SPECIES IDENTIFICATION OF *Cymbiola nobilis* (Gastropoda: Neogastropoda: Volutidae) IN HON THOM ISLAND, PHU QUOC USING COI BARCODING

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ABSTRACT

Cymbiola nobilis (Lightfoot, 1786) is a vulnerable gastropod species in the Indo–West Pacific and has become increasingly rare in Vietnam due to overharvesting for the ornamental shell trade and food consumption. Accurate species identification is essential for conservation management, yet traditional morphology can be challenging due to substantial variation in shell shape and coloration. In this study, we generated a DNA barcode record for *C. nobilis* collected from Hon Thom Island, Phu Quoc, Vietnam. A 5′ fragment of the mitochondrial cytochrome c oxidase subunit I gene (COI-5P) was amplified, sequenced, and compared with reference databases (GenBank and BOLD). BLAST and BOLD identification results showed 99–100% similarity to *C. nobilis*, and phylogenetic analysis recovered strong species-level monophyly. The validated sequence was assigned as the first barcode index number (BIN) for this species in BOLD database. This record contributes essential molecular data for taxonomy, conservation planning, and future barcoding efforts on marine gastropods in Vietnam.

XÁC ĐỊNH LOÀI *Cymbiola nobilis* (Gastropoda: Neogastropoda: Volutidae) Ở HÒN THƠM, PHÚ QUỐC BẰNG PHƯƠNG PHÁP MÃ VẠCH COI

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TỪ KHÓA

COI-5P
Cymbiola nobilis
Mã vạch DNA
Định danh loài
Ốc biển

TÓM TẮT

Cymbiola nobilis (Lightfoot, 1786) là một loài ốc biển có giá trị, phân bố ở vùng Ấn Độ – Tây Thái Bình Dương và hiện được xếp vào nhóm dễ bị tổn thương (vulnerable). Tại Việt Nam, loài này ngày càng hiếm gặp do bị khai thác quá mức phục vụ mục đích tiêu thụ, thương mại, mỹ nghệ và sưu tầm vỏ. Việc xác định loài chính xác là rất quan trọng cho công tác bảo tồn, tuy nhiên phương pháp hình thái học truyền thống có thể gặp nhiều khó khăn do sự biến đổi đáng kể về hình dạng và màu sắc của vỏ. Trong nghiên cứu này, chúng tôi xác định đoạn mã vạch DNA từ mẫu *C. nobilis* thu tại Hòn Thơm (Quần đảo Phú Quốc), Việt Nam. Đoạn 5′ của gen ty thể cytochrome c oxidase subunit I (COI-5P) đã được khuếch đại, giải trình tự và so sánh với các cơ sở dữ liệu tham chiếu (GenBank và BOLD). Kết quả phân tích sử dụng công cụ BLAST trên ngân hàng gen NCBI và cơ sở dữ liệu mã vạch thế giới BOLD cho thấy trình tự COI-5P của mẫu nghiên cứu có độ tương đồng 99–100% với loài *C. nobilis*. Phân tích phát sinh chủng loại khẳng định đơn ngành ở cấp độ loài. Trình tự COI hợp lệ này đã được gán mã BIN đầu tiên cho loài *C. nobilis* trong cơ sở dữ liệu BOLD. Kết quả này cung cấp dữ liệu phân tử quan trọng hỗ trợ công tác phân loại, bảo tồn và phát triển thư viện mã vạch DNA cho các loài ốc biển tại Việt Nam.

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1. Introduction

Marine gastropods constitute one of the most diverse groups of mollusks and play ecologically important roles across coastal and deep-sea ecosystems. The noble volute *Cymbiola nobilis* (Volutidae) is a large predatory gastropod distributed throughout the Indo–West Pacific, including the coastal waters of Vietnam [1]. It inhabits sandy substrates at depths of 5–80 m and is increasingly rare due to overharvesting for the ornamental shell trade and food consumption [1], [2]. Its distinctively patterned shell has made it highly prized among collectors, while ecologically, the species contributes to nutrient cycling and sediment turnover in sandy-bottom habitats. Recent mitochondrial genome data of *C. nobilis* [3] provides a foundation for molecular-based taxonomic studies. Although pharmacological studies on *C. nobilis* remain limited, investigations on related volutid species have identified peptides and lipid compounds with anti-inflammatory, antibacterial, and antioxidant activities [4], [5], suggesting its potential biomedical relevance.

Accurate identification of gastropods is essential for taxonomy, fisheries assessments, and biodiversity monitoring. However, morphological identification of *C. nobilis* can be unreliable due to substantial variation in shell shape, coloration, and ornamentation. In recent years, DNA barcoding has become a powerful tool for complementing traditional taxonomy. It provides a rapid, accurate, and standardized approach for species identification using short DNA sequences from specific gene regions [6]. This method is particularly useful for identifying cryptic species, larvae, damaged specimens, and processed materials where morphology-based identification is difficult [7]. The mitochondrial cytochrome c oxidase I (COI) gene, proposed by Paul Hebert and colleagues, is widely adopted as the standard barcode marker for animals [8]. Standardized protocols established by the Consortium for the Barcode of Life (CBOL, 2005) facilitate global comparability of barcode data [9].

Because *C. nobilis* is now rarely encountered in Vietnam, only a single voucher specimen could be obtained from Phu Quoc Island. In this study, we combined morphological identification with COI-5P sequencing to generate the first DNA barcode record for this species in Vietnam and globally. This reference sequence provides a validated molecular resource for future taxonomic, ecological, and conservation-based studies.

2. Materials and Methods

2.1. Study species and sampling

Voucher specimens comprising three individuals (TM02TT, TM02TT-2, and TM02TT-3) of the gastropod species *Cymbiola nobilis* was collected by scuba diving from the west coast of Hon Thom Island of Phu Quoc (9.9476° N, 104.0085° E) on 21 February 2025. All necessary permits were obtained for field collection, and the specimen was handled following ethical guidelines for research on marine invertebrates.

2.2. Morphological identification

Morphological identification of the specimen was conducted prior to DNA analysis. Shell characters including overall size, coloration, and pattern were examined. Photographs of each specimen were taken from dorsal, apertural, lateral, and detailed characteristic views. Taxonomic identification was performed following standard taxonomic keys [10], [11] and reference monographs for marine mollusks (MolluscaBase, WoRMS) [12].

2.3. DNA Extraction, PCR and Sequencing

Genomic DNA was extracted from approximately 50 mg of foot muscle tissue using a modified CTAB protocol [13]. The yield and purity of the extracted DNA were assessed using a NanoDrop™ 2000/200c spectrophotometer (Thermo Scientific, USA). The 5′ region of the cytochrome c oxidase subunit I gene (COI-5P) was amplified by polymerase chain reaction (PCR) using the

universal primers LCO1490 (5′-GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′-TAAACTTCAGGGTGACCAAAAAATCA-3′) [14]. Each 50 µL PCR reaction contained 25 µL of DreamTaq PCR Master Mix (2×) (Thermo Scientific, Lithuania), 2 µL of 25 mM $MgCl_2$, 0.5 µM of each primer, and approximately 10 ng of template DNA. The thermal cycling conditions consisted of an initial denaturation at 95°C for 3 min; 35 cycles of denaturation at 95°C for 30 s, annealing at 53°C for 30 s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min.

PCR amplicons (~5 µL of each reaction) were visualized on a 1% agarose gel stained with RedSafe (Intron). Amplified products were purified using the GeneJET Gel Extraction Kit (Thermo Scientific, Lithuania) according to the manufacturer's instructions, cloned into the pJET1.2 vector (Thermo Scientific, Lithuania), and bidirectionally sequenced by 1st BASE (Singapore). The raw COI-5′ sequences were assembled, and primer regions and ambiguous bases were trimmed using BioEdit 7.0 [15] and DNAMAN v10 (Lynnon Biosoft, USA).

2.4. Sequence Analysis

The obtained sequences were initially compared to reference sequences in GenBank and BOLD using BLAST and the BOLD Identification Engine (accessed in November 2025) to evaluate sequence identity. Species-level identification was considered reliable when the top BLAST hit showed ≥98% identity to a reference sequence of the same species. Accepted species names were standardized according to the World Register of Marine Species (WoRMS; accessed November 2025). Multiple sequence alignment was performed using BioEdit 7.0 [15]. The resulting sequence was deposited in GenBank, National Center for Biotechnology Information (NCBI) and in the Barcode of Life Data System (BOLD) under the project *'HMTM-Mollusk in Vietnam_2025'*. Barcode Index Numbers (BINs) were automatically assigned by BOLD Systems v5 as registry identifiers, enabling assessment of whether barcode clusters correspond to known species.

A Neighbor-Joining (NJ) tree of *Cymbiola* genus sequences (including our sample and related sequences from GenBank) was generated from the pairwise distance matrix using MEGA v11 under the Kimura 2-Parameter model (K2P) to visualize phylogenetic placement [16], [17]. Node support was assessed with 1000 bootstrap replicates.

3. Results

3.1. Morphological identification

The specimens TM-PQ 02, TM-PQ 02-2, and TM-PQ 02-3 were morphologically examined, and all were identified as *Cymbiola nobilis* (Lightfoot, 1786) (Volutidae). Diagnostic features including shell shape, sculpture, aperture characteristics, and coloration patterns were evaluated for consistency with the recognized morphological criteria of the species [1].

The three specimens show the typical shell morphology of *C. nobilis* but differ slightly in size and pattern intensity (Figure 1). The largest specimen (TM-PQ 02; shell length 68 mm) displays a strongly inflated fusiform–ovate shell with well-developed brown zigzag flames and reticulate markings over a bright yellow–orange background. The medium-sized specimen (TM-PQ 02-2; 40.1 mm) shows a similar pattern, though the reticulation appears finer and more fragmented, with darker apical pigmentation. The smallest specimen (TM-PQ 02-3; 32.5 mm) exhibits a paler background with reduced flame intensity and a more weathered appearance, reflecting juvenile or environmentally influenced variation.

All three shells are solid and robust, with a slightly elongate profile (length/width ratio ~1.5-1.7). The body whorl is inflated and accounts for approximately five-sixths of total shell length. The spire is low, blunt, and composed of five weakly expressed whorls separated by a shallow but distinct suture. The shell surface is smooth and glossy. The aperture is elongate and moderately wide, occupying more than half of the shell height, with a pale orange interior. The outer lip is

thick, smooth, and slightly flared. The columella bears four strong folds, the lowest being the most prominent. The siphonal canal is short, open, and slightly deflected to the right. These morphological characteristics closely match previously published descriptions of *C. nobilis* [1], [18]. Although the three specimens were collected from the same locality, they show minor differences in shell size, spire elevation, and coloration intensity. Such variation falls well within the known range of intraspecific morphological diversity and ontogenetic variation for *C. nobilis*.

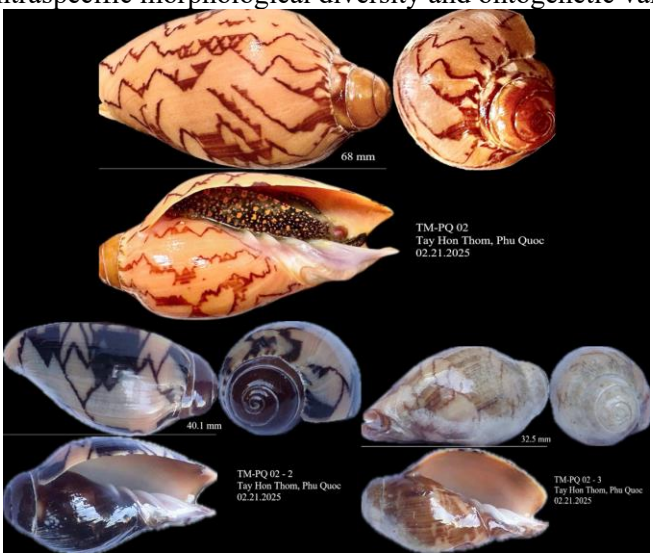

Figure 1. *Shell morphology of Cymbiola nobilis samples collected from the west coast of Hon Thom Island (Phu Quoc, Vietnam). Specimen voucher ID: TM-PQ 02 Tay Hon Thom, Phu Quoc*

Overall, the strong morphological congruence between our specimens and published references confirms their taxonomic placement within Mollusca (Phylum), Gastropoda Cuvier 1795 (Class), Neogastropoda Wenz, 1938 (Order), Volutidae Rafinesque, 1815 (Family), Amoriinae J. E. Gray, 1857 (Subfamily), Melonini Pilsbry & Olsson, 1954 (Tribe), *Cymbiola* Swainson, 1831 (Genus), and *Cymbiola nobilis* (Lightfoot, 1786) (Species). However, to confidently confirm the taxon, especially given potential morphological variation, molecular identification using COI barcoding was undertaken.

3.2. Amplification of COI-5P fragment and Sequence analysis

The COI-5P amplification of the three *C. nobilis* specimens produced a fragment of approximately 750 bp, as shown in Figure 2. A single, sharp band of the expected size on the agarose gel confirmed that the primers successfully amplified the target region without generating non-specific products or primer-dimers. The high-quality PCR product was later used for cloning and sequencing.

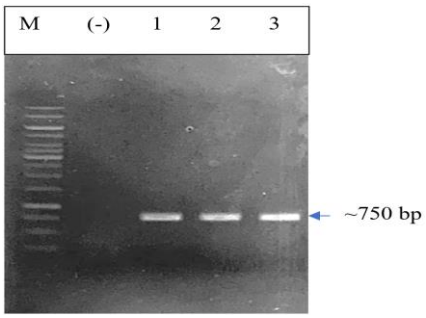

Figure 2. *Agarose gel electrophoresis of PCR-amplified COI-5P fragments. M: 1 kb DNA marker (Thermo); lanes 1–3: PCR products from specimens TM-PQ 02, TM-PQ 02-2, and TM PG 02-3; (-): negative control*

After sequence editing, a final alignment length of 677 bp was obtained, with no stop codons, insertions, or deletions. The COI-5P sequences of the three specimens were identical (100% similarity), confirming a single haplotype for the *C. nobilis* individuals collected from Tay Hon Thom Island. The resulting 677 bp sequence was deposited in GenBank, National Center for Biotechnology Information (NCBI) under accession number PX250366 and in the Barcode of Life Data System (BOLD) under the project *'HMTM-Mollusk in Vietnam_2025'* with sample ID TM02TT-53.

BLAST searches against GenBank showed that the obtained COI-5P sequence matched a COI-5P fragment within the complete mitochondrial genome sequence of *C. nobilis* (accession no. MN871953.1) and a COI fragment (accession no. LC738392) with 99.76% nucleotide similarity, differing by only two substitutions (C↔A and A↔C across 677 aligned positions (Figure 3). This strong correspondence confirms that the amplified fragment is the COI-5P barcode of *C. nobilis*.

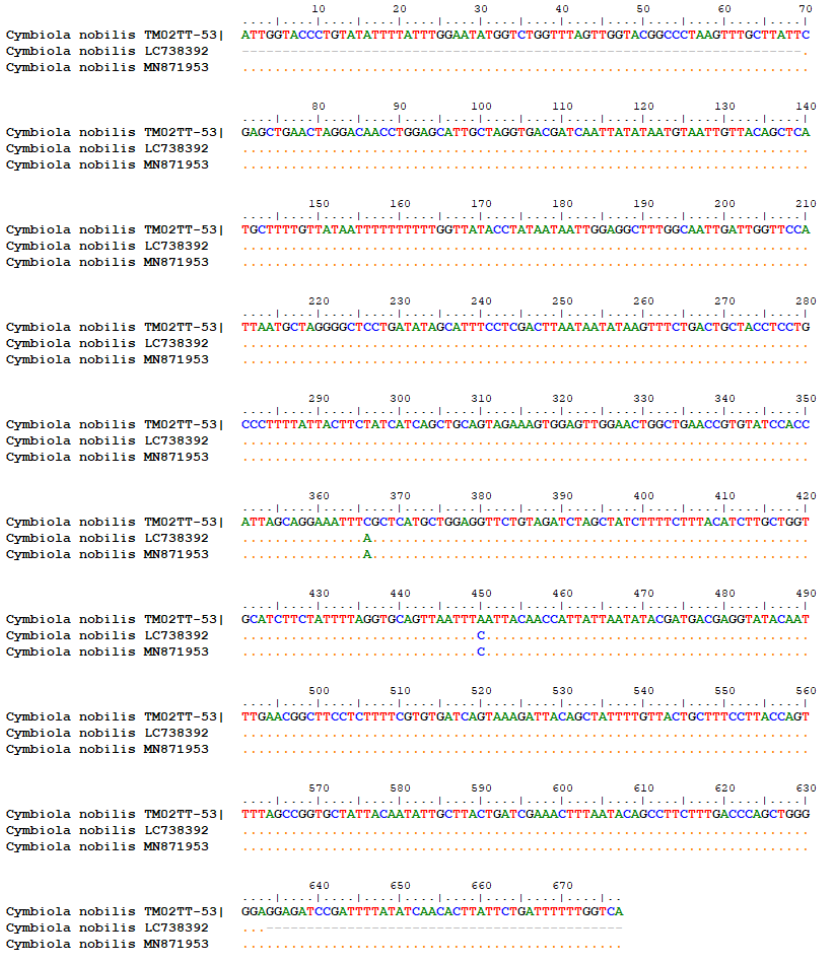

Figure 3. *Pairwise alignment between the COI-5P sequence obtained in this study (accs no. PX250366, Sequence sample ID: TM02TT-35) and the sequences of C. nobilis from GenBank (accs no. MN871953.1 and LC738392). Dots indicate identical nucleotides and letters indicate mismatches.*

The BOLD search returned no matching records for *C. nobilis,* suggesting that the *C. nobilis* sequence generated in this study represents the first validated DNA barcode COI record for this species in BOLD. The barcode sequence of *C. nobilis* in this study was assigned a Barcode Index Number BOLD:AGY7768 (Figure 4). Currently, it is the unique Bin in BOLD for *C. nobilis* species. BOLD:AGY7768 is a private BIN and will be public upon request.

3.3. Phylogenetic analysis

To further validate the usefulness of the obtained barcode sequence for *C. nobilis* species identification, a Neighbor-Joining (NJ) tree including studied COI-5P sequence and related sequences retrieved from GenBank. The resulting topology clearly resolved the target species into distinct and well-supported branches and clades (Figure 5). Genera within the family Volutidae formed a major branching lineage in the tree.

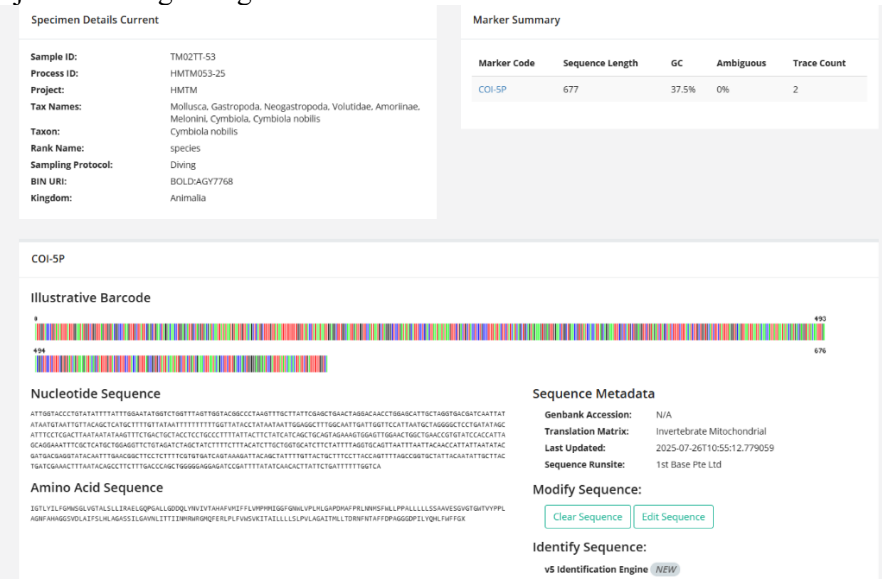

Figure 4. *Sequence view for Sample ID TM02TT-53 (BOLD Process ID: HMTM053-25). The COI-5P sequence of the Cymbiola nobilis specimen TM-PQ02 (Tay Hon Thom) was deposited in the BOLD database under the BIN BOLD:AGY7768. The corresponding barcode image is shown. The sequence length is 677 bp, and both the nucleotide and translated amino acid sequences are presented*

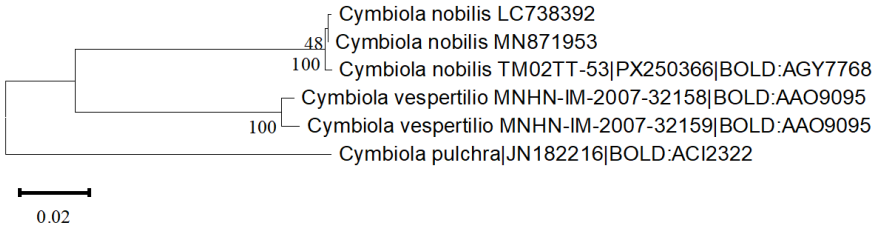

Figure 5. *Phylogenetic analysis using a neighbor Joining (NJ) tree was constructed using sequences retrieved from NCBI GenBank and BOLD (sample ID, accession numbers and BIN numbers are shown in the branch tips along with the sequence name). The tree was constructed using Kimura 2-parameter model in MEGA (Molecular Evolutionary Genetics Analysis)* [16]. *Bootstrap values (1000 replicates) are shown at branch nodes.*

The complete mitochondrial genome of *C. nobilis* (acc no. MN871953) and the 564 bp mitochondrial COI sequence (acc no. LC738392) obtained from a *C. nobilis* specimen collected from the commercial market [19] were retrieved from GenBank and included in the phylogenetic analysis. All *C. nobilis* sequences clustered together in a single, well-supported clade and were distinctly separated from the closely related species *C. vespertilio* (MNHN-IM-2007-32158, MNHN-IM-2007-32159) and *C. pulchra* (acc no. JN182216).

Pairwise genetic distances (K2P) within the Volutidae family are shown in Table 1. The intraspecific divergence within *C. nobilis* was very low (0.3%), indicating high genetic uniformity among samples. In contrast, the interspecific genetic distances between *C. nobilis* and its congeners, *C. vespertilio* and *C. pulchra*, were much higher. The genetic distance ranged from ~

13.6% between *C. nobilis* and *C. vespertilio* to ~19% between *C. nobilis* and *C. pulchra* (Table 1). These values were more than ten times greater than the intraspecific divergence. . This difference confirms clear species-level separation and a distinct barcode gap for *C. nobilis*.

Table 1. *Genetic distance between Cymbiola nobilis sequences and other species in Cymbiola genus available in GenBank*

	Sequence ID	1	2	3	4	5	6
1	Cymbiola_nobilis_TM02TT-53| PX250366| BOLD:AGY7768						
2	Cymbiola_nobilis_LC738392	0.0036					
3	Cymbiola_nobilis_MN871953	0.0036	0.0000				
4	Cymbiola_vespertilio_MNHN-IM-2007-32158|BOLD:AAO9095	0.1375	0.1356	0.1356			
5	Cymbiola_vespertilio_MNHN-IM-2007-32159|BOLD:AAO9095	0.1398	0.1378	0.1378	0.0108		
6	Cymbiola_pulchra_JN182216	0.1928	0.1884	0.1884	0.1831	0.1831	

3.4. Discussion

In this study, traditional morphological examination identified the specimens as *Cymbiola nobilis*, and this was independently confirmed by DNA barcoding using the COI-5P gene. The COI sequences obtained from our *C. nobilis* samples were nearly identical to each other (showing minimal intraspecific divergence) and clearly differentiated from those of other volute species, demonstrating that COI-5P is a suitable marker for species-level identification in this genus. In a neighbor-joining phylogenetic analysis, all *C. nobilis* sequences clustered together with strong support, forming a distinct clade that corroborates their correct classification. Notably, the COI sequences generated in this study represent the first validated DNA barcodes for *C. nobilis* by BOLD systems, providing a first BIN (barcode index number) accessed for this species. This result supports the identification of unknown or disputed specimens in the future.

The COI-5P sequences of the three specimens were identical (100% similarity), fully consistent with their morphological identification. These sequences also showed 99% similarity and formed a distinct clade with the complete mitochondrial genome of *C. nobilis* available in GenBank. Although only a single individual was analyzed, the genetic match and phylogenetic placement give confidence in the result. The assignment of these sequences as species-level barcodes is well justified because they meet the three essential criteria for DNA barcodes: low intraspecific genetic divergence, high interspecific variation, conserved flanking regions suitable for primer binding, and an amplicon length short enough (typically 200–900 bp) for efficient amplification and sequencing [18].

The clear clustering pattern and high bootstrap support indicate reliable species-level resolution. No overlap between interspecific and intraspecific branches was detected, reinforcing the accuracy of molecular identification. The topology of the NJ tree is also congruent with previous phylogenetic studies on Volutidae [3], confirming the genetic stability and taxonomic distinctness of *C. nobilis* from the southern coastal islands of Vietnam. The integration of morphological and molecular evidence in this study provides a robust framework for validating the taxonomic status of *C. nobilis* in the region. Morphological observations of shell structure and coloration were fully consistent with published descriptions, while the COI-5P sequence showed clear interspecific differentiation and strong monophyly.

Once reference DNA barcodes are available, DNA barcoding can be useful for specimen identification in museum collections, especially by comparing new samples to existing sequence databases [19]. It also aids in identification of specimens when morphological methods fail or to resolve taxonomic inconsistencies [8], [20]. DNA barcoding is also a powerful supplementary tool for delimiting closely related species and has the potential to reveal previously undescribed taxa [21],

[22]. In addition, the low cost and scalability of DNA barcoding make it highly suitable for high-throughput analysis of large numbers of samples [8], [23].

Cymbiola nobilis is known to inhabit shallow tropical marine environments, typically at depths of around 5–8 m on sandy seabeds or coral rubble substrates. This ecological preference is consistent with the conditions of our collection site in southern Vietnam's warm coastal waters. In Vietnam, *C. nobilis* has been recorded from the southern part of the central coast down to the country's southern tip, and our findings align with this known distribution. These observations of habitat and range not only provide context for our samples but also reinforce the accuracy of the species identification by matching established ecological data for *C. nobilis*. Due to intensive harvesting for food, the seashell trade, and ornamental use, *C. nobilis* populations have declined significantly and the species is now considered vulnerable. This overexploitation highlights the need for effective conservation and reliable species identification. The DNA barcode established in this study provides a valuable tool for biodiversity surveys, trade monitoring, and conservation programs. COI barcode data will enable rapid and accurate identification of *C. nobilis,* supporting future management and protection efforts.

4. Conclusion

Cymbiola nobilis is an economically valuable gastropod species in Vietnam, and its taxonomic identification has traditionally relied on morphological characteristics. In this study, we present the first DNA barcode records for this species. The validity of these barcodes was confirmed through sequence similarity searches (GenBank/BOLD), cluster-based analyses (NJ tree and BIN assignment), and genetic distance diagnostics. This DNA barcode now serves as a reference for *C. nobilis*, facilitating future studies in taxonomy, conservation, and resource management for this species in Vietnam and beyond.

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REFERENCES

[1] N. T. Nguyen, *New Mollusks: Seashells, Landshells, Cephalopods with 59 New Species*. Akron, OH, USA: 48HRBooks, 2014, p. 205.

[2] M. N. Noorsakinah, C. C. Zaidi, and A. G. Mazlan, "Development and growth of larvae of the volute *Cymbiola nobilis* Lightfoot," *AIP Conf. Proc.*, vol. 1571, no. 1, pp. 512–517, 2013.

[3] D. H. Nguyen, C. Lemieux, M. Turmel, T. B. Pham, V. D. Nguyen, J. L. Mouget, R. Tremblaye, and R. Gastineauf, "Complete mitogenome of the noble volute *Cymbiola nobilis* from the Vietnamese island of Phu Quoc," *Mitochondrial DNA Part B*, vol. 5, no. 2, pp. 1661–1662, 2020.

[4] J. Govindarajalu, M. Anand, G. Chelladurai, and A. K. Kumaraguru, "Bioactive potential of some economically important marine gastropods along the Gulf of Mannar region, southeast coast of India," *J. Coast. Life Med.,* vol. 4, pp. 608-611, 2016.

[5] A. Anita, D. Punitha, and D. Selvaraj, "Screening of marine gastropods for potential antibacterial sustances against pathogenic bacterial," *IJZAB,* vol. 5, no. 4, pp. 180-184, 2020.

[6] A. Sagar, T. Singh, Harsh, N. Jha, P. Dode, and A. Podder, "DNA barcoding: From taxonomy to technology A review," *J. Carcinog.,* vol. 24, no. 7, pp. 835-843, 2025.

[7] A. J. Failla, A. Vasquez, P. Hudson, M. Fujimoto, and J. Ram, "Morphological identification and COI barcodes of adult flies help determine species identities of chironomid larvae (Diptera, Chironomidae)," *Bull. Entomol. Res., vol*. 106, no. 1, pp. 1-13, 2015

[8] P. D. N. Hebert, A. Cywinska, S. L. Ball, and J. R. deWaard, "Biological identifications through DNA barcodes," *Proc. R. Soc. B Biol. Sci.*, vol. 270, no. 1512, pp. 313-321, 2003.

[9] Consortium for the Barcode of Life (CBOL), "CBOL homepage," 2005. [Online]. Available: https://www.ibol.org/phase1/cbol/. [Accessed Nov. 24, 2025].

[10] K. E. Carpenter and V. H. Niem, *FAO Species Identification Guide for Fishery Purposes: The Living Marine Resources of the Western Central Pacific. Volume 1: Seaweeds, Corals, Bivalves and Gastropods*. Rome, Italy: FAO, 1998, p. 700.

[11] E. N. Kozloff and L. H. Price, *Marine Invertebrates of the Pacific Northwest*., USA: University of Washington Press, 1987, p. 539.

[12] WoRMS, "World Register of Marine Species," 2025. [Online]. Available: https://www.marinespecies.org/. [Accessed Nov. 15, 2025].

[13] J. J. Doyle and J. L. Doyle, "A Rapid DNA isolation procedure for small quantities of fresh leaf tissue," *Phytochemical Bulletin*, vol. 19, pp. 11-15, 1987.

[14] O. Folmer, M. Black, W. Hoeh, R. Lutz, and R. Vrijenhoek, "DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates," *Mol. Mar. Biol. Biotechnol.*, vol. 3, pp. 294-299, 1994.

[15] T. A. Hall, "BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT," *Nucleic Acids Symp. Ser.*, vol. 41, pp. 95-98, 1999.

[16] K. Tamura, G. Stecher, and S. Kumar, "MEGA11: Molecular evolutionary genetics analysis version 11," *Mol. Biol. Evol.*, vol. 38, no. 7, pp. 3022-3027, 2021.

[17] M. Kimura, "A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences," *J. Mol. Evol.*, vol. 16, no. 2, pp. 111-120, 1980.

[18] W. J. Kress and D. L. Erickson, "DNA barcodes: genes, genomics, and bioinformatics," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 105, no. 8, pp. 2761–2762, 2008.

[19] D. H. E. Setiamarga, M. Shimizu, S. Nakashima, C. Osaki, K. Hirota, L. Anggraeni, D. V. Tu, and T. Sasaki, "DNA barcoding of museum-vouchered samples collected from fish markets reveals an unexpected diversity of consumed gastropods in Vietnam," *Future Foods*, vol. 12, 2025, Art. no. 100689.

[20] M. Casiraghi, M. Labra, E. Ferri, A. Galimberti, and F. Mattia, "DNA barcoding: theoretical aspects and practical applications," in *Tools for Identifying Biodiversity: Progress and Problems*, Nimis P. L., Vignes Lebbe R. (eds.), 2010, pp. 269–273.

[21] Sun, Q. Li, L. Kong, H. Yu, X. Zheng, R. Yu, L. Dai, Y. Sun, J. Chen, J. Liu, L. Ni, Y. Feng, Z. Yu, S. Zou, and J. Lin, "DNA barcoding reveal patterns of species diversity among northwestern Pacific molluscs," *Sci. Rep.*, vol. 6, no. 1, pp. 1-17, 2016.

[22] P. D. N. Hebert, E. H. Penton, J. M. Burns, D. H. Janzen, and W. Hallwachs, "Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*," *Proc. Natl. Acad. Sci.*, vol. 101, no. 41, pp. 14812-14817, 2004.

[23] M. Leray and N. Knowlton, "DNA barcoding and metabarcoding of standardized samples reveal patterns of marine benthic diversity," *Proc. Natl. Acad. Sci. U. S. A.*, vol. 112, no. 7, pp. 2076–2081, 2015.