



DNA Barcoding of Gastropods Gleaned from South Sulawesi Seagrass Beds

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ABSTRACT

Gastropods are an important component of seagrass ecosystems; however, this group is vulnerable to local pressures due to gleaning activities by coastal communities. Morphological-based identification is often hampered by phenotypic variation and the presence of cryptic species, so an integrative morphological and molecular approach is needed to improve identification accuracy. This study seeks to identify gastropods gleaned from seagrass beds in Labuange Village, Barru Regency, South Sulawesi, using an integrative approach using cytochrome c oxidase subunit I (COI) mitochondrial DNA barcoding and shell morphology. The combined morphological and molecular approach identified 16 gastropod specimens belonging to six species from four families: Strombidae (*Canarium urceus*, *Canarium cf. incisum*, *Gibberulus gibbosus*); Cypraeidae (*Cypraea cf. tigris*); Tegulidae (*Tectus cf. architectonicus*); and Volutidae (*Cymbiola cf. vespertilio*). Reference sequences showing a high level of similarity (98.99–100%) were available for most species from GenBank (families Strombidae and Cypraeidae), and from BOLD for *Cymbiola cf. vespertilio*, thereby confirming the effectiveness of this method. No similar sequences were available for *Tectus cf. architectonicus*, emphasizing the importance of enriching molecular barcode databases for tropical gastropods with vouchered reference sequences to support the use of DNA barcoding as an important tool in supporting the conservation and sustainable management of gastropod resources in seagrass ecosystems.

INTRODUCTION

Gastropods, the largest class of mollusks, comprise nearly 80,000 species globally and inhabit both terrestrial and marine ecosystems, including seagrass beds (Bieler, 1992). Seagrass ecosystems perform two key functions. First, they serve as important

nursery grounds that provide shelter and food for various fish and invertebrates (Carreras-Colom *et al.*, 2024; Heng *et al.*, 2025). Second, they play a vital role in mitigating the impacts of climate change by absorbing and storing large amounts of “blue carbon” in biomass and sediments (Quevedo *et al.*, 2023; Heng *et al.*, 2025). Gastropods also play an important ecological role in seagrass beds. They function as herbivores and detritivores that help control the growth of epiphytes on seagrass leaves (Heck & Valentine, 2006). Excessive epiphyte growth can block seagrass photosynthesis, thereby threatening the overall health of the ecosystem (Boyd *et al.*, 2022).

Seagrass ecosystems face numerous challenges, including pollution, coastal development, and climate change (Tang & Hadibarata, 2022; Mahjabin *et al.*, 2023). Local human activities pose additional threats, including gleaning, which refers to the collection of marine organisms in tidal areas (Stiepani *et al.*, 2023). Gleaning to collect shellfish, crustaceans, and other invertebrates is typically conducted by hand or with simple tools. This activity often represents an important form of small-scale fisheries that supports the food security of coastal communities and holds significant economic and cultural value (Wallner-Hahn *et al.*, 2022; Stiepani *et al.*, 2023). However, high gleaning intensity can threaten seagrass biodiversity, especially in densely populated areas with many gleaners. Contributing factors include physical disturbance to seagrass habitats caused by repeated trampling (Nadiarti *et al.*, 2021) and inappropriate species selection, which can lead to declines in target gastropod populations (Fanoro *et al.*, 2023).

Therefore, understanding the condition and diversity of gastropod populations is essential for maintaining the ecological functions of seagrass habitats. Furthermore, accurate species data are needed to support sustainable resource management (Costello *et al.*, 2010). Species identification based solely on morphological characteristics can be challenging, particularly for mollusks, which often exhibit high phenotypic plasticity and may include cryptic species that are genetically distinct but difficult to distinguish morphologically (Sturm *et al.*, 2006; Puillandre *et al.*, 2014). DNA barcoding has revolutionized the fields of taxonomy and ecology by using a standard gene fragment as an effective genetic marker for species identification. The most commonly used marker is the mitochondrial cytochrome c oxidase I subunit (COI) gene, which tends to be conserved within species but varies between species (Salis *et al.*, 2024). DNA barcoding has proven useful in aquatic ecosystems for detecting invasive species, monitoring marine populations for fisheries management, and discovering cryptic diversity (Salis *et al.*, 2024; Heng *et al.*, 2025). This is particularly evident in mollusks (Remigio & Hebert, 2003; Mai *et al.*, 2025).

Gleaning of mollusks and other invertebrates is common within the Coral Triangle, including the seagrass beds of Sulawesi (Allen & McKenna, 2001; Ambo-Rappe, 2020). From biodiversity conservation and resource management perspectives,

understanding the taxonomic diversity of gleaned gastropods is therefore essential. However, despite reports of high mollusk diversity in this region (Allen & McKenna, 2001), taxonomic data on harvested gastropods remain limited and rarely include DNA barcoding.

Therefore, the objective of this study is to determine which gastropod species are collected by local communities during gleaning activities in the seagrass beds of Labuange Village, Mallusetasi District, Barru Regency, South Sulawesi, Indonesia. This study used an integrative approach combining COI DNA barcoding with morphological observations. The results will provide vouchered DNA barcodes and data on the taxonomic composition of gleaned gastropods. These findings can contribute to the development of scientifically based management strategies for seagrass meadow ecosystems in South Sulawesi.

MATERIALS AND METHODS

Research Location and Time

The study site was the seagrass beds surrounding Batu Kalasi Island, located in Labuange Village, Mallusetasi District, Barru Regency, South Sulawesi, Indonesia (Fig. 1). Sampling was conducted in July–August 2025 during spring low tides, when the intertidal zone was exposed and local communities were actively gleaning in the seagrass beds.

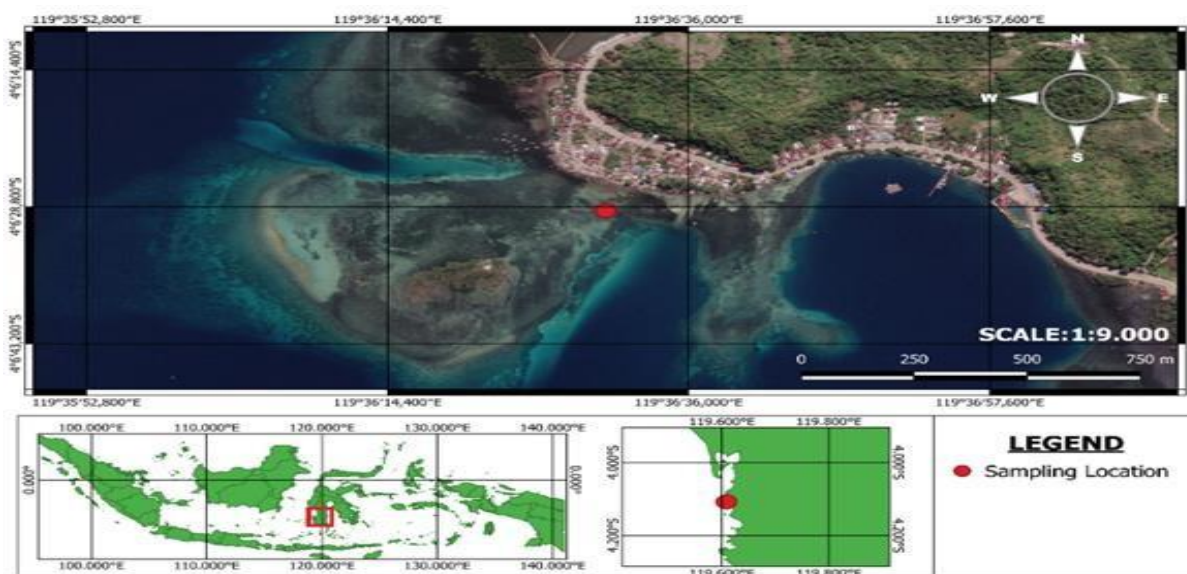


Fig. 1. Sample Collection Location in Batu Kalasi, Labuange Village, Mallusetasi-Barru District, South Sulawesi, Indonesia

Sample Collection and Morphological Analysis

Gastropod specimens were obtained from invertebrates collected by local gleaners. Individual gastropod samples were purposively selected to represent the taxa (morphotypes) present in the gleaning catches. These samples were grouped based on

morphological characteristics and photographed. Two to three specimens were selected from each morphological group for DNA analysis.

Muscle tissue from each specimen was extracted using sterile tweezers and immediately preserved in a 1.5mL microtube filled with 96% ethanol. The preserved samples were sent to the Indonesian Biodiversity Laboratory (BIONESIA), Ujung Kaja, Denpasar, Bali, Indonesia, for molecular analysis.

Morphological observations focused on external characteristics commonly used in mollusk identification, including shell shape, size, color, surface pattern, and structural features. These characteristics were compared with illustrations and descriptions in published references and online databases. Key references included studies on some gastropod taxa (**Passamonti, 2015; Arbi *et al.*, 2022; Maynawang *et al.*, 2023**) and the family Strombidae (**Maxwell *et al.*, 2020, 2023; Diamante *et al.*, 2024**). Online portals consulted included the World Register of Marine Species (**WoRMS Editorial Board, 2025**), the Global Biodiversity Information Facility (**GBIF, 2026**), Hardy's Internet Guide to Marine Gastropods (**Hardy, 2026**), MolluscaBase (**MolluscaBase eds., 2026**), and SeaLifeBase (**Palomares & Pauly, 2026**).

DNA Extraction, PCR Amplification, and COI Gene Sequencing

DNA extraction and PCR amplification were performed at the Bionesia Laboratory, Denpasar, Bali, Indonesia. Genomic DNA was extracted from each gastropod sample using Zymo and Qiagen DNA extraction kits following the manufacturers' protocols. The target fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI mtDNA) was amplified through polymerase chain reaction (PCR) using the forward primer LCO1490 (5'-GGTCAACAAATCATAAGATATTGG-3') and reverse primer HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (**Folmer *et al.*, 1994**).

The PCR reaction volume of 26µL consisted of 2µL of DNA template, 1.25µL of each primer (10mM), 12.5µL of ready mix, and 9µL of ddH₂O. The PCR thermal profile included an initial denaturation at 94°C for 3 minutes, followed by 38 cycles of denaturation at 94°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 60 seconds, with a final extension at 72°C for 2 minutes. PCR products were visualized by electrophoresis on 1% agarose gel stained with GelRed®.

Samples showing clear and bright DNA bands were sent to PT Genetika Science, Jakarta, for Sanger sequencing. The nucleotide sequences obtained were quality-checked and edited using MEGA 11 (**Tamura *et al.*, 2021**). Forward and reverse sequences were cleaned, assembled, and trimmed to produce high-quality DNA barcode sequences for each sample. The resulting DNA sequences were compared with reference sequences in the NCBI GenBank database using the Basic Local Alignment Search Tool (BLAST). Identification results were evaluated based on sequence similarity (% identity). The sequences generated in this study were submitted to the NCBI GenBank repository with accession numbers PX696984–PX696999.

Integrative Species Identification

The Basic Local Alignment Search Tool (BLAST) (Altschul *et al.*, 1990) was used with standard algorithm parameters for highly similar sequences (megablast) and a maximum of 100 target sequences to search the National Center for Biotechnology Information (NCBI) GenBank database for homologous sequences. BLAST results with the highest similarity (% identity) and high coverage (>90%) were used as preliminary references for specimen identification.

For each putative species, homologous sequences from BLAST results with high % identity and coverage greater than 90% were downloaded as FASTA files for phylogenetic analysis. When homologous sequences within the typical intraspecific similarity range of approximately 98–100% (Ward, 2009) were not available in the NCBI GenBank database, additional searches were conducted through the Barcode of Life Database (BOLD) online interface (Ratnasingham & Hebert, 2007), and relevant homologous sequences were downloaded as FASTA files.

Phylogenetic tree analysis was conducted using the Maximum Likelihood method with 1,000 bootstrap replicates in MEGA 11 (Tamura *et al.*, 2021). The resulting phylogenetic trees were exported as Newick files and edited using the Interactive Tree of Life (iTOL). *Clithon* sp. and *Cerithidea obtusa* were used as outgroup species. The results of the molecular identification were then compared with morphological observations to refine taxonomic identification to the lowest possible taxonomic level (species or genus). Taxonomic nomenclature was validated using the World Register of Marine Species (WoRMS Editorial Board, 2025).

RESULTS

1. Gleaning Observation and Morphological Analysis

Invertebrate collection activities by local community members in the study area revealed a pattern of resource utilization that was distributed across the seagrass beds and not concentrated in any particular location. During the low tide phase, local people consistently engaged in gleaning and moved around the seagrass beds exposed in the inter-tidal zone and shallow waters just below the inter-tidal zone around Batu Kalasi Island. The gleaning process was selective, focusing on certain mollusk species that are the main targets for home consumption and/or for sale. Observation of the mollusks collected by gleaners in the coastal waters of Batu Kalasi Island, Labuange Village, Barru Regency, South Sulawesi, Indonesia revealed a predominance of the family Strombidae (four morphotypes) as well as smaller numbers of individuals from three other morphotypes, each representing a different gastropod family (Table 1).

Table 1. Gastropod Specimens Collected from Gleaners at the Study Site in Batu Kalasi, South Sulawesi, and Identified Using Morphological Traits and DNA Barcoding

No.	Specimen details	Photograph
1	Voucher ID: UNH25-BKL001 Accession Number: PX696986 Family: Strombidae Genus: <i>Canarium</i>	
2	Voucher ID: UNH25-BKL002 Accession Number: PX696988 Family: Strombidae Genus: <i>Canarium</i>	
3	Voucher ID: UNH25-BKL003 Accession Number: PX696984 Family: Strombidae Genus: <i>Canarium</i>	
4	Voucher ID: UNH25-BKL004 Accession Number : PX696985 Family: Strombidae Genus: <i>Canarium</i>	
5	Voucher ID: UNH25-BKL005 Accession Number: PX696987 Family: Strombidae Genus: <i>Canarium</i>	
6	Voucher ID: UNH25-BKL006 Accession Number : PX696989 Family: Strombidae Genus: <i>Canarium</i>	
7	Voucher ID: UNH25-BKL007 Accession Number : PX696995 Family: Volutidae Genus: <i>Cymbiola</i>	

No.	Specimen details	Photograph
8	Voucher ID: UNH25-BKL008 Accession Number : PX696996 Family: Volutidae Genus: <i>Cymbiola</i>	
9	Voucher ID: UNH25-BKL009 Accession Number: PX696993 Family: Cypraeidae Genus: <i>Cypraea</i>	
10	Voucher ID: UNH25-BKL010 Accession Number: PX696994 Family: Cypraeidae Genus: <i>Cypraea</i>	
11	Voucher ID: UNH25-BKL011 Accession Number: PX696990 Family: Strombidae Genus: <i>Gibberulus</i>	
12	Voucher ID: UNH25-BKL012 Accession Number: PX696991 Family: Strombidae Genus: <i>Gibberulus</i>	
13	Voucher ID: UNH25-BKL013 Accession Number: PX696992 Family: Strombidae Genus: <i>Gibberulus</i>	
14	Voucher ID: UNH25-BKL014 Accession Number : PX696997 Family: Tegulidae Genus: <i>Tectus</i>	

No.	Specimen details	Photograph
15	Voucher ID: UNH25-BKL015 Accession Number : PX696998 Family: Tegulidae Genus: <i>Tectus</i>	
16	Voucher ID: UNH25-BKL016 Accession Number: PX696999 Family: Tegulidae Genus: <i>Tectus</i>	

2. DNA Barcoding and BLAST Results

Five of the 19 samples collected could not be identified because the Sanger sequence files were of extremely poor quality. These samples were excluded from further analysis and are not shown in Table (1).

BLAST analysis indicated that the remaining specimens with high-quality DNA barcode sequences belonged to five genera from four families and three orders. These included the order Littorinimorpha, represented by the genera *Canarium* and *Gibberulus* (family Strombidae) and *Cypraea* (family Cypraeidae); the order Trochida, represented by the genus *Tectus* (family Tegulidae); and the order Neogastropoda, represented by the genus *Cymbiola* (family Volutidae) (Table 2).

Table 2. GenBank Accession Numbers and BLAST Results for Gastropods Collected from Batu Kalasi, South Sulawesi

Specimen Sequence			Closest GenBank Accession			
Specimen ID	Accession Number*	Length Bp	Accession Number	Taxon	Query cover %	Identity %
UNH25-BKL001	PX696986	599	OR910731	<i>Canarium incisum</i>	100	99.50
UNH25-BKL002	PX696988	599	OR910731	<i>C. incisum</i>	100	99.50
UNH25-BKL003	PX696984	599	MN389049	<i>C. urceus</i>	100	99.50
UNH25-BKL004	PX696985	599	MN389051	<i>C. urceus</i>	100	100
UNH25-BKL005	PX696987	599	MN389051	<i>C. urceus</i>	100	100
UNH25-BKL006	PX696989	599	MN389051	<i>C. urceus</i>	100	99.67
UNH25-BKL007	PX696995	591	LC738392	<i>Cymbiola nobilis</i>	95	87.94
UNH25-BKL008	PX696996	591	LC738392	<i>C. nobilis</i>	95	87.94
UNH25-BKL009	PX696993	612	JF693369	<i>Cypraea tigris</i>	100	100
UNH25-BKL010	PX696994	612	JF693370	<i>C. tigris</i>	100	99.84

UNH25-BKL011	PX696990	609	OR910805	<i>Gibberulus gibbosus</i>	100	100
UNH25-BKL012	PX696991	609	OR910805	<i>G. gibbosus</i>	100	99.67
UNH25-BKL013	PX696992	596	OR910806	<i>G. gibbosus</i>	100	98.99
UNH25-BKL014	PX696997	602	EU530152	<i>Tectus tentorium</i>	100	91.17
UNH25-BKL015	PX696998	602	EU530152	<i>T. tentorium</i>	100	91.17
UNH25-BKL016	PX69699	602	EU530152	<i>T. tentorium</i>	100	91.17

The BLAST results showed a high degree of sequence identity with reference sequences for most taxa, except for the genera *Cymbiola* and *Tectus*.

For taxa without close matches in the GenBank database, additional searches were conducted using the Barcode of Life Data System (BOLD). These searches did not provide additional sequences for the genus *Tectus*. For the genus *Cymbiola*, however, the BOLD search returned several reference sequences of *Cymbiola nobilis* [Lightfoot] 1786 that had already been obtained through BLAST, as well as three additional sequences representing two species: two sequences of *C. vespertilio* Linnaeus 1758 and one sequence of *C. pulchra* Sowerby I 1825. These three sequences were added to the BLAST reference dataset prior to phylogenetic analysis.

2. Phylogenetic and Morphological Analysis

Phylogenetic analysis was performed after dividing the sequences obtained in this study into two groups. The first group included all sequences from the family Strombidae, using *Clithon* sp. as an outgroup (**Serdiati et al., 2023**) (Fig. 2). The second group included all remaining sequences, using *Cerithidea obtusa* Lamarck 1822 (accession HE680217.1) as the outgroup (Fig. 3).

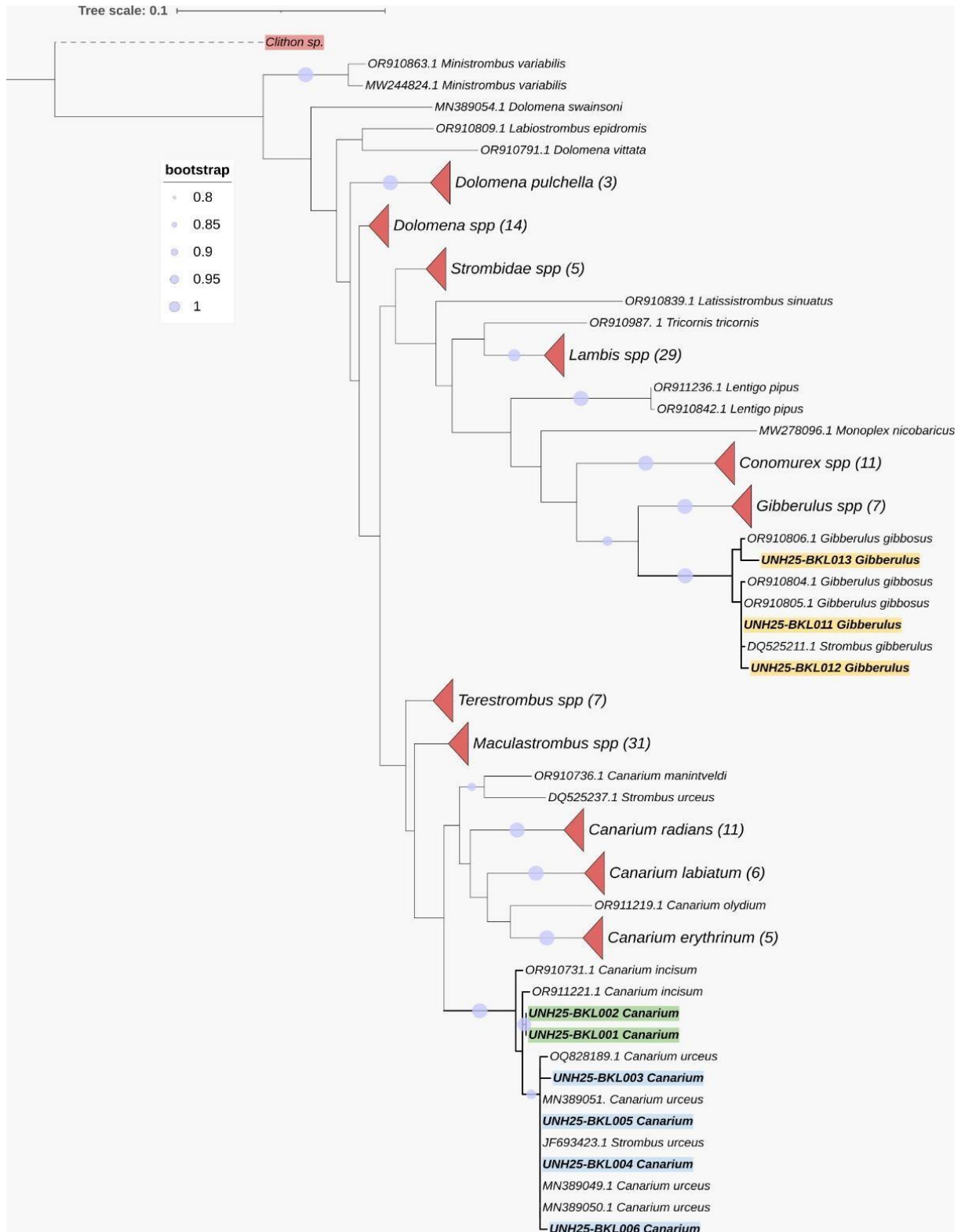


Fig. 2. Maximum Likelihood Phylogenetic Reconstruction of Strombidae Including Nine COI DNA Barcodes of Gastropods Gleaned from Batu Kalasi, South Sulawesi (1000 Bootstrap Iterations)

Fig. (2) shows strong bootstrap support for the clusters within the family Strombidae, which formed a major clade clearly separated from the outgroup. Three Strombidae specimens from this study resolved within the *Gibberulus gibberulus* Linnaeus 1758 clade, while six resolved within the *Canarium* clade. Both genera formed well-supported clades with high bootstrap values, indicating strong phylogenetic support for this tree structure.

Within the *Canarium* clade, two sequences clustered with *C. incisum* Wood 1828 and four with *C. urceus* Linnaeus 1758, providing strong support for the BLAST results (Table 2).

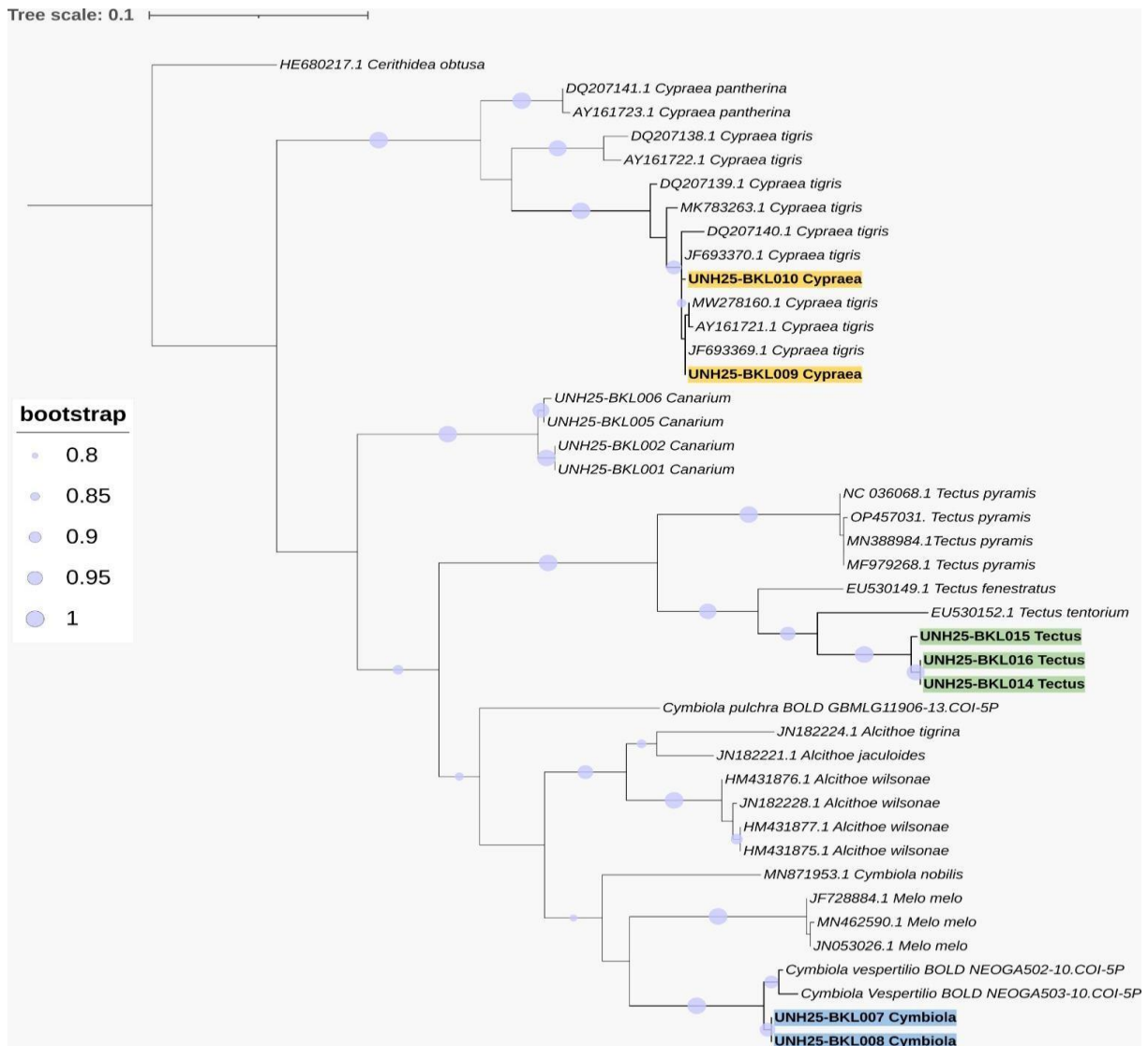


Fig. 3. Maximum Likelihood Phylogenetic Reconstruction Based on COI DNA Barcodes Including Seven Gastropods (Families Cypraeidae, Tegulidae and Volutidae) Gleaned from Batu Kalasi, South Sulawesi (1000 Bootstrap Iterations)

The phylogenetic tree for the remaining families (Fig. 3) showed grouping patterns consistent with the BLAST analysis. The major clades were clearly separated, and the tree structure was strongly supported by high bootstrap values, mostly ranging from 0.95 to 1.0.

Within the Cypraeidae clade, two sequences from this study (UNH25-BKL010 and UNH25-BKL009) clustered within the *Cypraea tigris* Linnaeus 1758 group, indicating high genetic similarity with the reference sequences and supported by strong bootstrap values. However, sequences submitted as *C. tigris* formed two clearly separated clades that appear to represent two species-level taxa. Consequently, it remains uncertain which clade corresponds to the true *C. tigris*. Therefore, despite the BLAST results (99.84–100% identity) shown in Table (2), this identification remains tentative and is reported as *Cypraea cf. tigris*.

The Tegulidae clade shows three sequences from this study (UNH25-BKL014, UNH25-BKL015, and UNH25-BKL016) forming a distinct clade nested within the *Tectus* subclade. The closest sister clade corresponds to *Tectus tentorium* Gmelin 1791 (accession EU530152.1). The tree structure indicates the absence of reference sequences for the species-level taxon of the genus *Tectus* sampled from Batu Kalasi. Based on morphological traits (Table 1), these samples most closely resembled *Tectus architectonicus* Adams 1853. However, no COI sequences for *T. architectonicus* were available in either the GenBank or BOLD databases.

Two samples (UNH25-BKL007 and UNH25-BKL008; Table 1) were nested within the Volutidae clade and formed a group with high similarity to the *Cymbiola vespertilio* clade containing reference sequences obtained from the BOLD database. In this case, using both databases allowed for a more accurate phylogenetic placement of the two samples than would have been possible using BLAST results alone.

Morphological observations also indicated that these specimens more closely resembled *C. vespertilio* compared with other Volutidae species, based on illustrations and descriptions available in online databases and reference literature. Based on the combined molecular and morphological evidence, the 16 specimens analyzed in this study were assigned to six species-level taxa. The designation *cf.* (confer) was used where taxonomic uncertainty remains (Table 2).

DISCUSSION

The taxonomic composition of the gastropods gleaned at the study site reveals a community dominated by the order Littorinimorpha, particularly the family Strombidae. Strombidae members are known to be strongly associated with sandy substrates, seagrass beds, and productive shallow waters, where habitat structures provide optimal feeding, shelter, and recruitment areas (Pangarungan *et al.*, 2022; Mustika *et al.*, 2025). Our

results are consistent with studies from the Philippines, which also reported Strombidae as a major component of gleaning catches and an important target of seagrass gleaning fisheries (**Diamante et al., 2024, 2025**). These findings support the view that seagrass beds represent key habitats for this group.

In addition to Strombidae, the presence of *Cypraea cf. tigris* indicates that gleaning activities are not limited to a single taxonomic group but also target other gastropods with high economic and aesthetic value. Species of the family Cypraeidae are frequently collected for their attractive shells and commercial value, and in some locations, overexploitation has been linked to declines in local abundance (**Nordlund et al., 2018; Jiang et al., 2020**). The low proportion of *Tectus cf. architectonicus* and *Cymbiola cf. vesperilio* in gleaning catches suggests that these species may be naturally rarer, have a narrower distribution, or occupy more specific microhabitats within the seagrass habitat mosaic compared with the strombid taxa collected (**Mustika et al., 2025; Sasmita et al., 2025**). Studies of gleaning fisheries in seagrass beds indicate that catch composition typically reflects a combination of organism availability and collector selectivity (**Furkon et al., 2020; Diamante et al., 2024**), and therefore does not always fully represent the structure of the benthic community.

COI DNA barcoding revealed that most mollusk samples, especially those from the families Strombidae and Cypraeidae, had very high sequence similarity with homologous reference sequences in the NCBI GenBank database (98.99–100%). This result is consistent with previous studies demonstrating the effectiveness of COI as a species identification marker for tropical marine gastropods (**Leray et al., 2012; Habib et al., 2021**). However, COI DNA barcode sequences diverging by 1% or less ($\geq 99\%$ identity) have a 98–99% probability of belonging to the same species, whereas divergences slightly above 2.1% ($< 98\%$ identity) indicate a very low probability of conspecificity but a very high likelihood ($> 99\%$) that sequences belong to the same genus (**Ward, 2009**). Therefore, low sequence similarity values (below 97–98%) in NCBI GenBank BLAST or BOLD search results likely reflect the absence of reference COI sequences in global databases for the sampled species-level taxon.

Several recent studies have highlighted that limitations in COI reference databases remain a major obstacle to accurate molecular identification, particularly for benthic invertebrates such as marine mollusks (**Gielings et al., 2021; Zhou et al., 2025**). Despite ongoing global initiatives to expand barcode libraries, **Weigand et al. (2019)** reported substantial gaps in taxonomic coverage within GenBank and BOLD, which often lead to difficulties in species-level identification. Furthermore, sequences may sometimes be submitted with outdated or inaccurate taxonomic information, including misidentified source specimens, as reported for marine taxa more generally (**Zhou et al., 2025**). This may be the case for some of the GenBank accessions used in this study, particularly several *Cypraea* spp. accessions shown in Fig. (3).

The limitations of COI reference data in public databases are reflected in the BLAST search results, particularly in the relatively low similarity (% identity) values observed for several taxa sequenced in this study. Low similarity values do not necessarily indicate the presence of new species. Although rare, intraspecific variation in COI DNA barcodes can reach 3% or even higher (**Ward, 2009**). However, for most taxonomic groups, such divergence more commonly reflects the lack of representative reference sequences in public databases such as GenBank and BOLD (**Zhou *et al.*, 2025**). In particular, recent studies indicate that the limited number of curated reference sequences for tropical marine mollusks means that many samples can only be identified to the genus or family level, thereby reducing the resolution of molecular identification and phylogenetic placement (**Mai *et al.*, 2025**; **Setiamarga *et al.*, 2025**). Consequently, the success of molecular identification is strongly dependent on the availability of comprehensive and geographically representative reference libraries. For example, **Mai *et al.* (2025)** demonstrated that the development of a regional barcode library substantially improved identification success (>98%) compared with relying solely on incomplete public databases.

Phylogenetic analyses in this study consistently reconstructed the gastropod relationships shown in Figs. (2, 3) at both family and genus levels, as indicated by high bootstrap support and confirmed through repeated analyses. The phylogenetic tree topology was generally consistent with morphological identification and BLAST results, indicating strong agreement between morphological and molecular approaches.

Within Cypraeidae, two sequences from this study clustered consistently within the *Cypraea tigris* group with high bootstrap values. This supports the use of mitochondrial data for phylogenetic reconstruction in cowries, with clear species boundaries based on the COI marker, as demonstrated by mitogenomic analyses identifying monophyletic clades within this family (**Qingxia *et al.*, 2023**). However, the ambiguity observed in Fig. (3) arises because sequences submitted as *C. tigris* form two clearly separated terminal clades, each supported by 100% bootstrap values. This pattern could indicate that the nominal taxon *C. tigris* represents a species complex containing cryptic diversity, similar to patterns observed in freshwater mollusks from the Caucasus (**Bikashvili *et al.*, 2022**). Alternatively, some sequences deposited in the GenBank database may have been derived from incorrectly identified specimens, highlighting the importance of database curation for many taxa (**Mugnai *et al.*, 2021**).

Within the family Tegulidae, the three sequences obtained in this study consistently formed a single clade within the genus *Tectus*. The closest sister clade corresponded to *Tectus tentorium*, which was clearly separated from the *Tectus* sequences obtained from the study site. This phylogenetic structure suggests that the Tegulidae specimens collected at Batu Kalasi belong to a species of *Tectus* for which no COI barcode sequences have yet been deposited in GenBank or BOLD. Based on

morphological characteristics, these specimens were identified as *Tectus cf. architectonicus*. The use of the **cf.** notation (Table 3) reflects the tentative nature of this identification. If this interpretation is correct, the sequences generated in this study represent the first COI barcode records deposited in GenBank for this species.

Table 3. Mollusk Taxa Identified from Gleaning Catches in Batu Kalasi, South Sulawesi Using a Combined Molecular and Morphological Approach

Order	Family	Species	Sample count (n)	Common name
Littorinimorpha	Strombidae	<i>Canarium urceus</i>	4	Little bear conch
		<i>Canarium cf. incisum</i>	2	Incised canarium
		<i>Gibberulus gibbosus</i>	3	Gibbose conch
	Cypraeidae	<i>Cyprea cf. tigris</i>	2	Tiger cowrie
Trochida	Tegulidae	<i>Tectus cf. architectonicus</i>	3	Top shell (genus)
Neogastropoda	Volutidae	<i>Cymbiola cf. vespertilio</i>	2	Bat volute

The Volutidae sequences consistently formed a cluster within the genus *Cymbiola*. Although no closely related sequences were found in the GenBank database, *Cymbiola vespertilio* sequences from BOLD formed a sister clade to the sample cluster. Despite the clear separation of the two clusters (bootstrap values close to 100%), the genetic distance between them was relatively low and comparable to the intraspecific variation observed within the *C. vespertilio* clade. However, representation of Volutidae in GenBank and BOLD remains limited, particularly for Indo-Pacific taxa, including species of *Cymbiola*. Morphological characteristics of the Volutidae specimens collected in this study correspond closely to published descriptions of *C. vespertilio*, such as those provided by **Arbi et al. (2022)** and various online databases. However, available images of this species show considerable morphological variation.

Considering both the molecular and morphological evidence, it is therefore possible that the sequences obtained in this study represent either intraspecific genetic variation within *C. vespertilio* or a closely related species lacking reference sequences in public databases. These findings reinforce the conclusion that COI-based species delimitation is highly dependent on the completeness and curation of reference databases and benefits greatly from integrative taxonomic approaches. At the same time, they highlight the need for continued expansion and validation of molecular reference databases.

Overall, these results confirm that COI is an effective marker for evaluating evolutionary relationships among gastropods at the family and genus levels. However, its taxonomic resolution may vary among groups depending on the availability of reference data and the degree of intra- and interspecific divergence within the COI barcode region (**Ward, 2009**). This highlights the importance of enriching molecular sequence databases—particularly for tropical gastropods—and integrating morphological and

molecular data within an integrative taxonomy framework, as recommended by **Furfaro and Mariottini (2021)** for nudibranchs.

CONCLUSION

This study shows that various gastropod groups are gleaned in the seagrass beds of Labuange Village. The dominance of the Strombidae family reflects the important role of seagrass beds as a major habitat for this group. The integrative morphological and molecular (COI DNA barcoding) approach to identification confirmed the genus-level identity of 16 gastropod specimens from five genera (*Canarium*, *Gibberulus*, *Cyprea*, *Tectus*, and *Cymbiola*) and four families (Strombidae, Cypraeidae, Tegulidae, and Volutidae), enabling the identification of two species with high confidence. Ambiguities remain for four species due to gaps in DNA barcode sequence coverage and metadata curation issues. The lack of high similarity sequences for *Tectus cf. architectonicus* highlights the patchy representation of Indo-Pacific gastropods in global sequences databases (GenBank and BOLD). Although the results confirm that COI gene-based DNA barcoding can be an effective approach for the identification of gleaned gastropods, especially when combined with classical morphology-based methods. These findings also highlight the importance of developing comprehensive and well-curated molecular barcode libraries with sequences from reliably identified voucher specimens as well as accessible collections in highly biodiverse regions, in particular for marine mollusks. Such databases and collections would support biodiversity exploration, conservation, and sustainable management of gastropods in tropical seagrass ecosystems.

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