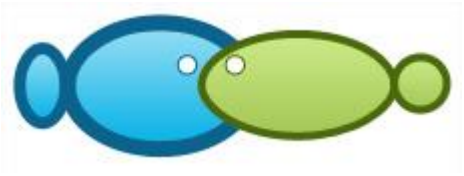

DNA barcoding of Gonggong sea snails (*Laevistrombus canarium* Linnaeus, 1758) from Batam waters, Indonesia

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Abstract. The accurate identification of aquatic resources for the gastropod sea snail Gonggong (*Laevistrombus canarium* Linnaeus, 1758) is essential for the sustainable management of *L. canarium* fisheries. DNA barcoding techniques for species determination have become a mainstay, since their introduction into molecular taxonomy based on the cytochrome c oxidase subunit I (COI) gene. In this study, DNA amplification was performed by polymerase chain reaction (PCR). For extraction and PCR, genomic samples were collected at the Oceanogen Environmental Biotechnology Laboratory, Bogor Agricultural University (IPB), Bogor, Indonesia. The PCR product, in the form of DNA, was transferred to a PCR plate and sent to First Base, Malaysia. Specimens from different habitats were labeled as BTM-L1 and BTM-L2 for species living on muddy substrates, and BTM-P1 and BTM-P2 were living on sandy substrate habitats. Specimens from the two habitats are considered different species, because they have different morphometric characteristics. *L. canarium* from muddy habitats is larger than in sandy habitats. This study conclusively confirms that the species *L. canarium* from Batam waters, which lives on muddy and sandy substrates, is the same species from the Srombidae family. The length of DNA for each specimen was: BTM-P1 629 bp, BTM-P2 630 bp, BTM-L1 625 bp, and BTM-L2 625 bp. Phylogenetic tree analysis showed a very high confidence value for the clades formed in the four samples of *L. canarium*.
Key Words: bioinformatics, dog conch, genome, Strombidae, taxonomy.

Introduction. In scientific studies that focus on taxa, the precise and accurate identification of the taxon holds significant importance (Fontanilla et al 2014). Knowledge of the correct identification of fishery resources (including gastropod species) is important for sustainable fisheries management (Fadli et al 2020). Furthermore, Fadli et al (2020) stated that Without knowledge of the species involved, it becomes impracticable to formulate an efficient management strategy and conservation plan. Inaccurate species identification inevitably affects inventory accuracy. This has severe implications for the ability to sustainably manage highly exploited species when combined with non-threatened species. Thus, a high proportion of misidentifications in catch statistics can have considerable economic consequences.

Molecular taxonomic methods have been widely used to complement morphological approaches in species identification and to establish phylogenetic relationships (Galan et al 2018). DNA barcoding techniques for species determination have become a mainstay since the introduction of molecular taxonomy based on the cytochrome c oxidase subunit I (COI) gene (Hebert et al 2003a). DNA barcoding is a new identification technique using a molecular approach (Pramono et al 2017), characterizing an organism's species using short sequences of mitochondrial DNA (Sulandari et al 2013).

DNA barcoding is an efficient method for identification at species level (Sun et al 2012), being precise and accurate (Fontanilla et al 2014; Galan et al 2018; Fadli et al 2020). It has wide applications in identifying individuals in different developmental stages

(Hubert et al 2010), species with similar morphological characters (Bingpeng et al 2018; Ran et al 2020), food forensics (Madduppa et al 2020), cryptic species and discovery of new species (Lim et al 2016; Samsudin et al 2018; Ran et al 2020). This technique has been used in the identification of marine fish species globally (Sembiring et al 2015; Prehadi et al 2015; Jefri et al 2015; Madduppa et al 2016; Yulianto et al 2020), as well for species of the gastropod group (Smith et al 2011; Appeltans et al 2012; Sun et al 2012; Chee & Nor 2016; Borges et al 2016; Leatemia et al 2018; Setiamarga et al 2019).

Gastropods are among the most studied classes of marine invertebrates. Most species are identified based only on their morphology. DNA barcoding has proven to be very useful for identifying species (Borges et al 2016). The estimated range of described marine gastropod species is approximately 32000 to 40000. However, this figure may account for only 23-32% of the overall estimated total number of marine gastropod species (Appeltans et al 2012). This diversity, among other factors, has made gastropods one of the most studied animal groups (Smith et al 2011). Identification based only on morphological characteristics is very susceptible to identification errors because of the similarities in shape and color. Although mollusks are the most diverse marine species, studies on their genetic and phylogenetic structures are still rare (Tindi et al 2017). This also occurs in the marine gastropod class, especially in the species of Gonggong sea snails in Indonesian waters. In line with this, Ran et al (2020) argued that traditional morphological methods should be combined with DNA barcoding for classification and identification. Threats to species from the Strombidae family have occurred worldwide. Marquez et al (2016) reported the scarcity of the marine gastropod species *Strombus gigas* due to overfishing and reduced natural population recovery. The lack of genomic knowledge on this gastropod species limits the use of polymorphic mitochondrial and nuclear sequences for their preservation. In line with this, sustainable management must be based on detailed information about genetic characteristics (Suppapan & Supmee 2016). According to Setiamarga et al (2019), DNA barcodes can serve as a reference for future DNA-based environmental and biodiversity monitoring in addition to providing data sequences for future systematic studies on gastropod groups.

The DNA barcoding technique was designed to identify quickly and accurately a species based on the nucleotide base sequence of a standardized short marker gene, the Cytochrome Oxidase Subunit I (COI) gene. The COI gene is one of the coding genes in the mtDNA genome, known to have many advantages, one of which it has very few deletions and insertions in its sequence and many parts are conserved, so that it can be used as a DNA barcode as an identifier for each species (Hebert et al 2003b).

Each species has a unique ecological niche. Although, in general, the waters are muddy and overgrown with seagrass vegetation, many are touted as the main habitat of the Gonggong sea snail (*Laevistrombus canarium*). However, in several locations in Batam waters, these sea slugs are also found in sandy waters. The empirical experience of Gonggong sea snails fishermen in the Batam area notes that there are two types of *L. canarium* sea slugs, namely "Gonggong mud" and "Gonggong sand". Both have different sizes: *L. canarium* living in habitats with muddy substrates has a red chest color and is larger than *L. canarium* living on sandy substrates. Samples from the genus *Strombus* have also been tested through DNA analysis based on the thickness of the shells of *L. canarium* snails from Bintan waters and Madong-Tanjungpinang waters, Riau Islands (Viruly 2019; Muzahar 2019). However, the reports also raise the following questions: What are the taxon similarities with species from other waters? What are the differences in living habitat and body size differences at the same maturity level?

This study aimed to examine the molecular taxonomy of *L. canarium* in Batam waters using DNA barcoding. This study further aims to reveal if the species living on muddy substrates with larger morphology is the same with that living on sandy substrates with smaller morphology.

Material and Method

Specimen collection and handling. Specimen collection was conducted in August 2020 in Batam waters, Riau Islands Province, Indonesia, from seven locations of fishing centers for sea snails *L. canarium*. Specimens were collected as follows: 2 individuals from the mud substrate habitat in the waters of Temoyong and Jaloh Islands, and 2 individuals from the sandy substrate habitats in the waters of Sembur and Panjang Islands (Figure 1). The shell of the *L. canarium* sea snail was broken, and then part of its body was extracted and placed into a 1.5 mL micro centrifugation tube filled with 1 mL 100% ethanol, labeled, and stored in a cool box. The specimens were transported to the laboratory. Specimens from each habitat were labeled as BTM-L1 (Temoyong Island waters), BTM-L2 (Jaloh Island waters), BTM-P1 (Sembur Island waters), and BTM-P2 (Panjang Island waters).

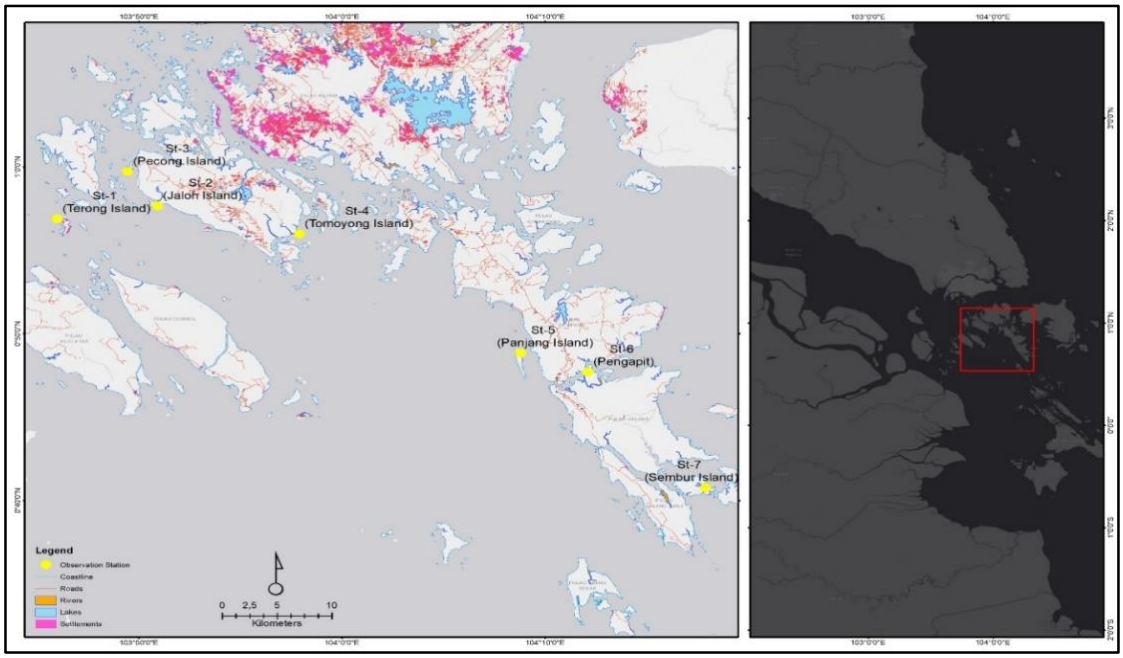


Figure 1. Map of research locations.

For extraction and Polymerase Chain Reaction (PCR), genomic samples were collected at the Oceanogen Environmental Biotechnology Laboratory, Bogor Agricultural University (IPB), Bogor, Indonesia. The PCR product, in the form of DNA, was transferred to a PCR plate and sent to First Base, Malaysia.

DNA extraction and PCR. DNA extraction aims to destroy cells and separate the DNA from the sample. The extraction kit (Qiagen) was used at 56°C until lysis. This stage must be sterile to prevent sample contamination. DNA amplification using PCR enzymatically amplifies (replicates) DNA. The primers used were HCO-2198 and LCO 1490 (Geller et al 2013). The PCR components were ddH_2O, myTaq HS Redmix 2x, primer, and DNA template. Separation of double stranded DNA (pre-denaturation) at 94°C for 4 min, denaturation at 94°C for 15 s, annealing at 50°C for 15 s, DNA segment elongation (extension) at 72°C for 15 s, and a final extension at 72°C for 7 min took place during 35 cycles.

DNA electrophoresis. Electrophoresis is the purification of molecules by separating chemical compounds based on the rate of movement of molecules in an electric current, and aims to determine the quality of DNA from PCR results (Madduppa et al 2016). The electrophoresis process was started by preparing a 1% agarose gel using DNA Gel Red dye (2 µL) as the electrophoretic medium. The PCR results were prepared in agarose

wells, and electrophoresis was performed using a voltage of 100 volts for 25 min. A low DNA mass leader (Invitrogen) was used to determine the length of the DNA strands, which could be seen in Geldoc using a UV transilluminator. A good PCR result shows a clear band with a product size of 500-700 bp.

DNA sequencing. The nucleotide sequencing cycle (DNA sequencing) was used to determine the order of nucleotide bases in the DNA (Madduppa et al 2016). DNA base sequences represent various biological phenomena that can be projected as a result of hereditary genetic information originating from the tissues of an organism (Richterich et al 1989). The PCR product, in the form of DNA, was transferred to a PCR plate and sent to First Base, Malaysia for DNA sequencing using the Sanger method (Sanger et al 1977).

Results and Discussion. In this study, specimens of *L. canarium* from Batam waters, muddy and sandy substrate habitats, were analyzed using the DNA barcoding technique. The results of sequence bioinformatics analysis showed the identity of the species, namely *L. canarium*, from 4 specimens, BTM-P1, BTM-P2, BTM-L1, and BTM-L2, as presented in Table 1. Visual morphology of *L. canarium* based on habitat can be seen in Figure 2.

Table 1

BLAST (Basic Local Alignment Search Tool) results based on the NCBI (National Center for Biotechnology Information) database

No	*Sample ID*	*DNA length (bp)*	*Query cover (%)*	*Per Ident (%)*	*Identified species*
1	BTM-P1 (sandy substrate)	629	100	99.52	*Laevistrombus canarium*
2	BTM-P2 (sandy substrate)	630	100	99.20	*Laevistrombus canarium*
3	BTM-L1 (muddy substrate)	625	100	99.52	*Laevistrombus canarium*
4	BTM-L1 (muddy substrate)	625	100	99.68	*Laevistrombus canarium*

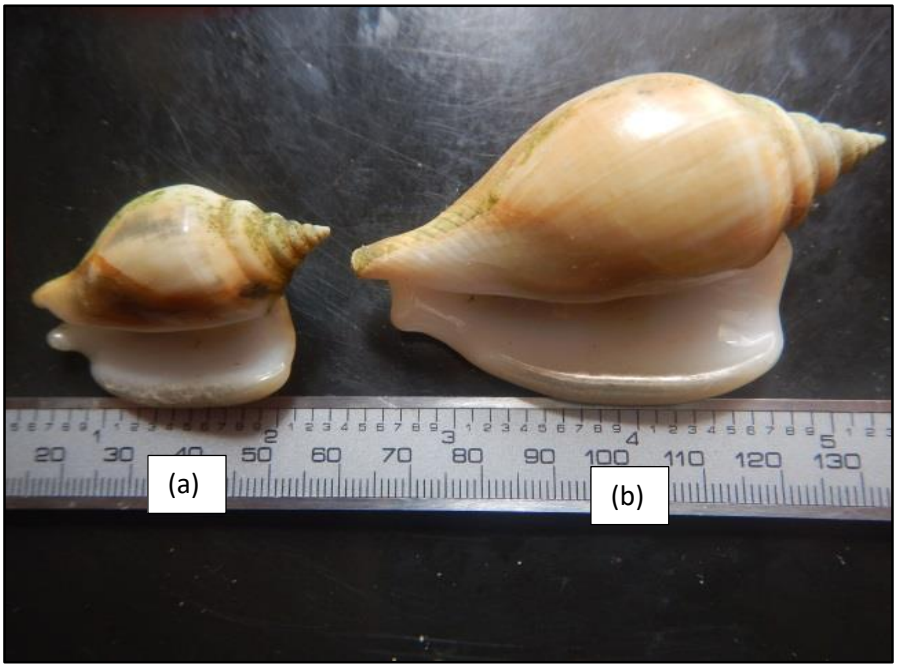


Figure 2. Adult *Laevistrombus canarium* from Batam waters: a - living in sandy habitat; b - living in muddy habitat.

COI gene amplification from *L. canarium* in Batam water produced a high-quality PCR product. A good PCR result shows a clear band with a product size of 500-700 bp. Query coverage represents the percentage of nucleotide length aligned with BLAST (Basic Local Alignment Search Tool) on NCBI (National Center of Biotechnology Information). Per ident represents the percentage indicating the level of confidence in high-similarity sequences.

Based on the results of the study, the DNA length was 629 bp for BTM-P1, with a nucleotide composition of thymine 249 (39.59%), cytosine 104 (16.53%), adenine 153 (24.32%), and guanine 123 (19.56%). In BTM-P2, the length of the DNA was 630 bp, with a nucleotide composition of thymine 249 (39.59%), cytosine 104 (16.53%), adenine 154 (24.32%), and guanine 123 (19.56%). Thymine nucleotide base content was the highest, reaching 39.59% for both *L. canarium* specimens in sandy substrate habitats. In specimens of *L. canarium* from the muddy substrate habitat, DNA length was 625 bp and the nucleotide composition of BTM-L1 specimens, namely thymine, was 154 (24.64%), cytosine 123 (19.68%), adenine 245 (39.2%), and guanine 103 (16.48%). In the BTM-L2 specimen, a DNA length of 625 bp was also obtained with almost the same nucleotide composition, namely thymine 153 (24.48%), cytosine 123 (19.68%), adenine 245 (39.2%), and guanine 104 (16.64%). The adenine nucleotide base content was the highest, reaching 39.2% for both *L. canarium* specimens in the muddy substrate habitats.

In this study, the amplified COI gene had a length of approximately 700 bp using a 10000 bp DNA ladder as a comparison (Figure 3). The most similar gene bank sequences were characterized by the same maximum score and total score, query coverage close to 100%, E-value close to 0, and Ident close to 100% in each database.

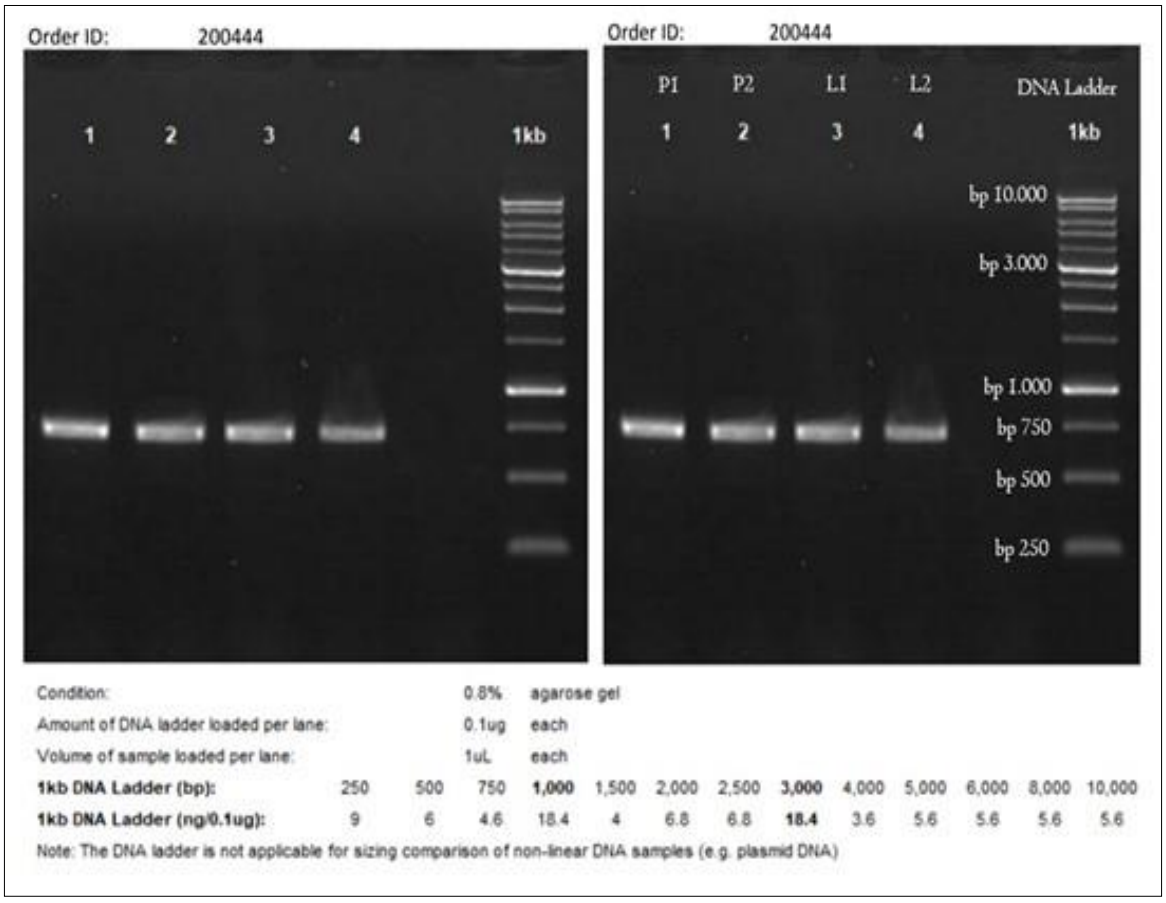
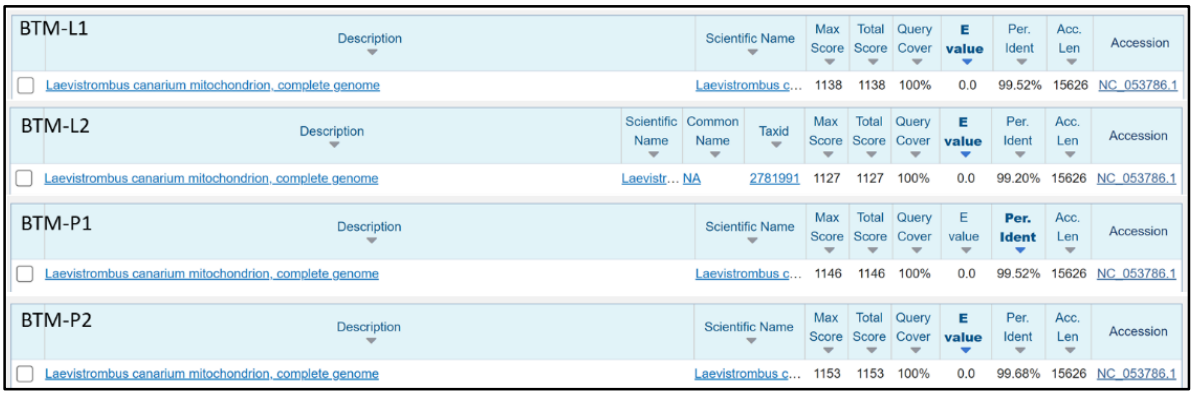


Figure 3. The results of DNA amplification (PCR) of the four samples were analyzed at the electrophoresis stage.

L. canarium has the highest similarity level with the same max score and total score of 1153, 100% query cover, 0.0 E-value, and 99.68% Ident (BTM-P2). The E-value of 0.0 obtained indicates a significant juxtaposition, meaning that the search for the sequence of specimens in this study is identical, being from the same genus, even at the species level. This study confirmed that the 4 specimens of the Gonggong sea snails from Batam waters were of the species *L. canarium*, with the following similarity values: BTM-L1 99.52%, BTM-L2 99.20%, BTM-P1 99.52%, and BTM-P2 99.68% (Figure 4).



BTM-L1

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Laevistrombus canarium mitochondrion, complete genome	Laevistrombus c...	1138	1138	100%	0.0	99.52%	15626	NC_053786.1

BTM-L2

Description	Scientific Name	Common Name	Taxid	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Laevistrombus canarium mitochondrion, complete genome	Laevistr...	NA	2781991	1127	1127	100%	0.0	99.20%	15626	NC_053786.1

BTM-P1

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Laevistrombus canarium mitochondrion, complete genome	Laevistrombus c...	1146	1146	100%	0.0	99.52%	15626	NC_053786.1

BTM-P2

Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per. Ident	Acc. Len	Accession
Laevistrombus canarium mitochondrion, complete genome	Laevistrombus c...	1153	1153	100%	0.0	99.68%	15626	NC_053786.1

Figure 4. BLAST (Basic Local Alignment Search Tool) results from the four samples at NCBI (source: www.blast.ncbi.nlm.nih.gov).

The results of the phylogenetic tree analysis showed that the samples BTMP1, BTMP2, BTML1, and BTML2 formed the same clade as *L. canarium* species with a bootstrapping value of 100, using the neighbor-joining approach, and a bootstrapping value of 99 using the maximum-likelihood approach (Figure 5). This shows a very high confidence value for the clade formed in the four samples with *L. canarium* species.

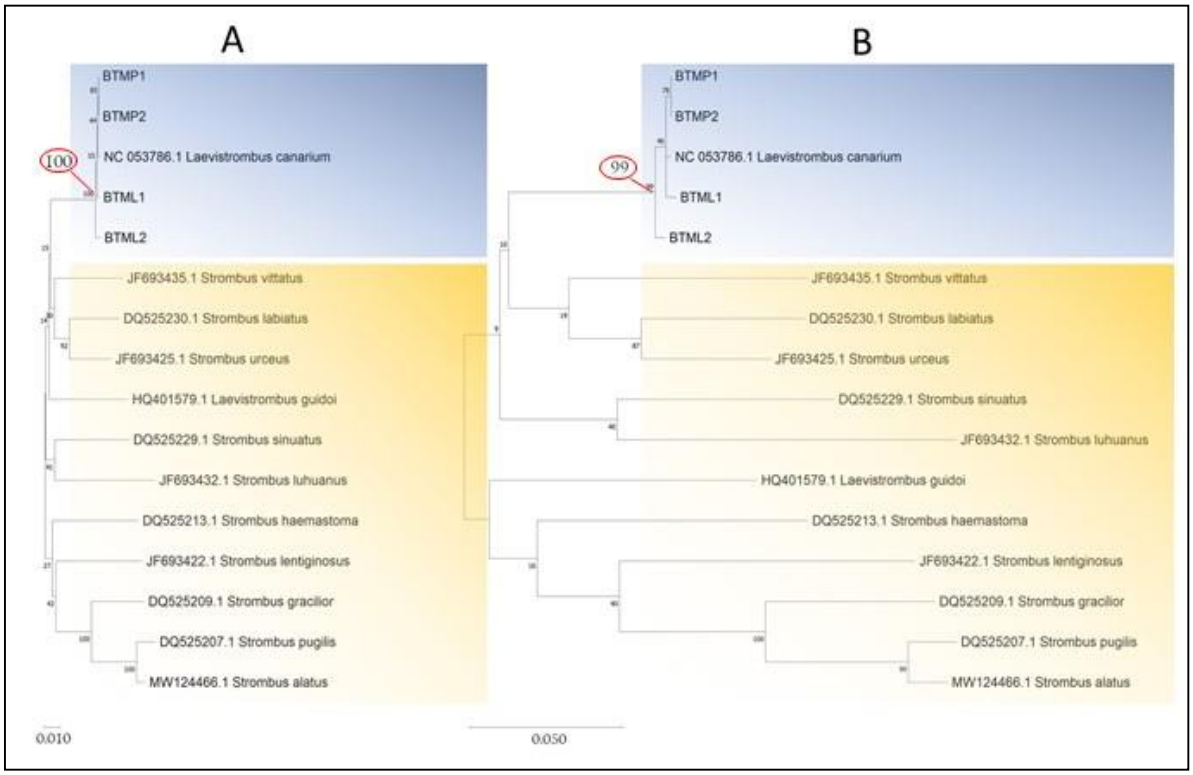


Figure 5. Reconstruction of phylogenetic trees by comparing samples with sequences from Genbank NCBI using: A - the Neighbor-Joining approach; B - the Maximum Likelihood with Bootstrap 1000x.

This study reinforces the results of previous research on the bioinformatics of sea snails of the species *L. canarium* in the waters of Kepulauan Riau Province. This study confirms that *L. canarium* from Batam waters that lives in sandy and muddy substrate habitats is, in fact, the same species, although it has different morphometry and meristic characteristics. Viruly et al (2019) stated that differences in the thickness of the shells of *L. canarium* from Bintan waters did not prove the existence of species differences. Muzahar (2019) reported that differences in morphometry and meristic characteristics could be observed in the notching characters, in the widening of the posterior shell lip, in the thickening of the outer shell lip and in the lateral dilatation of Gonggong sea snails from Modang-Tanjungpinang waters, which were subsequently identified as *Laevistrombus turturella*.

Morphologically, according to Dharma (2005) and Dody (2012), the sea snail Gonggong was also identified as *L. turturella*. Cob et al (2008) refer to this species as *Strombus canarium*. Because of the complexity of the morphologically distinguishing characteristics of the genus *Strombus*, Latiolais et al (2006) proposed a cladogram (descendant tree) that attempted to show the phylogenetic relationships of 34 species in the family Strombidae (Figure 6).

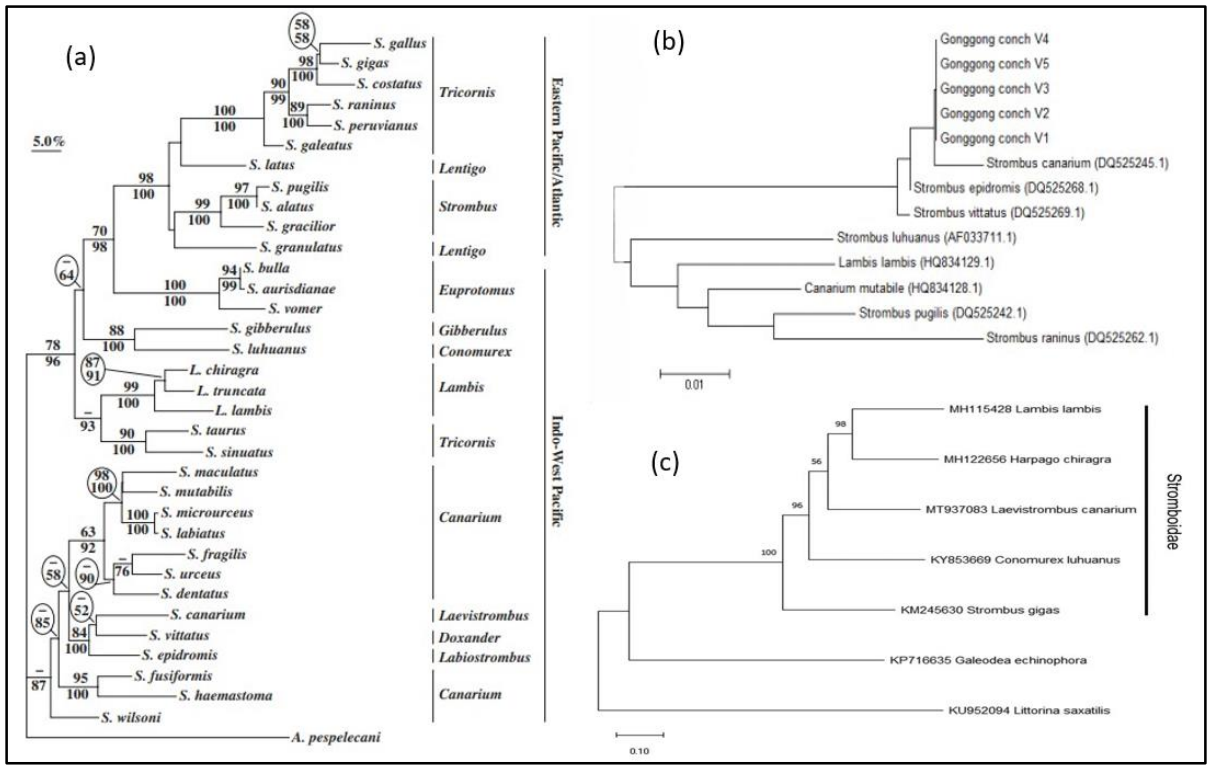


Figure 6. Strombidae phylogenetic tree: (a) Strombus species relationships (Latiolais et al 2006); (b) phylogenetic tree of the "Gonggong" Madong-Tanjungpinang sea snails as *Laevistrombus turturella* (Muzahar 2019); (c) mitochondrial genome phylogenetic tree of *Laevistrombus canarium* and four other Stromboidae species (Lee et al 2021).

The authors analyzed 31 species in the genus *Strombus* (including *Strombus canarium*), and three species in the genus *Lambis*. The cladogram is based on the histone H3 DNA sequence and mitochondrial cytochrome c oxidase I gene. In the proposed phylogeny, *Strombus phylonia*, *Strombus vittatus* (a synonym for *Doxander vittatus*) and *Strombus epidromis* (*Labiostrombus epidromis*) are closely related and appear to have a common ancestor. The phylogenetic positions of some species appear to be unstable. This can occur because of differences in the number of small nucleotides, thereby minimizing the genetic variation between each specimen and species (Chee & Nor 2016).

Conclusions. This study conclusively confirmed that the samples of sea snails Gonggong from Batam, Riau Islands, which live in muddy and sandy substrates, are from the same species from the Srombidae family, namely *L. canarium*. For the 4 specimens tested, the Per Ident value of BTM-P1 was 99.52%, for BTM-P2 it was 99.20%, for BTM-L1 it was 99.52%, and for BTM-L2 it was 99.68%. The DNA lengths of the BTM-P1 specimen was 629 bp, of BTM-P2 was 630 bp, of BTM-L1 was 625 bp, and of BTM-L2 was 625 bp. The phylogenetic tree analysis showed that the samples BTMP1, BTMP2, BTML1, and BTML2 formed the same clade as the *L. canarium* species and showed a very high confidence value of the clade with a bootstrapping value of 100 with the Neighbor-Joining approach. The bootstrap value was 99 using the Maximum Likelihood approach. The findings and results of the bioinformatics analysis of *L. canarium* in this study are expected to support the sustainable management of sea snails fisheries and species conservation efforts.

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Conflict of Interest. The authors declare that there is no conflict of interest.

References

Appeltans W., Ahyong S. T., Anderson G., Angel M. V., Artois T., Bailly N., Bamber R., Barber A., Bartsch I., Berta A., Blazewicz-Paszkowycz M., Bock P., Boxshall G., Boyko C. B., Brandao S. N., Bray R. A., Bruce N. L., Cairns S. D., Chan T. Y., Cheng L., et al., 2012 The magnitude of global marine species diversity. Current Biology 22(23):2189-2202.

Bingpeng X., Heshan L., Zhilan Z., Chunguang W., Yanguo W., Jianjun W., 2018 DNA barcoding for identification of fish species in the Taiwan Strait. PLoS ONE 13(6):e0198109.

Borges L. M. S., Hollatz C., Lobo J., Cunha A. M., Vilela A. P., Calado G., Coelho R., Costa A. C., Ferreira M. S. G., Costa M. H., Costa F. O., 2016 With a little help from DNA barcoding: investigating the diversity of Gastropoda from the Portuguese coast. Scientific Reports 6:20226.

Chee S. Y., Nor S. A. M., 2016 DNA barcoding reveals neritid diversity (Mollusca: Gastropoda) diversity in Malaysian waters. Mitochondrial DNA Part A DNA Mapping, Sequencing, and Analysis 27(3): 2282-2284.

Cob Z. C., 2008 Biology and ecology of dog conch (*Strombus canarium* Linnaeus, 1758) (Gastropoda: Strombidae) from Merambong Shoal, Johor Straits, Malaysia. PhD Thesis, Universiti Putra Malaysia, Serdang, Malaysia, 377 p.

Dharma B., 2005 Recent & fossil Indonesian shells. ConchBooks, 424 p.

Dody S., 2012 [Spawning and larval development of dog conch (*Strombus turturella*)]. Jurnal Ilmu dan Teknologi Kelautan Tropis 4(1):107-113. [In Indonesian].

Fadli N., Nor S. A. M., Othman A. S., Sofyan H., Muchlisin Z. A., 2020 DNA barcoding of commercially important reef fishes in Weh Island, Aceh, Indonesia. PeerJ 8:e9641.

Fontanilla K. I., Torres A. F., Cañasa J. A. D. G., Yap S. L., Ong P. S., 2014 State of animal DNA barcoding in the Philippines: A review of COI sequencing of the Philippine native fauna. Philippine Science Letters 7(1):104-137.

Galan G. L., Mendez N. P., Delacruz R. Y., 2018 DNA barcoding of three selected gastropod species using cytochrome oxidase (COI) gene. Annals of West University of Timişoara. Series of Biology 21(1):93-102.

Geller J., Meyer C., Parker M., Hawk H., 2013 Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. Molecular Ecology Resources 13(5):851-861.

Hebert P. D. N., Cywinska A., Ball S. L., deWaard J. R., 2003a Biological identifications through DNA barcodes. Proceedings of the Royal Society of London. Series B 270:313-321.
Hebert P. D. N., Ratnasingham S., deWaard J. R., 2003b Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. Proceedings of teh Royal Society of London. Series B 270:96-99.
Hubert N., Delrieu-Trottin E., Irisson J. O., Meyer C., Planes S., 2010 Identifying coral reef fish larvae through DNA barcoding: a test case with the families Acanthuridae and Holocentridae. Molecular Phylogenetics and Evolution 55(3):1195-1203.
Jefri E., Zamani N. P., Subhan B., Madduppa H. H., 2015 Molecular phylogeny inferred from mitochondrial DNA of the grouper *Epinephelus* spp. in Indonesia collected from local fish market. Biodiversitas 16(2):254-263.
Latiolais J. M., Taylor M. S., Roy K., Hellberg M. E., 2006 A molecular phylogenetic analysis of strombid gastropod morphological diversity. Molecular Phylogenetics and Evolution 41(2):436-444.
Leatemia S. P. O., Manumpil A. W., Saleky D., Dailami M., 2018 [DNA barcode and molecular phylogeny of *Turbo* sp. in Manokwari waters, West Papua]. Prosiding Seminar Nasional MIPA UNIPA, Manokwari, pp. 103-114. [In Indonesian].
Lee H. T., Liao C. H., Huang C. W., Chang Y. C., Hsu T. H., 2021 The complete mitochondrial genome of *Laevistrombus canarium* (Gastropoda: Stromboidae). Mitochondrial DNA Part B, Resources 6(2):591-592.
Lim H. C., Abidin M. Z., Pulungan C. P., de Bruyn M., Nor S. A. M., 2016 DNA barcoding reveals high cryptic diversity of the freshwater halfbeak genus *Hemirhamphodon* from Sundaland. PLoS ONE 11(9):e0163596
Madduppa H., Ayuningtyas R. U., Subhan B., Arafat D., Prehadi, 2016 Exploited but unevaluated: DNA barcoding reveals skates and stingrays (Chordata, Chondrichthyes) species landed in the Indonesian fish market. Ilmu Kelautan 21(1):29-36.
Madduppa H., Sani L. M. I., Mursawal A., 2020 [eModul DNA barcoding for beginners online course]. Laboratorium Biodiversitas dan Biosistematika Kelautan IPB, Oceanogen Laboklinikum Bogor, Bogor, Indonesia, 125 p. [In Indonesian].
Marquez E. J., Castro E. R., Alzate J. F., 2016 Mitochondrial genome of the endangered marine gastropod *Strombus gigas* Linnaeus, 1758 (Mollusca: Gastropoda). Mitochondrial DNA Part A, DNA Mapping, Sequencing, and Analysis 27(2):1516-1517.
Muzahar, 2019 [Reproductive physiology on gonad maturation and spawning of the Gonggong snail (*Laevistrombus turturella*) from Tanjungpinang in cultivation containers]. Dissertation, Sekolah Pascasarjana, Institut Pertanian Bogor (IPB), Bogor, Indonesia, 79 p. [In Indonesian].
Pramono T. B., Arfiati D., Maheno S., Widodo M. S., Yanuhar U., 2017 [Identification of genus *Mystus* in fish with a genetic approach]. Jurnal Sumberdaya Akuatik Indopasifik 1(2):123-131. [In Indonesian].
Prehadi P., Sembiring A., Kurniasih E. M., Rahmad R., Arafat D., Subhan B., Madduppa H. H., 2015 DNA barcoding and phylogenetic reconstruction of shark species landed in Muncar fisheries landing site in comparison with Southern Java fishing port. Biodiversitas 16(1):55-61.
Ran K., Li Q., Qi L., Li W., Kong L., 2020 Molecular identification of Cerithiidae (Mollusca: Gastropod) in Hainan island, China. Mitochondrial DNA Part A, Mapping, Sequencing, and Analysis 31(2):57-63.
Richterich P., Heller C., Wurst H., Pohl F. M., 1989 DNA sequencing with direct blotting electrophoresis and colorimetric detection. BioTechniques 7(1):52-59.
Samsudin N. F., Abidin M. Z., Yen D. T., Tanyaros S., Page L. M., Zhao Y., Adamson E. A. S., Zain K. M., de Bruyn M., Nor S. A. M., 2018 Exploring hidden diversity in Southeast Asia's Dermogenys spp. (Beloniformes: Zenarchopteridae) through DNA barcoding. Scientific Reports 8:10787.

Sanger F., Nicklen S., Coulson A. R., 1977 DNA sequencing with chain-terminating inhibitors. Proceedings of the National Academy of Sciences of the United States of America 74(12):5463-5467.
Sembiring A., Pertiwi N. P. D., Mahardini A., Wulandari R., Kurniasih E. M., Kuncoro A. W., Cahyani N. K. D., Anggoro A. W., Ulfa A., Madduppa H., Carpenter K. E., Barber P. H., Mahardika G. N., 2015 DNA barcoding reveals targeted fisheries for endangered sharks in Indonesia. Fisheries Research 164:130-134.
Setiamarga D. H. E., Nakaji N., Iwamoto S., Teruya S., Sasaki T., 2019 DNA barcoding study of shelled gastropods in the intertidal rocky coasts of central Wakayama Prefecture, Japan, using two gene markers. International Journal of GEOMATE 17(62):9-16.
Smith S. A., Wilson N. G., Goetz F. E., Feehery C., Andrade S. C. S., Rouse G. W., Giribet G., Dunn C. W., 2011 Resolving the evolutionary relationships of molluscs with phylogenomic tools. Nature 480:364-367.
Sulandari S., Zein M. S. A., Sutrisno H., Dharmayanthi A. B., Natalia I., 2013 [Working stages in DNA barcoding. 1st Edition]. Zein S. A., Prawiradilaga D. M. (eds), Prenadamedia Group, Jakarta, 242 p. [In Indonesian].
Sun Y., Li Q., Kong L., Zheng X., 2012 DNA barcoding of Caenogastropoda along coast of China based on the COI gene. Molecular Ecology Resources 12(2):209-218.
Suppapan J., Supmee V., 2016 [Population genetic structure of wing shell (*Strombus canarium*) along the Andaman Sea coast]. Burapha Science Journal 21(3):138-150. [In Thai].
Tindi M., Mamangkey N. G. F., Wullur S., 2017 [DNA barcodes and molecular phylogenetic analysis of several species of bivalves from North Sulawesi waters based on COI genes]. Junal Pesisir dan Laut Tropis 1(2):32-38. [In Indonesian].
Viruly L., 2019 [Characterization of antimicrobial peptides (AMPs) from the barking sea slug (*Strombus* sp.) from Bintan Island, Riau Archipelago]. Dissertation, Sekolah Pascasarjana, Institut Pertanian Bogor (IPB), Bogor, Indonesia, 69 p. [In Indonesian].
Viruly L., Andarwulan N., Suhartono M., Nurilmala M., 2019 Morphologycal and molecular partial histone-H3 characterization of Bintan sea snail Gonggong (*Strombus* sp.) as a species validation. Hayati Journal of Biosciences 26(2):56-62.
Yulianto D., Indra I., Batubara A. S., Fadli N., Nur F. M., Rizal S., Siti-Azizah M. N., Muchlisin Z. A., 2020 Morphometrics and genetics variations of mullets (Pisces: Mugilidae) from Aceh waters, Indonesia. Biodiversitas 21(8):3422-3430.
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