The role of wildlife DNA forensics in combatting rhino horn trafficking: Lessons from Indonesian cases

Dwi Sendi Priyono[1], Hapiz Al Khairi[1], Andryan Andryan[1], Tuty Arisuryanti[1], Rury Eprilurahman[1], Muhammad Abdul Latiff Abu Bakar[2]

[1]Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sinduadi. Mlati, Sleman, 55281. Special Region of Yogyakarta, Indonesia.
[2]Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia

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Dwi Sendi Priyono - 0000-0001-5828-7559
Hapiz Al Khairi - 0009-0008-7845-7533
Tuty Arisuryanti - 0000-0001-5786-2937
Rury Eprilurahman - 0000-0002-1786-6201
Muhammad Abdul Latiff Abu Bakar - 0000-0001-9551-8571

Abstract:
Indonesia is home to two critically endangered rhinoceros species, the Javan rhinoceros (*Rhinoceros sondaicus*) and the Sumatran rhinoceros (*Dicerorhinus sumatraensis*), which face severe threats from illegal wildlife trade, particularly poaching driven by the demand for rhino horn. DNA forensics offers a promising approach to combat this illicit trade, enabling precise species identification and reducing the risk of misidentification in confiscated wildlife products. This study explores the application of wildlife DNA forensics in Indonesia, with a focus on recent case studies of confiscated rhino horn samples. Using the 12S rRNA marker, the research demonstrates the effectiveness of DNA analysis in confirming the authenticity of rhino horn samples and identifying counterfeit items. Phylogenetic analysis of DNA sequences from suspected rhino horns reveals distinct genetic clades corresponding to different rhinoceros species, providing strong forensic evidence for species identification. This research underscores the potential of DNA forensics to enhance law enforcement's ability to prosecute wildlife traffickers and protect endangered rhinoceros populations. To further strengthen forensic investigations, the development of population-specific DNA databases and standardized field procedures could improve the accuracy of species and geographic assignment while minimizing contamination. Rapid molecular detection tools, such as qPCR or genomics approaches, may also facilitate timely authentication of rhino horn samples in both laboratory and field settings. By integrating genetic technologies into conservation and legal frameworks, this study contributes to advancing wildlife forensics as a critical tool in global conservation strategies.

Keywords: Indonesia, *Rhinoceros*, Illegal Wildlife Trade, Wildlife DNA Forensic.

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Corresponding author
Dwi Sendi Priyono
Department of Tropical Biology, Faculty of Biology, Universitas Gadjah Mada. Jl. Teknika Selatan, Sinduadi. Mlati, Sleman, 55281. Special Region of Yogyakarta, Indonesia.; email: dwisendipriyono@ugm.ac.id

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Indonesia is home to two critically endangered rhinoceros species, the Javan rhinoceros (*Rhinoceros sondaicus*) and the Sumatran rhinoceros (*Dicerorhinus sumatraensis*), which face severe threats from illegal wildlife trade, particularly poaching driven by the demand for rhino horn. DNA forensics offers a promising approach to combat this illicit trade, enabling precise species identification and reducing the risk of misidentification in confiscated wildlife products. This study explores the application of wildlife DNA forensics in Indonesia, with a focus on recent case studies of confiscated rhino horn samples. Using the 12S rRNA marker, the research demonstrates the effectiveness of DNA analysis in confirming the authenticity of rhino horn samples and identifying counterfeit items. Phylogenetic analysis of DNA sequences from suspected rhino horns reveals distinct genetic clades corresponding to different rhinoceros species, providing strong forensic evidence for species identification. This research underscores the potential of DNA forensics to enhance law enforcement's ability to prosecute wildlife traffickers and protect endangered rhinoceros populations. To further strengthen forensic investigations, the development of population-specific DNA databases and standardized field procedures could improve the accuracy of species and geographic assignment while minimizing contamination. Rapid molecular detection tools, such as qPCR or genomics approaches, may also facilitate timely authentication of rhino horn samples in both laboratory and field settings. By integrating genetic technologies into conservation and legal frameworks, this study contributes to advancing wildlife forensics as a critical tool in global conservation strategies.

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1. Introduction

Indonesia is home to the critically endangered Javan rhinoceros (*Rhinoceros sondaicus*) and the Sumatran rhinoceros (*Dicerorhinus sumatraensis*). These species are vital components of Indonesia's rich biodiversity and serve as indicators of the health of their ecosystems. However, the illegal trade of rhinoceros horns poses a significant threat to their survival. The demand for rhino horn, primarily driven by traditional medicine and status symbols in certain cultures, has led to rampant poaching and trafficking activities, particularly in Southeast Asia, where Indonesia has become a critical transit point for illegal wildlife trade (Ewart et al., 2018; Greenfield & Veríssimo, 2019; Haas & Ferreira, 2016). The illegal rhino horn trade in Indonesia is alarming, with reports indicating that rhino horns can fetch prices exceeding USD 35,000 per kilogram on the black (Haas & Ferreira, 2016). Both Indonesian rhinoceros species persist in extremely small populations (Fig. 1), with the Sumatran rhinoceros (*Dicerorhinus sumatrensis*) estimated at ~34–47 individuals and the Javan rhinoceros (*Rhinoceros sondaicus*) at ~50 individuals (International Rhino Foundation, 2025; Save the Rhino International, 2025). Under such low population sizes, recorded poaching losses represent a severe additional pressure, including ≥135 Sumatran rhinos poached historically (1980–1995) and up to 26 Javan rhinos poached in recent years (2019–2023) (International Rhino Foundation, 2024, 2025; Yayasan Badak Indonesia, 2010).

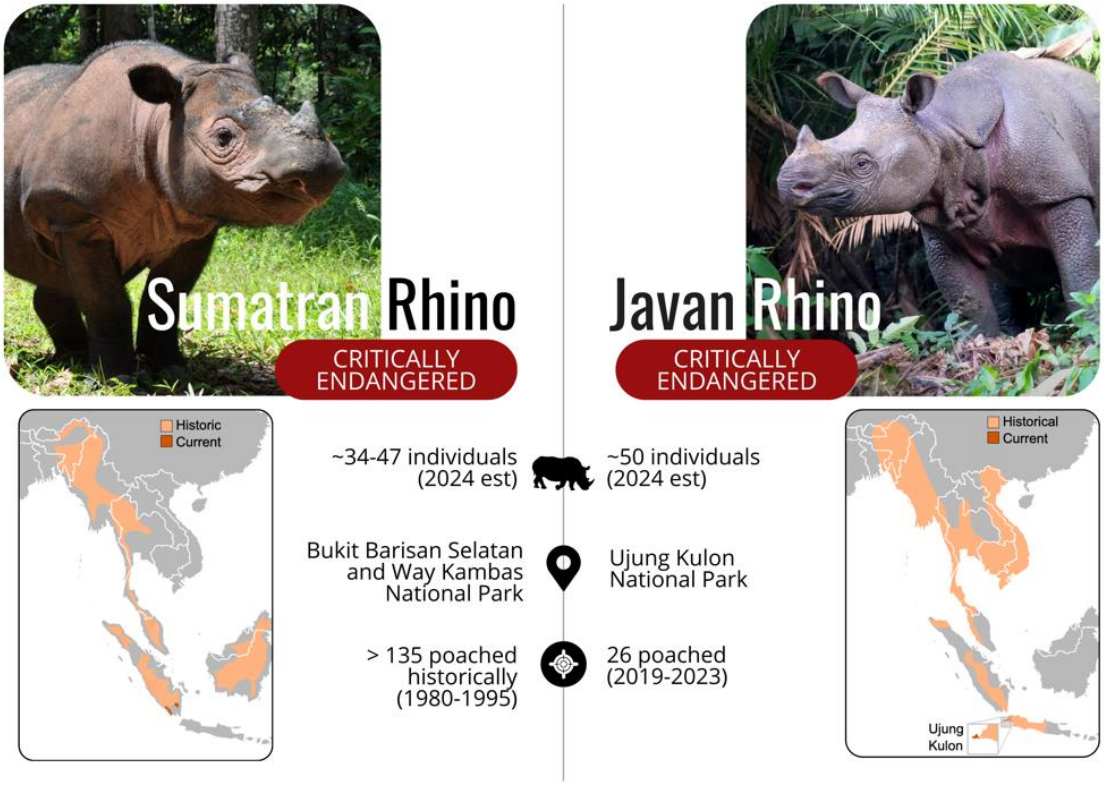

Figure 1. Conservation status, distribution, and poaching pressure for Indonesia's two extant rhinoceros species (photo: Yayasan Badak Indonesia; Tobias Nowlan).

Despite the critical situation, the application of wildlife DNA forensics presents a promising avenue for combating the illegal rhino horn trade in Indonesia. Recent studies have demonstrated the effectiveness of DNA-based techniques in identifying species and tracing the origins of confiscated wildlife products. For example, Ewart et al. developed an internationally standardized species identification test for suspected seized rhinoceros horn, which employs mitochondrial DNA markers to ensure accurate species identification (Ewart et al., 2018). This method has been validated across multiple laboratories, providing law enforcement agencies with a reliable tool to prosecute wildlife traffickers effectively. Moreover, the integration of genetic technologies, such as DNA barcoding (Dalton & Kotze, 2011; Priyono et al., 2023), has shown potential in wildlife forensics. These techniques have been successfully applied in various contexts, including the identification of seized wildlife products and the monitoring of endangered species (Lavenia et al., 2024; Priyono et al., 2025). For instance, a research study in Australia highlighted the role of DNA identification in prosecuting wildlife traffickers, demonstrating the importance of genetic evidence in legal proceedings (Johnson, 2010). Similarly, the work of Harper et al. on forensic matching of confiscated horns to individual poached African rhinoceroses illustrates the potential of DNA forensics in linking poaching incidents to specific individuals, thereby enhancing the ability to hold traffickers accountable (Harper, 2023).

In Indonesia, the establishment of a robust forensic framework that includes the development of a comprehensive DNA database for rhinoceros species could significantly enhance efforts to combat the illegal rhino horn trade. This database would facilitate the identification of illegally traded rhino horn and improve prosecution rates of wildlife traffickers. Furthermore, the use of advanced genetic techniques, such as single nucleotide polymorphisms (SNPs) and microsatellite analysis (Dawnay et al., 2008; Ghosh et al., 2021; Harper et al., 2018; Jan & Fumagalli, 2016), has been shown to provide detailed genetic profiles that can assist in tracing the geographic origins of seized wildlife products (Ogden, 2010).

The aim of this study was to apply a standardized wildlife DNA forensic workflow to species-identify 17 seized horn-like using mitochondrial 12S rRNA marker. Specifically, we sought to (i) determine whether each item originated from a protected rhinoceros species or from non-rhinoceros materials used as counterfeits, and (ii) report sample-by-sample assignments that can support investigation and prosecution in rhino horn trafficking cases.

2. Materials and Methods

A total of 17 horn-like samples seized as suspected rhinoceros horn were submitted for species identification from criminal investigations handled by the West Java Regional Police and Civil Servant Investigators (PPNS) of the Ministry of Environment and Forestry. The samples originated from five cases from Palembang (South Sumatra), Padang (West Sumatra), Surabaya (East Java), and Cirebon (West Java). Prior to DNA work, samples were documented and inspected morphologically; however, because horn-like materials from different mammals can be visually similar, genetic species confirmation was requested to support law-enforcement casework and court proceedings. All analytical steps followed SWFS Standards and Guidelines for Wildlife Forensic Analysis (Moore et al., 2021). To minimize contamination, sample processing, DNA extraction, PCR setup, and post-PCR procedures were conducted in physically separated work areas using dedicated consumables; extraction blanks and no-template controls were included in each batch/run.

DNA isolation and PCR amplification

For each sample, the outer surface was cleaned using 10% bleach followed by aquadest rinse, air-dried, and the inner portion was scraped using a sterile scalpel to obtain approximately 100 mg of keratin shavings. DNA was extracted using the QIAamp DNA Investigator Kit (QIAGEN), following the manufacturer’s protocol, with one modification: the lysis incubation was extended to 24 h to improve DNA yield from keratinous material.

Species identification targeted a 215-bp fragment of the mitochondrial 12S rRNA gene using universal vertebrate primers L1085 and H1259 (Kitano et al., 2007). Purified DNA was used as a template to amplify a 215-bp fragment using universal 12S rRNA primers: L1085 (5′-CCC AAA CTG GGA TTA GAT ACC C-3′) and H1259 (5′-GTT TGC TGA AGA TGG CGG TA-3′) (Kitano et al., 2007). Negative controls were included in each PCR run to monitor for potential contamination. Polymerase chain reaction (PCR) amplification was carried out in a total volume of 25 µL containing KOD One™ PCR Master Mix, 1.5 mM $MgCl_2$, and 10 µM of each primer. The thermocycling conditions consisted of an initial denaturation at 94°C for 3 minutes, followed by 34 cycles of denaturation at 94°C (15 s), annealing at 57°C (5 s), and extension at 68°C (5 s), with a final extension at 68°C for 5 minutes. PCR amplicons were separated by electrophoresis on a 1% agarose gel in 1× Tris-Borate-EDTA (TBE) buffer at 100 V for 25 minutes. PCR amplicons were purified using the QIAquick PCR Purification Kit (QIAGEN) following the manufacturer's protocol. Purified amplicons were quantified using a Qubit fluorometer and then submitted to the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada, for bidirectional Sanger sequencing on an ABI 3500 capillary sequencer.

Data Analysis

The obtained sequences were analyzed bioinformatically. Chromatograms were inspected in Geneious Prime 2025 (https://www.geneious.com; Kearse et al., 2012) and trimmed to a minimum base quality threshold of QV > 20; forward and reverse reads were assembled to generate consensus sequences. Species assignments were first obtained using BLASTn against GenBank, recording the top hit, percent identity, and accession number for each sample (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Edgar, 2010). For each consensus sequence, BLASTn searches were performed against GenBank and the top-ranking matches were evaluated based on percent identity, query coverage, and E-value. Species identification was assigned when the best hit showed high query coverage and high sequence identity, and when alternative hits showed a clear drop in similarity; ambiguous results were flagged for further verification by phylogenetic clustering. Although BLASTn provides a primary species assignment, phylogenetic reconstruction was used as an independent validation to confirm that each seized sample clustered with the expected reference clade with statistical support.

Phylogenetic validation was performed using both Bayesian inference (BI) and maximum-likelihood (ML) approaches. Bayesian phylogenetic reconstruction was conducted in BEAST 2.4 (Bouckaert et al., 2014). The best-fitting nucleotide substitution model was selected using jModelTest 2.1.7 7 (Darriba et al., 2012) based on the Akaike information criterion, and BI was run under the GTR + I + G model (pinv = 0.6222; alpha = 0.2561) using Markov chain Monte Carlo (MCMC) for 10,000,000 generations with sampling every 1,000 generations; the first 25% of samples were discarded as burn-in, and posterior probabilities were calculated on the consensus tree. ML analysis and bootstrap support were estimated using IQ. Trees were visualized and edited in FigTree 1.4.4 (Rambaut, 2009).

3. Results

Seventeen samples suspected to be rhinoceros horns were collected from various cases handled by the West Java Regional Police and the Ministry of Environment and Forestry. Upon morphological examination, the samples closely resembled the horns of the Sumatran rhinoceros (*Dicerorhinus sumatraensis*), the Javan rhinoceros (*Rhinoceros sondaicus*), and the African black rhinoceros (*Diceros bicornis*) (Figure 2). Of the 17 samples analyzed, all yielded successful PCR amplification of the 12S rRNA gene fragment (~215 bp), producing single, bright bands on agarose gel electrophoresis. All successfully amplified samples produced high-quality sequences with quality values (QV) above 20 in Geneious Prime

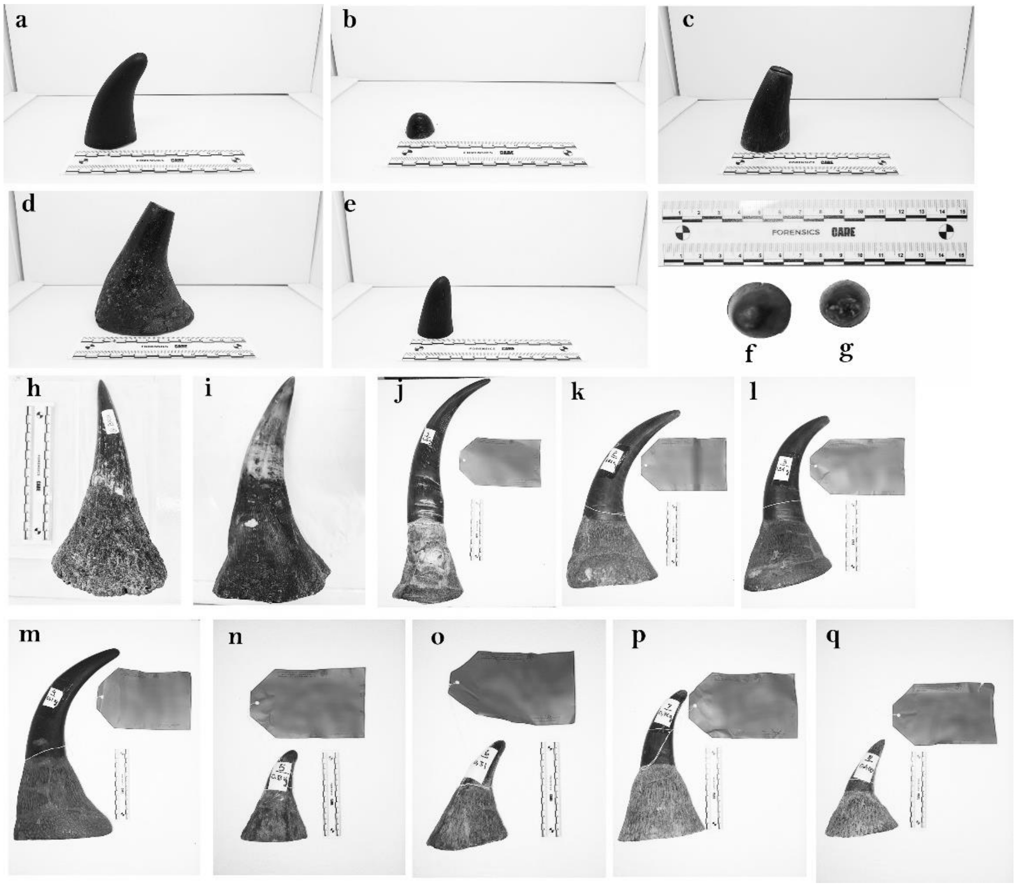

Figure 2. Morphological of the rhino horn samples collected for forensic analysis.

BLASTn analysis of the 12S rRNA sequences revealed four distinct species among the analyzed samples (Table 1). Six samples (a, b, c, d, e, and i) showed the highest sequence identity (99.4–100%) to *Bos taurus* (GenBank accession MZ901612.1), indicating that these items were derived from cattle horn. Two samples (f and g) matched *Bubalus bubalis* with 99.4% identity (accession OR766453.1), confirming a water buffalo origin. Four samples (j, k, l, and m) matched *Diceros bicornis* with 100% identity (accession AJ245721.1), while five samples (h, n, o, p, and q) matched *Dicerorhinus sumatrensis* with 100% identity (accession NC_012684.1), confirming them as genuine rhinoceros horn from African black rhinoceros and Sumatran rhinoceros, respectively.

Table 1. Species assignment of 17 suspected rhinoceros horn samples based on 12S rRNA BLASTn analysis

Sample Code	Origin	BLASTn top hit	Identity (%)	GenBank accession
a	Cirebon, West Java	*Bos taurus*	100	MZ901612.1
b	Cirebon, West Java	*Bos taurus*	100	MZ901612.1
c	Cirebon, West Java	*Bos taurus*	99.4	MZ901612.1
d	Cirebon, West Java	*Bos taurus*	100	MZ901612.1
e	Cirebon, West Java	*Bos taurus*	100	MZ901612.1
f	Cirebon, West Java	*Bubalus bubalis*	99.4	OR766453.1
g	Cirebon, West Java	*Bubalus bubalis*	99.4	OR766453.1
h	Padang, West Sumatra	*Dicerorhinus sumatrensis*	100	NC_012684.1
i	Padang, West Sumatra	*Bos taurus*	100	MZ901612.1
j	Palembang, South Sumatra	*Diceros bicornis*	100	AJ245721.1
k	Palembang, South Sumatra	*Diceros bicornis*	100	AJ245721.1
l	Palembang, South Sumatra	*Diceros bicornis*	100	AJ245721.1
m	Palembang, South Sumatra	*Diceros bicornis*	100	AJ245721.1
n	Palembang, South Sumatra	*Dicerorhinus sumatrensis*	100	NC_012684.1
o	Palembang, South Sumatra	*Dicerorhinus sumatrensis*	100	NC_012684.1
p	Palembang, South Sumatra	*Dicerorhinus sumatrensis*	100	NC_012684.1
q	Palembang, South Sumatra	*Dicerorhinus sumatrensis*	100	NC_012684.1

The phylogenetic analysis, presented in Figure 3, was performed to examine the authenticity of the collected rhino horn samples. The tree was constructed using DNA sequences from several species, including Bos taurus (cattle), Bubalus bubalis (water buffalo), and the rhinoceros species *Rhinoceros sondaicus* (Javan rhinoceros), *Diceros bicornis* (black rhinoceros), and *Dicerorhinus sumatrensis* (Sumatran rhinoceros). Both posterior probabilities and bootstrap values were employed to assess the robustness of the tree topology. High posterior probabilities and bootstrap values were observed for several clades, indicating strong support for these groupings, particularly in the *Dicerorhinus sumatrensis* and *Diceros bicornis* clusters.

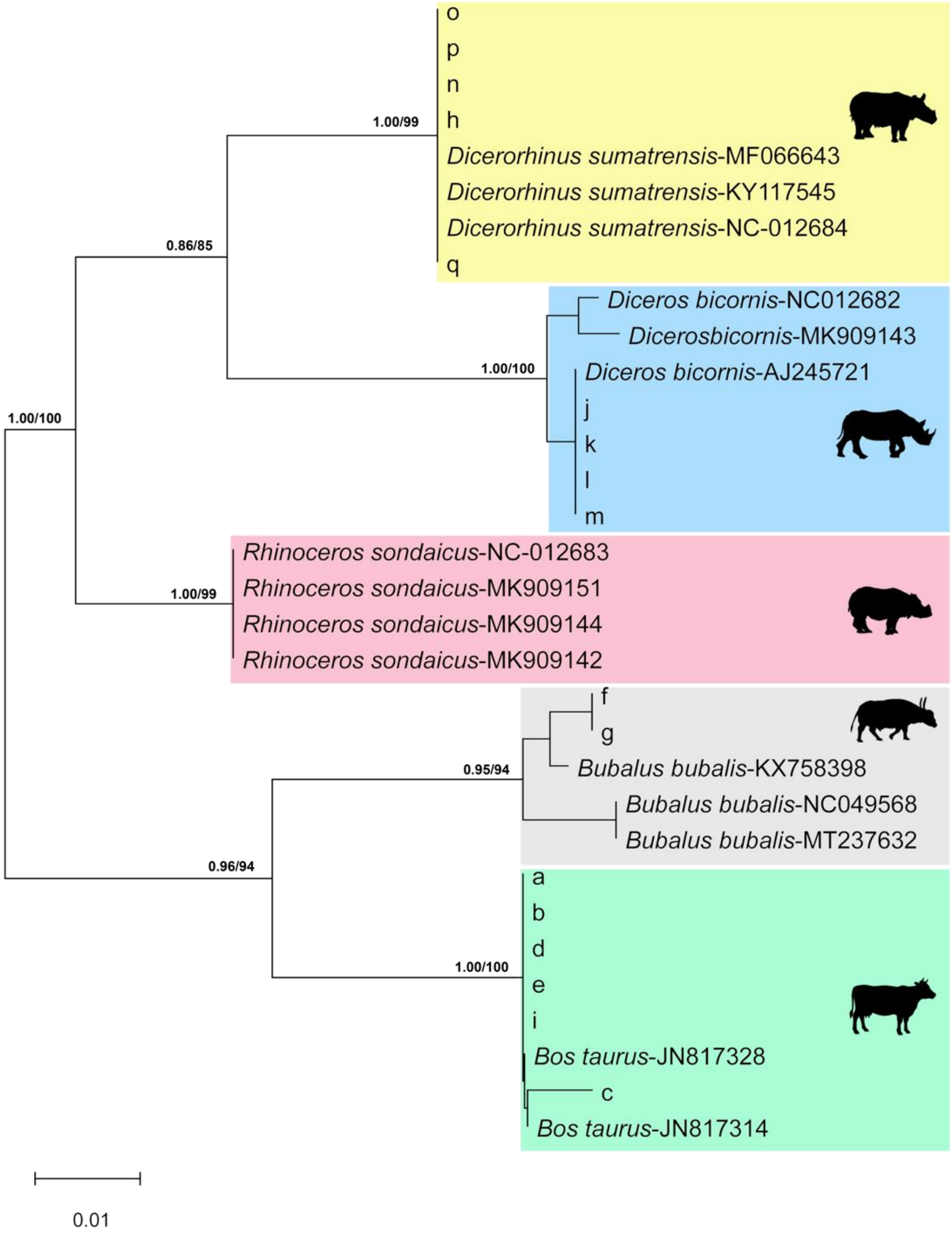

Figure 3. Phylogenetic tree of rhino horn samples and related species.

The phylogenetic analysis placed the water buffalo (*Bubalus bubalis*) and cattle (*Bos taurus*) outside the rhinoceros clades. Notably, some samples that were initially suspected to be rhinoceros horns, such as samples f and g, grouped within the buffalo clade, while other samples (a, b, d, e, i, c) were positioned within the cattle clade. This unexpected clustering of rhinoceros-suspected samples within non-rhinoceros clades indicated that these items were not genuine rhinoceros horns, suggesting either intentional counterfeiting or misidentification. The posterior probabilities and bootstrap values across the tree were exceptionally high, providing strong statistical support for the tree topology and the reliability of the observed clustering patterns.

4. Discussion

Illegal wildlife trade involving suspected rhinoceros horn poses a major challenge for law enforcement because morphological inspection alone is often insufficient to verify species identity, particularly when items are processed, fragmented, or intentionally modified. The morphological appearance of seized horn samples can closely resemble rhinoceros horn, and horn-like structures from other large mammals—such as cattle (*Bos taurus*) and water buffalo (*Bubalus bubalis*)—may mimic genuine rhinoceros horn, creating a high risk of misidentification (Harper et al., 2018). Therefore, integrating morphological examination with DNA-based species identification is essential to substantiate claims of rhinoceros horn authenticity and to support legal and conservation actions against trafficking (Ewart et al., 2018; Greenfield & Veríssimo, 2019; Haas & Ferreira, 2016; Harper et al., 2013).

In the present casework, 12S rRNA sequencing provided objective evidence to distinguish genuine rhinoceros horn from visually similar materials derived from domestic livestock, thereby reducing the risk of misclassification in criminal investigations. DNA analysis showed that a substantial proportion of seized horn items originated from *Bos taurus* and *Bubalus bubalis*, particularly in seizures from Cirebon (West Java) and partly from Padang (West Sumatra), whereas genuine Sumatran and African black rhinoceros horn was predominantly associated with cases from Palembang (South Sumatra) and Padang. This clear separation between counterfeit livestock horns and genuine rhinoceros horn, combined with the documented geographic origin of seizures, illustrates how wildlife DNA forensics can both prevent the laundering of non-protected species as “rhino horn” and provide prosecutors with robust, location-specific genetic evidence relevant to trafficking investigations. Notably, the occurrence of both African black rhinoceros and Sumatran rhinoceros horn in Indonesian seizures is consistent with seizure intelligence linking Indonesia to rhino horn seizures involving both African and Sumatran rhinoceros horn. Seizure analyses further indicate that rhino horn trafficking routes are often dominated by a limited set of source, transit, and destination locations, with southern Africa frequently implicated at the supply end and several Asian hubs (e.g., Malaysia, Viet Nam, China and Hong Kong SAR) repeatedly implicated as transit/destination nodes (Milliken et al., 2009; Wildlife Justice Commission, 2022). Although DNA provided definitive species identification in this study, purely morphological screening in the field can be unreliable because seized horn items may be fragmented, processed, or intentionally modified to mimic rhinoceros horn. However, preliminary inspection may still be aided by a small number of practical cues (when present), such as the characteristic filamentous/hair-pattern appearance of genuine rhinoceros horn and differences in internal structure when a clean cross-section is available (Jha & Kshetry, 2015), while recognizing that these indicators are not diagnostic and should be confirmed by genetic testing.

Genetic evidence is increasingly used in wildlife enforcement because it provides objective species identification and can contribute to prosecutorial efforts against traffickers (Bourret et al., 2020; Ogden, 2010; Priyono et al., 2025). In this study, the phylogenetic analysis clearly separated seized samples into rhinoceros and non-rhinoceros clades, supporting the BLASTn-based assignments and strengthening confidence in the forensic identifications. High posterior probability and bootstrap support across the main clades further indicated that the 12S rRNA marker provided consistent and repeatable species discrimination for the examined horn materials. To maximize the legal and operational value of such casework across jurisdictions, future efforts should prioritize workflow standardization and inter-laboratory harmonization for wildlife forensic investigations (Alacs et al., 2010; Ewart et al., 2018; Nishant et al., 2017). In addition, developing validated reference resources and higher-resolution genetic markers would enable stronger geographic attribution (when required) and help connect seizures to potential poaching or trafficking routes (Iyengar, 2014; Olmedo et al., 2018).

While 12S rRNA sequencing provided reliable species identification in this casework, it does not enable fine-scale geographic attribution of rhinoceros horn origin. Therefore, establishing a validated Indonesian reference database for rhinoceros populations and adopting higher-resolution markers (e.g., microsatellites, SNPs, or haplotype-based approaches) would be essential to support individual assignment of future seizures; geographic assignment to pinpoint poaching origins would be expected to be most informative for Sumatran rhinoceros populations, whereas for Javan rhinoceros the main applications would be individual identification and forensic matching/monitoring (Ghosh et al., 2021; Jan & Fumagalli, 2016; Sukawutthiya et al., 2017). In parallel, standardized field collection procedures and capacity building for enforcement officers remain critical to minimize contamination and preserve evidentiary value in court. Finally, rapid molecular screening tools (e.g., qPCR-based assays) could support timely authentication of suspected horn materials, particularly when rapid triage is needed in enforcement settings (Mallorie et al., 2024; Ogden, 2011; Rodionov et al., 2021).

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