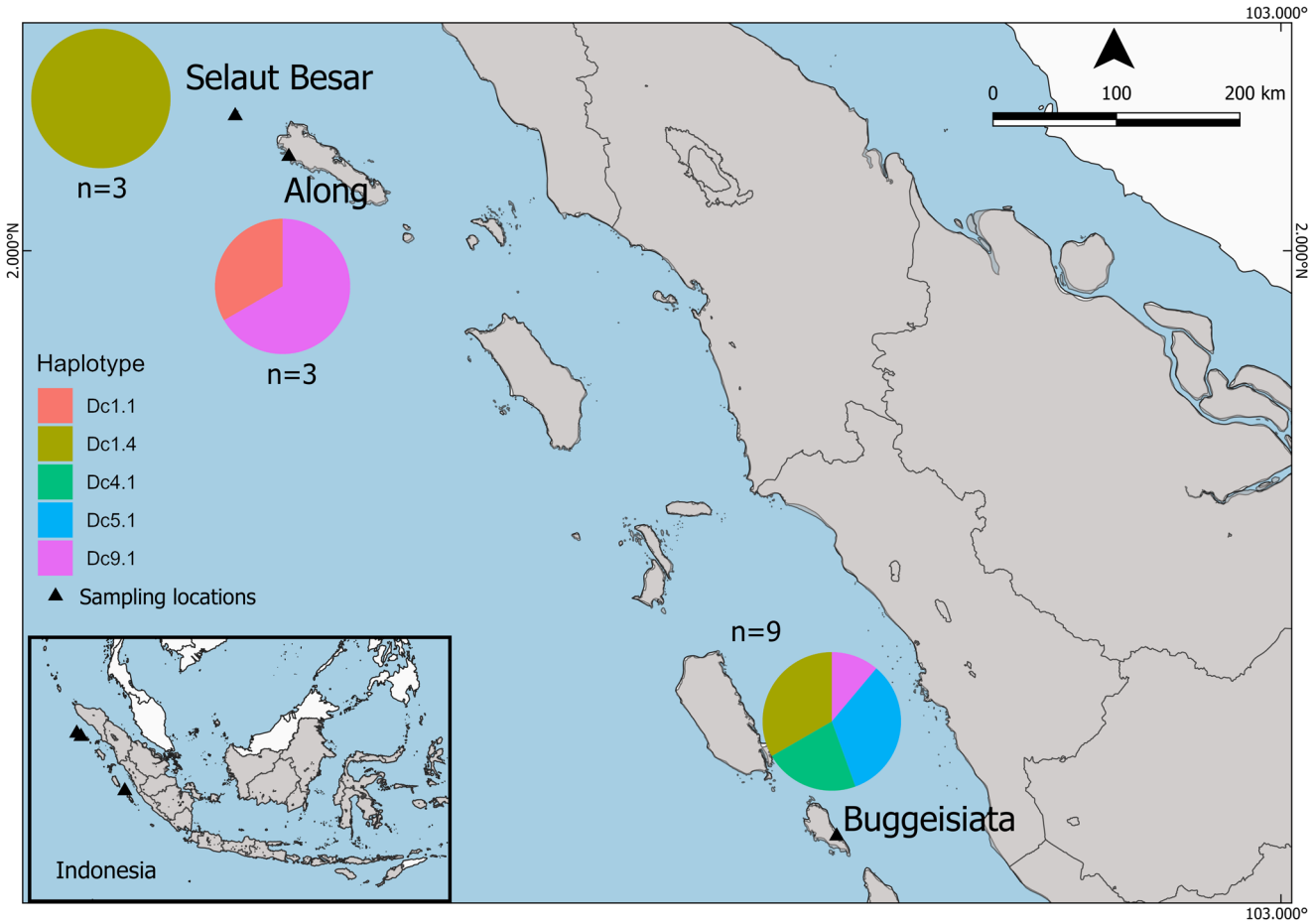


**FIGURE 5** | Haplotype composition across three sampling locations in Sumatra (Along, Selaut Besar, and Buggeisiata) based on eDNA seawater samples ($n$ = number of individuals per location).

night or in the morning can reliably capture the haplotypes of the nesting individuals from the previous night.

The geographical distribution of haplotypes across three nesting sites in Sumatra (Buggeisiata, Selaut Besar, and Along) reveals distinct patterns of haplotype composition based on eDNA samples representing individual turtles (Figure 5). Buggeisiata exhibits the highest diversity, with four haplotypes detected (Dc1.4, Dc4.1, Dc5.1, and Dc9.1), while Along is characterized by two haplotypes (Dc1.1 and Dc9.1), and Selaut Besar contains only one haplotype (Dc1.4). This higher diversity observed in Buggeisiata may be attributed to the larger number of individuals sampled (nine individuals) compared with the other two locations (three individuals each from Along and Selaut Besar). The presence of multiple haplotypes in Along and Buggeisiata highlights the genetic diversity of the leatherback turtle populations across these Sumatran rookeries, effectively captured using eDNA samples.

Several of the haplotypes identified in this study have previously been reported in Indo-Pacific leatherback populations. For instance, Dc1.1 has been recorded in Atlantic rookeries (Dutton et al. 2013) and Dc1.4 has been observed in Thailand (Wongfu et al. 2022), while Dc4.1, Dc5.1, and Dc9.1 have also been documented in Papua Barat, Indonesia (Dutton et al. 2007). These observations help contextualize the genetic composition of the Sumatran populations.

### 3.3 | Connecting the Haplotypes From eDNA to the Global Database

The phylogenetic analysis illustrates the relationships between global leatherback turtle haplotypes and those identified using eDNA samples in this study (Figure 6). It reveals genetic connectivity between the Sumatran leatherback turtle population and global populations in the Pacific, Indian, and Atlantic Oceans, suggesting the presence of regional genetic diversity. Two haplotypes (Dc5.1 and Dc9.1) correspond to those documented in Pacific populations, one haplotype (Dc1.4) aligns with the Indian Ocean and West Pacific populations, and another haplotype (Dc4.1) aligns with Atlantic and Pacific populations. Additionally, one haplotype (Dc1.1), although commonly observed in Atlantic populations, is also detected in the Pacific and Indian Ocean (Toha et al. 2025; Dutton et al., unpublished). These alignments contribute to current knowledge of haplotype distributions, which are the focus of ongoing research efforts to better understand their global patterns.

## 4 | Discussion

In this study, we assessed the use of leatherback turtle eDNA from seawater to identify the haplotypes of nesting females.

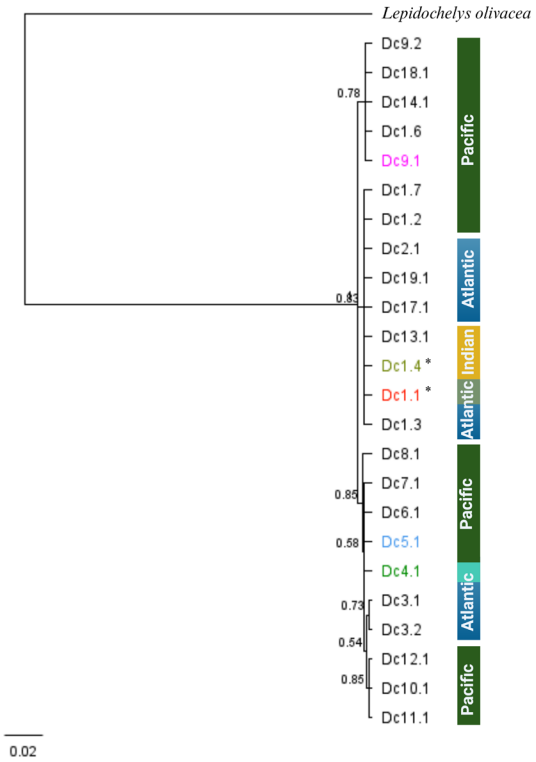


**FIGURE 6** | Bayesian phylogenetic tree of global leatherback turtle haplotypes published by Dutton et al. (2007, 2013), including those identified from eDNA seawater samples using shorter markers (CR1, CR2, and CR3). Color-highlighted haplotypes represent those detected in the Sumatran rookeries, while the boxes with ocean names indicate the regions where the haplotypes have been found. The mixed colors in the box indicate that the haplotypes are present in both regions. *Also found in Pacific (Toha et al. 2025; Dutton et al., unpublished).

We successfully detected haplotypes in eDNA that matched those captured from tissue samples of nesting females in the field. Furthermore, we also demonstrated the feasibility of obtaining haplotypes from water samples that were collected 12 h after the nesting sea turtle had left. Our results show that haplotypes of the individuals that nested the previous night can be collected during the common practice of monitoring sea turtle nests at dawn. In addition, our panel of primer sets allowed the entire 763 bp mtDNA CR fragment to be sequenced and thus identify five haplotypes from Sumatran rookeries that correspond to those found in other global populations.

### 4.1 | eDNA From Seawater for Haplotype Identification

We successfully identified all haplotypes present in individual tissue samples taken from nesting females at the three study locations using eDNA extracted from seawater. This finding highlights the ability of seawater eDNA to accurately capture genetic material from nesting females, even hours after they have departed the nesting site. Our findings complement previous studies on the use of eDNA for sea turtle detection, as demonstrated by Farrell et al. (2022) and Harper et al. (2020), who employed species-specific primers to detect green turtles (*Chelonia mydas*) in seawater and by Farrell et al. (2022) for loggerheads (*Caretta caretta*) from sand and seawater samples using non-targeted Illumina shotgun for pathogen detection. Similar approaches have been applied in other taxa, such as Pacific salmon (*Oncorhynchus clarkii clarkii*, *O. mykiss*, *O. kisutch, O. tshawytscha*), where mitochondrial markers (COI, ND2, and 12S ribosomal DNA) were used to identify haplotypes using Illumina Miseq (Weitemier et al. 2021). Additionally, in round goby (*Neogobius melanostomus*), eDNA mesocosm experiments demonstrated a strong correlation between eDNA read frequencies and field samples ($r = 0.84$), as well as with tissue-based allele frequencies ($r = 0.69$, $p < 0.001$) across 28 microsatellite loci (Andres et al. 2021; Andres, Lodge, and Andrés 2023; Andres, Lodge, Sethi, and Andrés 2023). These findings underscore the potential of eDNA as a reliable tool for genetic monitoring.

We also demonstrated further application of the eDNA in detecting specific haplotypes of nesting females using shoreline seawater, which was successfully achieved with simple amplification and sequencing methods accessible to most laboratories, namely targeted PCR and Sanger sequencing. Our results suggest that the new primers were effective in targeting leatherback turtles, even though our study sites are also used by green and olive ridley turtles. Although the newly designed primers were not experimentally tested against DNA or eDNA from other sea turtle species, they consistently yielded clean leatherback haplotype sequences confirmed by Sanger sequencing, and no other sea turtle species were observed at or near the sampling sites during the study period. Nonetheless, we acknowledge the need for further validation using controlled multispecies eDNA samples to definitively confirm their specificity and rule out potential crossamplification. Future laboratory testing with known mixed-species pools or field validation in areas with sympatric nesting species will be essential to fully establish the robustness of these primers for broader application. Given the fact that genetic stock population data is typically retrieved from the control region of mitochondrial DNA, our findings provide additional evidence that eDNA techniques show promise as a non-invasive alternative when tissue sampling is not feasible (Couton et al. 2023; Farrell et al. 2022; Komoroske et al. 2017; McCauley et al. 2024). Environmental DNA methods offer the advantage of sampling from the environment without direct interaction with individuals, which could be particularly useful in situations where nesting events are infrequent or difficult to observe.

### 4.2 | Application of eDNA-Based Monitoring

In the present study, we examined the persistence of eDNA in seawater collected immediately after a female left the beach (night sample) and 12 h later (morning sample). We did not observe significant differences in captured haplotypes across these collection times, indicating that eDNA persisted in seawater for at least 12 h after females had left the beach. Although eDNA, a mixture of DNA from multiple sources, is known to degrade more rapidly in aquatic environments (Barnes and Turner 2016; Collins et al. 2018), our findings showed that samples taken 12 h later still contained the genetic material of specific individuals exposed to the nearshore seawater. By using targeted markers, it is possible to obtain haplotypes of particular individuals from these samples (Couton et al. 2023; Suarez-Bregua et al. 2022;

Westgaard et al. 2024). In addition to the high specificity of the primers, the coastal conditions and geomorphology at the sampling site in Western Sumatra during the nesting season (October–March) may have contributed to the persistence of eDNA in this environment (Haditiar et al. 2024). There may be less water exchange in this area due to calm waters, minimal tidal variation, low waves, and weak currents. Furthermore, females may stay resident in the coastal waters between clutches (Tapilatu et al. 2013), allowing for possibilities of continued eDNA detection.

Our findings validate the use of eDNA for mtDNA-based assessments and demonstrate its potential as a noninvasive tool for complementing traditional monitoring techniques. The identification of mitochondrial haplotypes through eDNA provides a rapid method for assessing the minimum number of haplotypes present at nesting sites (Seymour et al. 2021). This approach could also be valuable for initial genetic assessments in understudied populations or remote rookeries where traditional methods, such as tagging or nest monitoring, are difficult to implement (Hamann et al. 2010; Omeyer et al. 2022; Wallace et al. 2011; Willis-Norton et al. 2015). By offering an additional layer of genetic insight, eDNA can enhance existing monitoring programs, such as nest counts or censuses of nesting activity, to better inform conservation and management strategies (Dutton et al. 2007).

### 4.3 | Haplotypes From Sumatra: Diversity and Global Connectivity

We identified five mtDNA control region haplotypes from tissue and eDNA samples collected at three newly monitored nesting sites in Sumatra (Selaut Besar, Along, and Buggeisiata) in Indonesia, providing the first genetic insights into these previously unmonitored rookeries. These haplotypes correspond to those found in the Pacific, Atlantic, and Indian Oceans (Dutton et al. 2007, 2013; Vargas et al. 2019; Wongfu et al. 2022). Our preliminary findings suggest relatively high haplotypic diversity within the Sumatran population. However, to gain a more comprehensive understanding of the population stock structure in Sumatra, additional samples representing each rookery are needed. While these findings offer an initial insight into the leatherback turtle population in Sumatra, data on population structure and migration patterns remain scarce compared to other Indo-Pacific populations, such as those in Malaysia, Papua, and the Solomon Islands (Bailey et al. 2012; Benson et al. 2011; Dutton et al. 2007; Hamann et al. 2010; Mazaris et al. 2014; Wallace et al. 2011). The phylogeographic links observed with other global populations suggest that Sumatran leatherback turtles may be part of a broader source of global diversity found within the Indo-Pacific (Dutton et al. 1999). These preliminary results emphasize the potential conservation importance of the Sumatran leatherback populations for maintaining genetic diversity for leatherbacks globally.

## 5 | Conclusion

This study demonstrates the potential of eDNA as a noninvasive tool for monitoring leatherback turtles, offering a practical alternative for genetic sampling at nesting sites. We successfully detected mitochondrial haplotypes from seawater samples and confirmed eDNA persistence in seawater. However, this study is limited to mitochondrial DNA and was conducted in a small population with low nesting density. Further research is required to validate the use of eDNA for broader genomic applications, such as whole-genome sequencing or SNP analyses, which demand higher-quality DNA samples. Additionally, evaluating the effectiveness of eDNA in high-density nesting areas is crucial, as overlapping genetic material from multiple individuals could complicate haplotype resolution. By addressing these challenges and refining eDNA methodologies, this approach could become an invaluable tool for assessing genetic diversity and population genomics in sea turtles and supporting conservation strategies, particularly in regions where logistical or ethical considerations limit the use of traditional methods.

#### Author Contributions

M.A.-s., R.N., I.B., and L.E.B. were responsible for conceptualizing and designing the research. M.A. carried out the literature review, data analysis, and the initial drafting of the manuscript. M.A.-s, R.N., I.B., P.H.D., and L.E.B. contributed to refining and revising the manuscript throughout its development, from inception to completion. M.A. created the figures and tables with contributions from all authors.

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#### Conflicts of Interest

The authors declare no conflicts of interest.

#### Data Availability Statement

All the data used in the article are available in the Supporting Information (Data S1, Tables S1 and S2).

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## Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Data S1:** edn370160-sup-0001-DataS1.pdf. **Table S1:** edn370160-sup-0002-TableS1.csv. **Table S2:** edn370160-sup-0003-TableS2.pdf.