



DNA Barcoding and Phylogenetic Analysis of the Gastropod *Terebralia semistriata* from the Coastal Waters of Merauke, South Papua Province

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Abstract: This study aimed to identify the gastropod species *Terebralia* sp. from the coastal waters of Merauke, South Papua Province, using a molecular identification approach. The Cytochrome Oxidase Subunit I (COI) gene was amplified through the Polymerase Chain Reaction (PCR) method using the forward primer LCO-1490 (5'-GGTCAACAAATCATAAAAGATATTGG-3') and the reverse primer HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3'). DNA sequence analyses were performed using MEGA X software. A total of five samples were successfully amplified, producing DNA fragments with a length of 658 bp. Identification based on the DNA barcoding approach confirmed that all analyzed specimens belonged to *Terebralia semistriata*, with sequence similarity values ranging from 98.94% to 100% among samples. The genetic distance values obtained ranged from 0.003 to 0.012. Phylogenetic reconstruction using the Neighbor-Joining method with the Kimura 2-Parameter model revealed clustering patterns of sequences based on genetic similarity and interspecific genetic distances. Molecular identification using DNA barcoding provides an effective approach for accurate species identification and supports sustainable management and utilization of gastropod resources.

Keywords: *Terebralia semistriata*; DNA barcoding; Cytochrome Oxidase Subunit I gene; phylogenetics

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INTRODUCTION

Terebralia is a genus within the family Potamididae that is widely distributed and abundant in brackish and marine environments (Noersativa et al., 2015; Saleky et al., 2023). This genus has important ecological and economic roles, particularly in mangrove and intertidal ecosystems. However, the high diversity of *Terebralia* has resulted in persistent taxonomic challenges and frequent species misidentification (Layton et al., 2014). These difficulties are mainly associated with the presence of morphologically similar (*cryptic*) species within this group (Ulfa et al., 2020; Saleky & Merly, 2021). Traditionally, gastropod identification relies heavily on shell morphology; however, shell-based identification can be problematic due to variations in morphological characteristics and differences in taxonomic interpretation (Merly & Saleky, 2021). Therefore, an accurate and reliable identification approach is required to overcome taxonomic uncertainty, particularly for closely related gastropod species.

DNA barcoding has emerged as an effective molecular approach for species identification by utilizing specific DNA fragments as genetic markers (Achmad et al., 2020). Beyond species discrimination, this approach can also provide insights into species origin, evolutionary history, population connectivity, and genetic structure (Madduppa et al., 2017). The mitochondrial Cytochrome Oxidase Subunit I (COI) gene

is one of the most widely used molecular markers in animal DNA barcoding due to its effectiveness in distinguishing closely related species. Although DNA barcoding has been widely applied for species identification, its accuracy depends strongly on the availability and completeness of reference barcode sequences in databases such as GenBank (Antil et al., 2022). This molecular approach has contributed to resolving long-standing taxonomic uncertainties and improving knowledge of the geographical distribution of fauna associated with mangrove ecosystems (Bravo et al., 2021).

The coastal region of Merauke, South Papua, is predominantly characterized by mangrove ecosystems that support diverse aquatic organisms, including fish, bivalves, crabs, and gastropods (Eddiwan et al., 2017; Syahril et al., 2018). Among the abundant gastropods found in this ecosystem is *Terebralia* sp., which has ecological and economic importance and is commonly utilized as an environmental bioindicator. In addition, *Terebralia* sp. is harvested by local communities in Merauke as a food resource and source of income. Previous research by Saleky & Merly (2021) identified *Terebralia* sp. collected from Payum Beach, Merauke Regency, as *Terebralia semistriata*. However, molecular information regarding *Terebralia semistriata* populations from the coastal waters of Merauke remains limited. The previous study was conducted at a single location, whereas broader sampling across multiple locations is required to provide a more comprehensive understanding of the distribution and genetic characteristics of this species in the region.

In this study, sampling was expanded to five coastal locations in Merauke to represent a wider distribution area of *Terebralia semistriata*. The limited availability of molecular reference data for the genus *Terebralia* in Merauke coastal waters highlights the importance of generating additional genetic information to support accurate species identification and sustainable resource management. Molecular characterization of *Terebralia semistriata* from this region is expected to contribute valuable baseline data for future studies on biodiversity, population genetics, and conservation of mangrove-associated gastropods.

Therefore, this study aimed to identify *Terebralia* sp. from the coastal waters of Merauke, South Papua Province, using a molecular approach based on DNA barcoding and phylogenetic analysis of the Cytochrome Oxidase Subunit I (COI) gene. This molecular identification approach is expected to provide accurate species-level identification and support sustainable management and utilization of gastropod resources.

METHOD

Sample Collection

This study employed an exploratory survey approach to describe natural conditions without any experimental manipulation. Sampling was conducted from August to September 2025 at five different locations along the coastal waters of Merauke, South Papua Province, including Payum Beach, Buti Beach, Biangkuk, Urumb Beach, and Kumbe Beach (Figure 1).

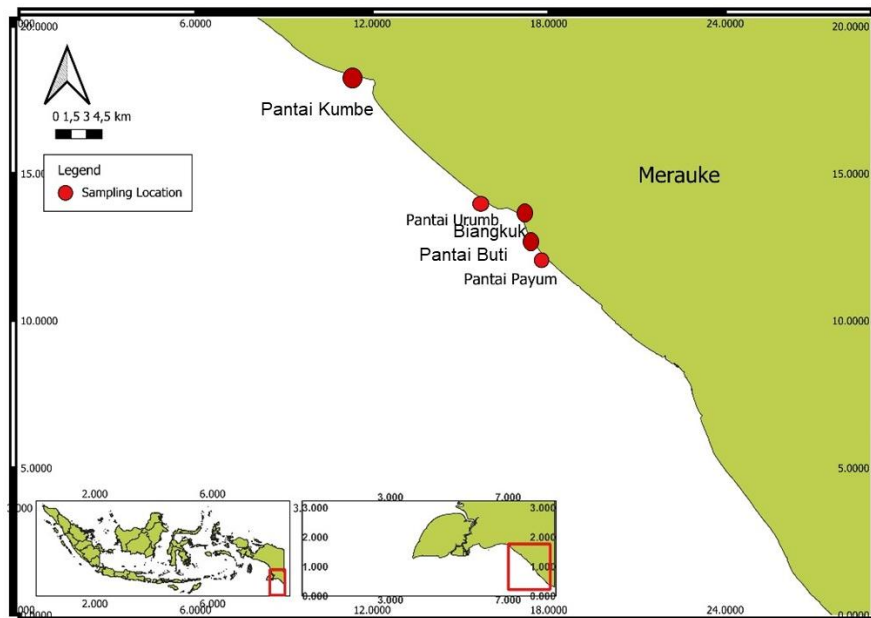


Figure 1. Study location

The research procedures consisted of four main stages: sample collection, morphological identification, genetic analysis, and data analysis. Preserved specimens were identified based on gastropod and bivalve identification guides (Dharma, 1988; Dharma, 2005). Species identification was conducted at the Aquatic Resources Management Laboratory, Faculty of Agriculture, Universitas Musamus. Tissue samples from the soft body of *Terebralia* sp. were preserved in 95% ethanol for subsequent molecular analysis.

Laboratory Analysis

The preserved tissue samples were subsequently sent to the BIONESIA Laboratory for molecular analysis and DNA data acquisition. The mitochondrial Cytochrome Oxidase Subunit I (COI) gene was amplified using universal primers, namely the forward primer LCO-1490 (5'-GGTCAACAAATCATAAAAGATATTGG-3') and the reverse primer HCO-2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer *et al.*, 1994).

The total PCR reaction volume was 26 μ L, consisting of 2 μ L extracted DNA template, 1.25 μ L of each primer (10 mM concentration), 9 μ L ddH₂O, and 12.5 μ L Ready Mix. PCR amplification was performed using an Applied Biosystems™ 2720 Thermal Cycler. The PCR thermal profile consisted of an initial denaturation step at 94°C for 3 min, followed by 38 cycles of denaturation at 94°C for 30 s, annealing at 50°C for 30 s, and extension at 72°C for 60 s. The final extension step was conducted at 72°C for 2 min.

PCR products were visualized through electrophoresis on a 1% agarose gel stained with Nucleic Acid Gel Stain (GelRed®). Samples showing positive amplification, indicated by visible DNA bands, were subsequently subjected to DNA sequencing using the Sanger dideoxy sequencing method at PT. Genetika Science Jakarta.

Data Analysis

The obtained DNA sequences were edited and aligned using the MUSCLE alignment method implemented in MEGA X software. Each nucleotide sequence was

manually inspected to ensure the quality and accuracy of the dataset. Sequences with poor quality were reprocessed through PCR amplification and sequencing.

The obtained sequences were compared with reference sequences available in the GenBank database using the Basic Local Alignment Search Tool (BLAST) available through the NCBI website (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>). The percentage of sequence similarity and identification accuracy were recorded for each sample.

Phylogenetic analysis was subsequently performed to evaluate genetic relationships among samples and to confirm species identification based on BLAST results. Phylogenetic trees were constructed using the Neighbor-Joining (NJ) method with 1,000 bootstrap replications in MEGA X software. Reference DNA sequences representing ingroup and outgroup taxa were obtained from the GenBank database for phylogenetic reconstruction. Genetic distances among sequences were calculated using the p-distance method to compare genetic divergence among samples. All molecular analyses were performed using MEGA X software (Kumar et al., 2018).

RESULTS AND DISCUSSION

Molecular Characteristics

A total of five *Terebralia semistriata* specimens were successfully amplified using the COI gene marker. Electrophoresis analysis showed clear DNA bands corresponding to approximately 700 bp based on the DNA ladder reference (Figure 2). The obtained COI sequences had a final length of 658 bp. This fragment size is consistent with the expected amplification target of the universal COI primers LCO1490 and HCO2198, which generally amplify approximately 700 bp of the mitochondrial COI region (Triandiza et al., 2022).

Although DNA barcoding has been widely applied for species identification, amplification of mitochondrial markers in marine organisms can be challenging due to their high taxonomic diversity and variation in primer efficiency among different animal groups. Several universal primers have demonstrated broad applicability; however, their amplification success is not consistent across all marine taxa (Lobo et al., 2013). The successful amplification obtained in this study indicates that the LCO1490 and HCO2198 primer combination was effective for generating COI sequences of *Terebralia semistriata* from Merauke coastal waters.

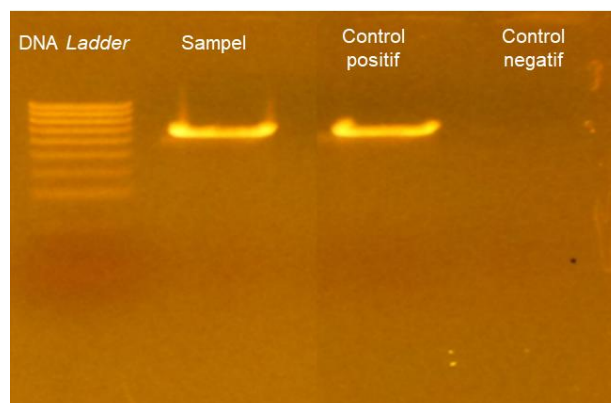


Figure 2. Amplification results of the COI gene from the gastropod *Terebralia semistriata*

The mitochondrial COI analysis successfully identified all five specimens collected from Payum Beach, Buti Beach, Biangkuk, Urumb Beach, and Kumbe Beach as *Terebralia semistriata*. All sequences showed an identical fragment length of 658

bp. The effectiveness of the COI marker for gastropod identification is related to its relatively conserved primer-binding regions and sufficient interspecific variation, allowing discrimination among closely related taxa.



Figure 3. Morphological characteristics of the gastropod *Terebralia semistriata*

Terebralia semistriata belongs to the family Potamididae (Class Gastropoda) and is commonly distributed in mangrove forests and intertidal mudflats throughout the Indo-Pacific region (Saleky et al., 2023). This species is abundant in Indonesian coastal ecosystems, including Papua, where it contributes to ecosystem functioning through organic matter processing. Ecologically, *T. semistriata* functions as a herbivore and deposit feeder, utilizing organic detritus as a food source. Its role in mangrove ecosystems is important because it contributes to the decomposition of fallen mangrove leaves and facilitates nutrient recycling within coastal ecosystems (Merly et al., 2023).

Molecular Identification Based on BLAST Analysis

The COI sequences obtained from the five *Terebralia* specimens were subjected to BLAST analysis against the NCBI nucleotide database to determine their taxonomic identity. Species identification was assessed based on sequence similarity, query coverage, and percentage identity with available reference sequences. The BLAST-based identification results are summarized in Table 1.

Table 1. Molecular identification results using BLAST analysis in the National Center for Biotechnology Information (NCBI)

No. Species	bp	Gene	Accession Number	Query Cover (%)	Identity (%)
1 <i>Terebralia semistriata</i>	658	COI	AM932805.1	100%	98.94%
2 <i>Terebralia semistriata</i>	658	COI	AM932805.1	100%	98.94%
3 <i>Terebralia semistriata</i>	658	COI	AM932805.1	100%	98.94%
4 <i>Terebralia semistriata</i>	658	COI	AM932805.1	100%	100%
5 <i>Terebralia semistriata</i>	658	COI	AM932803.1	100%	99.39%

The genus *Terebralia* includes several morphologically similar species, including *Terebralia semistriata*, *Terebralia palustris*, and *Terebralia sulcata*. These species share similar ecological habitats, particularly mangrove ecosystems, which may complicate species identification based solely on shell morphology. Therefore,

molecular identification provides an important complementary approach for accurate species discrimination.

BLAST analysis confirmed that all analyzed specimens belonged to *Terebralia semistriata*, with sequence identity values ranging from 98.94% to 100% and 100% query coverage. These results support the reliability of the COI marker for distinguishing *Terebralia* species from Merauke coastal waters.

The nucleotide composition analysis showed that the COI sequences consisted of thymine (T) (23.28%), cytosine (C) (18.12%), adenine (A) (19.57%), and guanine (G) (39.03%). The average GC content was 57.15%, while the average AT content was 42.85% (Table 2). These findings confirm that the COI marker successfully generated informative molecular data for identifying the analyzed specimens as *Terebralia semistriata*.

Nucleotide Composition Analysis

The nucleotide composition analysis was conducted to determine the proportion of each nucleotide base within the COI gene fragment of *Terebralia semistriata*. The percentage composition of G, C, A, and T nucleotides, together with the GC and AT content, is summarized in Table 2.

Table 2. Nucleotide composition of the COI gene fragment of *Terebralia semistriata*

Sample	G (%)	C (%)	A (%)	T (%)	G+C (%)	A+T (%)	Length (bp)
<i>Terebralia semistriata</i>	39.06	18.09	19.45	23.40	57.15	42.85	658
<i>Terebralia semistriata</i>	38.75	18.39	19.45	23.40	57.14	42.85	658
<i>Terebralia semistriata</i>	39.06	18.09	19.45	23.40	57.15	42.85	658
<i>Terebralia semistriata</i>	39.51	17.63	19.76	23.10	57.14	42.86	658
<i>Terebralia semistriata</i>	38.75	18.39	19.76	23.10	57.14	42.86	658
Average	39.03	18.12	19.57	23.28	57.15	42.85	658

The nucleotide composition analysis showed that the COI sequences of *Terebralia semistriata* had an average nucleotide composition of 39.03% guanine (G), 18.12% cytosine (C), 19.57% adenine (A), and 23.28% thymine (T). The average GC content was 57.15%, while the AT content was 42.85%.

The nucleotide composition results indicated that the GC content was higher than the AT content in the analyzed samples. This nucleotide composition may influence DNA stability and amplification characteristics during the PCR process. The relatively higher AT proportion facilitates sequence denaturation during amplification (Saleky et al., 2021). In this study, the denaturation temperature applied during PCR was 94°C, which is considered relatively low.

Genetic Distance and Phylogenetic Analysis

Genetic distance represents a numerical measurement of genetic differences or similarities among individuals, populations, or species based on DNA/genomic variation. Lower genetic distance values indicate closer genetic relationships (Fatmarischa et al., 2014). This parameter is commonly used in phylogenetic reconstruction and biodiversity assessment.

The genetic distance analysis of *Terebralia semistriata* populations from Merauke coastal waters ranged from 0.003 to 0.012. These values were below 4%, indicating that all analyzed samples belonged to the same species. Intraspecific genetic distances are generally less than 4%, whereas interspecific distances are typically greater than 4% (Madduppa et al., 2017). Genetic distance reflects the degree of similarity in DNA composition among individuals, populations, or species; therefore,

smaller genetic distance values indicate higher genetic similarity (Doğan & Doğan, 2016).

Table 3. Genetic distance among *Terebralia semistriata*, *Terebralia palustris*, *Terebralia sulcata*, and *Telescopium telescopium*

No	Species	1	2	3	4	5	6	7	8	9	10
1	<i>Terebralia semistriata</i> (Pantai Payum)	*	*	*	*	*	*	*	*	*	*
2	<i>Terebralia semistriata</i> (Pantai Buti)	0,003	*	*	*	*	*	*	*	*	*
3	<i>Terebralia semistriata</i> (Biangkuk)	0,003	0,003	*	*	*	*	*	*	*	*
4	<i>Terebralia semistriata</i> (Pantai Urumb)	0,011	0,011	0,011	*	*	*	*	*	*	*
5	<i>Terebralia semistriata</i> (Pantai Kumbe)	0,012	0,012	0,012	0,011	*	*	*	*	*	*
6	<i>Terebralia semistriata</i> (HE680658.1. Australia)	0,075	0,079	0,079	0,066	0,079	*	*	*	*	*
7	<i>Terebralia semistriata</i> (HE680659.1. Australia)	0,012	0,012	0,012	0,014	0,006	0,075	*	*	*	*
8	<i>Terebralia sulcata</i> (MZ832021.1. Hongkong)	0,170	0,170	0,170	0,164	0,172	0,144	0,166	*	*	*
9	<i>Terebralia palustris</i> (PV034246.1. India)	0,174	0,174	0,174	0,167	0,176	0,148	0,170	0,176	*	*
10	<i>Telescopium telescopium</i> (MT034052.1. Pakistan)	0,242	0,245	0,245	0,234	0,245	0,203	0,237	0,223	0,228	*

Comparison with reference sequences from Australia showed higher genetic distance values for some *Terebralia semistriata* sequences. Such variation may reflect geographic structuring among populations, potentially associated with spatial separation, environmental differences, or limited gene flow. However, additional population-level analyses using more specimens and additional molecular markers are required to confirm evolutionary processes such as population differentiation or possible geographic subdivision.

Evolutionary relationships among taxa can be better understood through phylogenetic analysis, which provides information regarding lineage relationships and genetic divergence. This approach is particularly useful for taxa with morphological similarities or unresolved taxonomic relationships (Lestari et al., 2018).

Reference Sequences Used for Phylogenetic Reconstruction

Reference sequences were obtained from the NCBI GenBank database to reconstruct the phylogenetic relationships of *Terebralia semistriata* and related taxa. The selected sequences included closely related *Terebralia* species and an outgroup taxon, with their corresponding locations, accession numbers, and references summarized in Table 4.

Table 4. DNA sequences of *Terebralia semistriata* used for phylogenetic reconstruction, including location, accession numbers, and references from the National Center for Biotechnology Information (NCBI)

No Species	Location	Accession No.	Source
1 <i>Terebralia semistriata</i>	Australia: Charles Darwin National Park	HE680658.1	Reid et al., 2008
2 <i>Terebralia semistriata</i>	Australia: Charles Darwin National Park	HE680659.1	Reid et al., 2008
3 <i>Terebralia sulcata</i>	Hong Kong, Nam Chung	MZ832021.1	Bravo et al., 2021
4 <i>Terebralia sulcata</i>	Hong Kong, Nam Chung	MZ832014.1	Bravo et al., 2021
5 <i>Terebralia palustris</i>	India	PV034246.1	Unpublished
6 <i>Terebralia palustris</i>	India	PQ856153.1	Unpublished
7 <i>Telescopium telescopium</i>	Pakistan	MT034052.1	George et al., 2021
8 <i>Telescopium telescopium</i>	India	PP499016.1	Bhaumik et al., 2025

The phylogenetic tree topology (Figure 4) was used to evaluate evolutionary relationships and clarify patterns of species differentiation based on molecular characteristics. Molecular markers can be combined with other genetic markers to improve the accuracy of phylogenetic reconstruction and species grouping.

In the phylogenetic reconstruction of *Terebralia semistriata*, several taxa were selected as outgroups, including *Terebralia sulcata*, *Terebralia palustris*, and *Telescopium telescopium*. An outgroup is a distantly related taxon used as a reference group in phylogenetic analysis. The inclusion of an outgroup allows the determination of the root of the phylogenetic tree and facilitates interpretation of evolutionary direction from ancestral to derived lineages.

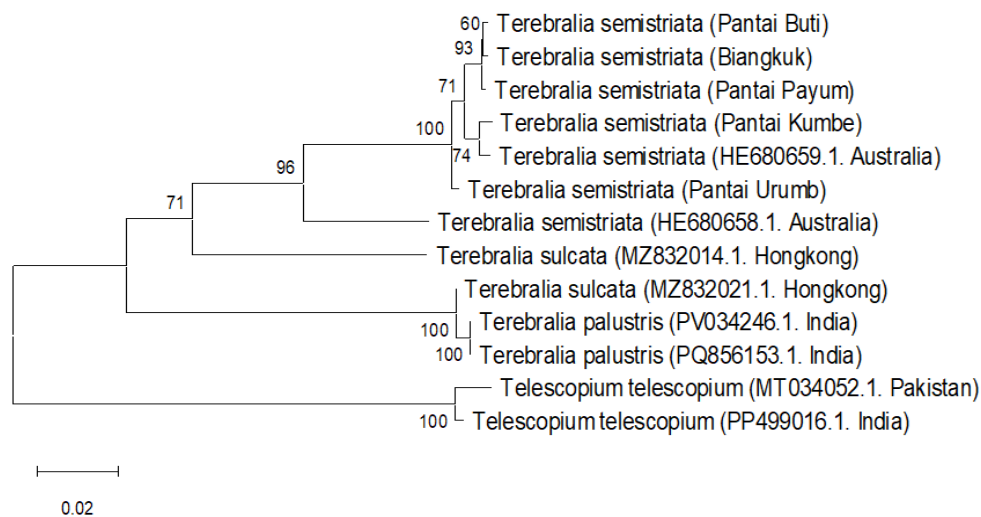


Figure 4. Phylogenetic tree reconstruction of *Terebralia semistriata*

The phylogenetic reconstruction showed that *Terebralia semistriata* specimens from Merauke clustered within the same clade as *Terebralia semistriata* (HE680659.1) from Australia, indicating a close genetic relationship between these populations. In contrast, other *Terebralia* species formed separate clades, demonstrating clear genetic differentiation among species.

The phylogenetic grouping obtained in this study supports the effectiveness of molecular markers in resolving taxonomic uncertainty that may arise from morphology-based identification. The clustering pattern generated using the Neighbor-Joining method with the Kimura 2-Parameter model indicates that *Terebralia semistriata* from Merauke shares a close evolutionary relationship with Australian populations.

The genetic similarity observed between geographically separated populations may be influenced by historical connectivity, environmental similarity, and species dispersal mechanisms (Saleky et al., 2016). Nevertheless, further investigations involving population genetics approaches, larger sample sizes, and additional molecular markers are recommended to better understand population structure and evolutionary connectivity of *Terebralia semistriata* across its distribution range.

CONCLUSION

The present study confirms that the gastropod specimens collected from the coastal waters of Merauke were identified as *Terebralia semistriata* based on COI gene-based DNA barcoding analysis. The obtained COI sequences had a length of 658 bp, with sequence identity values ranging from 98.94% to 100% compared with reference sequences available in the NCBI database. Phylogenetic analysis further

supported the species identification by demonstrating the clustering of *Terebralia semistriata* sequences based on genetic similarity and genetic distance patterns. These findings highlight the effectiveness of the COI gene as a molecular marker for accurate species identification and provide baseline genetic information for *Terebralia semistriata* populations in the coastal waters of Merauke.

RECOMMENDATION

Based on the findings of this study, further research is recommended to investigate the genetic population structure of *Terebralia semistriata* distributed along the coastal waters of Merauke. Such studies would provide additional insights into population connectivity, genetic variation, and evolutionary patterns of this mangrove-associated gastropod species.

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