HAYATI
Journal of Biosciences

Vol. 26 No. 2, April 2019 56-62
DOI:10.4308/hjb.26.2.56
ISSN: 1978-3019
EISSN: 2086-4094

# Morphologycal and Molecular Partial Histone-H3 Characterization of Bintan Sea Snail Gonggong (*Strombus* sp.) as a Species Validation

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ARTICLE INFO

*Article history:*
Received July 24, 2018
Received in revised form January 15, 2019
Accepted March 18, 2019

*KEYWORDS:*
Sea snail gonggong,
morphology,
molecular identification,
*Strombus turturella*

**ABSTRACT**

**Sea snail gonggong is an icon of Tanjungpinang-Riau Islands Province. It is a favorite seafood item in Riau Islands Province, and is high economic value but not known widely yet. Until now, sea snail gonggong has been highly exploited but the research on this snail is very limited. The aim of this study was to morphology and molecular characterization of Bintan gonggong snail (*Strombus* sp.) as a species validation. Bintan gonggong snail included thick-shelled gonggong and thin-shelled gonggong. Morphology identification of species Bintan gonggong snail was based on morphometric variability. Molecular identification used partial Histone-H3, MEGA version 6.06, and bioinformatics analysis. The result showed that the morphological identification of thick-shelled and thin-shelled gonggong based on shell width, the lip thickness, and total weight significantly different, but other variables (i.e shell length, shell depth, aperture length, and gonggong weight) were not significantly different ($p<0.05$). Resulted of a molecular identification with phylogenetic analysis that the thin-shelled and thick-shelled Bintan gonggong snails were 1 species and a genetic distance of 1%. They were not species *Strombus canarium*, *Strombus vitatus,* and *Strombus epidromis*. Bintan gonggong snails were *Strombus turturella* (*Leavistrombus turturella*). DNA sequences of Bintan gonggong have been registered in Gen-Bank with registration numbers MH348131 (thin-shelled gonggong) and MH348132 (thick-shelled gonggong).**

## 1. Introduction

Gonggong is one type of marine biota in Riau Islands Province with good market value and it is known as "siput gonggong" (*Strombus* sp.) (Cob *et al.* 2009). Gonggong is an endemic biota that lives on Bintan Island and its surroundings, such as Dompak, Lobam, Mantang Island, Senggarang, and Tanjung Uban. Gonggong became the icon of Tanjungpinang, Riau Islands Province (Viruly 2012). Empirically of gonggong is known as vitality, enhanching foods and healthy foods, because it is believed to contain high protein. Gonggong is boiled water and it is eaten with sauce or peanut sauce (Amini 1986; Viruly 2012).

Studying gonggong is least. Preliminary study of gonggong is focused on proximation composition by Amini (1986), and amino acid, heavy metal by Viruly (2012); Muzahar and Viruly (2013). Biological diversity can be measured in ways ranging from simple counts of species or measured morphological diversity (Amini and Pralampita 1987). Until now, species gonggong has not been identified morphologically, because morphological of characterization gonggong is very complex. Morphological gonggong influenced by environment (habitat). Habitat's gonggong was the coastal areas and was highly associated with seagrass (*Enhallus* sp.) (Amini 1986). Information regarding morphology of gonggong is very limited and currently there is no regulation concerning the fishery of this species, whereas they are included ancient animals (Cob *et al.* 2008). Natural mortality rate of gonggong is the highest; this rate indicated that has been overexploited. The exploitation rate of gonggong (E=F/Z) were 0.68 for males and 0.63 for females, which were higher than the optimum level of exploitation (E=0.50) (Cob *et al.* 2009; Cob *et al.* 2009b). According to Latiolais *et al.* (2006) that

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molecular phylogenetic of sea snail *Leavistrombus* (*Strombus* sp.) can be used primer histones subunit 3 (H3A and H3B), because primer COI proved problematic to sequence across full length. Therefore, the aim of this study was to identify morphology and molecular partial histone-H3 characterization of Bintan sea snails gonggong (*Strombus* sp.) to validate species identification.

## 2. Materials and Methods

### 2.1. Sample Collection

Live sea snail gonggong were collected from Madong village in Bintan Island, Riau Islands Province. They were collected from 4 stations (Figure 1). They were transported to laboratory in Bogor at August 2017. Gonggong from Bintan Island included thick-shelled and thin-shelled sea snails gonggong as many as 200 tails to morphological and molecular characterization of gonggong. In the laboratory, they were cleaned and separated to shape their shell (Cob *et al.* 2008).

### 2.2. Morphometric Variability

Samples of gonggong divided into different to shape their shell were thick-shelled and thin-shelled gonggong each 30 tails. Morphometric variability included shell length, shell width, shell depth, aperture length, lip thickness, gonggong weight, shell weight, and total weight. Morphometric characteristics of this spesies gonggong can known morphometric variability (Figure 2). Differences in specific morphometric parameters between two group gonggong analyzed via one-way ANOVA at p<0.05 probability levels (Cob *et al.* 2008; Cob *et al.* 2009a, 2009b).

### 2.3. Molecular Characterization of Bintan Gonggong Snail

#### 2.3.1. DNA Extraction and Isolation

DNA extraction from gonggong samples (thick-shelled and thin-shelled) was randomly carried out on 50 gonggong samples. 10-20 mg of each sample were taken then placed into Eppendorf tubes. Afterward, 250 µl of lysis cell solution and 1.5 µl K proteinase solution were added and homogenized for 3 sec. The sample was then vortexed for 1 second and incubated with a wobble at 55°C for 24 hours. The RNA was eliminated by adding 1.5 RNase (4 mg/ml) into Eppendorf and vortexed (in tubes) times, incubated at 37°C for 60 minutes, then incubated at room temperature. The protein sample was precipitated using a protein precipitation solution of 200 µl; vortexed for 30 seconds to homogenize and incubated in the freezer for 10-15 minutes. The sample was centrifuged at 12,000 rpm for 10 minutes. 100% isopropanol (0.8 x DNA fluid volume) was added to the sample, and the tube was reversed 50 times, before being centrifuged at 12,000 rpm for 10 minutes. The supernatant was removed, and 300 µl of cold ethanol was added (70%). The sample was centrifuged again at 12,000 rpm for another 10 minutes, and ethanol was discarded and dried for 30 minutes. After that, the sample was added 50 µl Nuclease-Free Water (NFW) and heated to 50°C for 2 minutes. The solution was diluted 10 times (Latiolais *et al.* 2006).

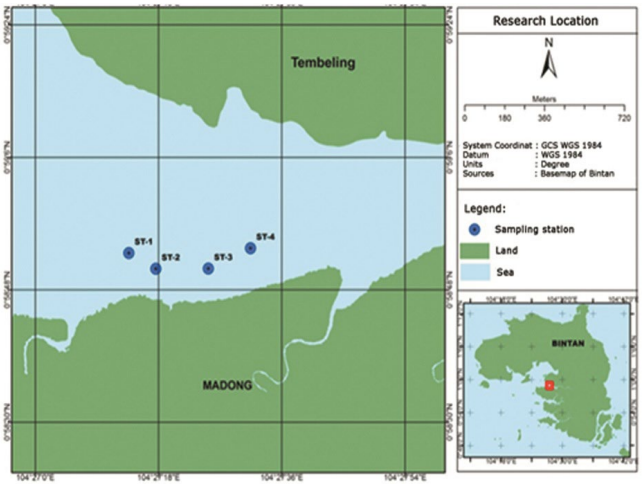

Figure 1. Map of sampling station

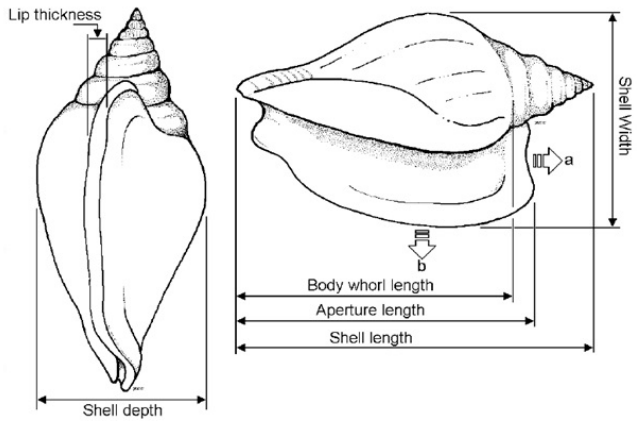

Figure 2. Morphometric variability of sea snail gonggong (Cob *et al.* 2008)

### 2.3.2. Amplification and Sequensing

This study used 2 µl DNA from each sample extraction. The intensification of sample was carried out using Polymerase Chain Reaction (PCR). All reactions were performed at a volume of 25 µl, consisting of 10 µl mixture (Mytaq, dNTP, DNA polymerase, and buffer), Histone H3 Primer (F) and Histone 141 H3 Primer 2 µl, NFW (ddH2O), respectively with a volume of 9 µl. The PCR was carried out under the following conditions: initial temperature of 94°C for 5 minutes, pre-denaturation at 94°C for 5 minutes, DNA denaturation at 94°C for 25 seconds, annealing at 57°C for 25 seconds, and extension at 72°C for 25 seconds, the final elongation temperature was 72°C for 5 minutes, and the total reaction was 40 cycles. The target amplification is at 350 bp from the H3 histone protein. The sequencing using histone primer H3A is ATGGCTCGTACCAAGCAGACVGC-3', while the sequence of the H3B protein is 5'ATATCCTTRGGCATRATRGTGAC-3' (Colgan *et al.* 1998). The PCR reaction was visualized using 2% agarose gel and electrophoresis lasting for 30 minutes at 200 V. This reaction produces a single band of the expected size (350 bp). All products are sequenced in both directions using fluorescently labeled dye-terminators (ABI, Foster City, CA) (Latiolais *et al.* 2006).

### 2.3.3. Phylogenetic and Bioinformation Analysis

All the nucleotide sequences are compared with other sequences from GenBank using the Basic Local Alignment Tool nucleotide (BLASTn) at NCBI (http://www.ncbi.nlm.nih.gov/blast). The BLASTn program is used to analyze the nucleotide similar to *Strombus* sp. All sequences are available at Gen Bank-H3 for *Strombus* sp., *Littorina* sp., *Biomphalaria* sp., and *Haliotis* sp. Pairwise and multiple sequence alignment were analyzed using the ClustalW program. Phylogenetic tree was performed using the Neighbor-Joining from Mega version 6.06 (Latiolais *et al.* 2006).

## 3. Results

### 3.1. Morphology Identification of Bintan Gonggong Snail

Various morphometric parameters of spesies Bintan gonggong snail, Riau Islands Province were measured and analyzed. The parameters included shell length, shell width, shell depth, aperture length, lip thickness, gonggong weight, shell weight, and total weight (Table 1). Comparisons between thick-shelled and thin-shelled sea snails gonggong were based on morphology indicated that thick-shelled and thin-shelled sea snails gonggong had shell width, lip thickness, and total weight significantly different ($p<0.05$), which shell length, shell depth, aperture length, and gonggong weight were not significantly different (Table 1 and Figure 3).

Comparison of body, digestive, adult operculum, and male reproductive organs (penis) of the species were presented in Figure 3. The difference of morphology also occurred in body, digestive, adult operculum, and male reproductive organs (penis) (Figure 4), although they were very similar.

Morphologycal identification based of shell sharp were presented in Figure 5. Figure 5 showed that thin shell gonggong (gonggong tipis) and thick shell gonggong (gonggong tebal) were *Laevistrombus turturella*, but they were not *Strombus canarium*.

### 3.2. Molecular Identification of Bintan Gonggong

The identification of gonggong species based on morphology is complicated, leading to the need for gonggong molecular characterization. Thus, the molecular identification of gonggong used H3 Histone primers. The result of gonggong DNA extract amplification using PCR with Histone primer (Figure 6).

Table 1. Morphological comparisons between thick-shelled and thin-shelled of gonggong

| Parameters | Thick-shelled gonggong | Thick-shelled gonggong |
|---|---|---|
| Shell length (cm) | 6.58±0.15[a] | 6.69±0.42[a] |
| Shell width (cm) | 4.05±0.09[b] | 3.86±0.32[c] |
| Shell depth (cm) | 2.95±0.10[a] | 2.99±0.25[a] |
| Aperture length (cm) | 5.030±0.11[a] | 5.07±0.34[a] |
| Lip thickness (cm) | 0.21±0.01[b] | 0.05±0.02[c] |
| Gonggong weight (g) | 9.84±0.52[a] | 8.87±2.57[a] |
| Total weight (g) | 28.34±2.02[b] | 24.69±4.60[c] |

Value with different superscript in the same column is significantly different ($p<0.05$)
*Average of ten data (duplicate in triple measurements)

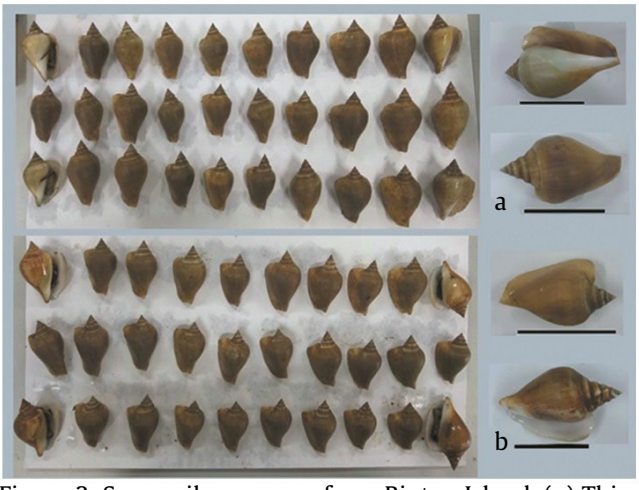


Figure 3. Sea snail gonggong from Bintan Island. (a) Thin-shelled gonggong, (b) thick-shelled gonggong. Scale bars: A, B=5 cm


Figure 4. Physically comparison of between sea snails gonggong, thick-shelled gonggong (a, c, e, g); thin-shelled gonggong (b, d, f, h); body (a, b); digestive (c, d); adult operculum (e, f); penis (g, h). Scale bar: 1 cm

a. Thin shelled gonggong b. Thick shelled gonggong c. *Laevistombus turturella*

d. *Strombus canarium* e. *Strombus vitatus* f. *Strombus epidromis*

Figure 5. Comparison of morphologycal identification based of shell sharp; Bintan gonggong snail: thin shelled gonggong (5a), thick shelled gonggong (5b); Mollusca Base 2018 (5c, 5d, 5e, 5f)

Nucleotide base sequence after alignment used BLASTn program (Table 2 and Figure 7). The construction of the phylogenetic tree (Figure 8).

## 4. Discussion

Differences in morphology of thick-shelled and thin-shelled sea snails gonggong were influenced by environment such as temperature, pH, salinity, water depth, natural nursery, food, and level of pollution (Cob *et al.* 2010). Morphological of sea snails gonggong were strongly influenced by environment and food that largely the protein's gonggong so it was contributed to their phenotype. Furthermore, they are intertidal benthic organisms that protect them against pathogens with peptides protein in tissue of meat, so they have been antimicrobial compound. Those facts caused differences in their morphology (Cob *et al.* 2009a; Duval *et al.* 2009; Nam *et al.* 2015). So, the metamorphosis responses their larvae were influenced by the sediment and detrital substrata taken from their natural habitat (Cob *et al.* 2010).

ANOVA resulted that the species are different in shell width, lip thickness, and total weight; however, the two of sea snails species were still difficult to differentiate, because there were played that the difference other morphometric variables (shell length, shell depth, aperture length, and weight), more important also occurred in species identification based on morphology. Shell length is determinants on morphological identification. Shell length gonggong from researchers were variously, for example: 3.1-9.7 cm (Abbott 1960), 6.5 cm (Poutiers 1998), and 3.6-7.2 cm (Amini 1986). The sea snail gonggong of Bintan Island in this study is similar shell length studied by Amini (1986).

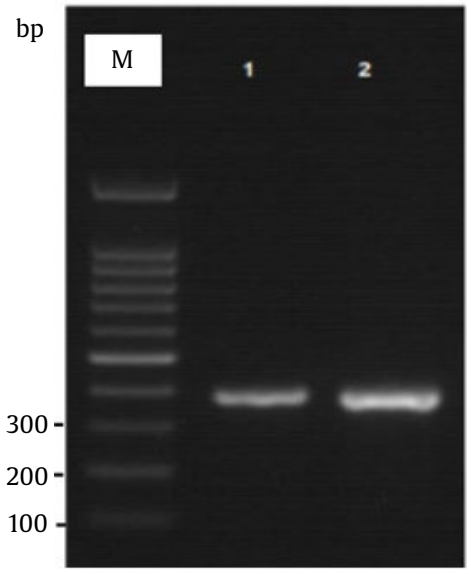


Figure 6. Visualisation of amplification genomic gonggong extraction. (1) Genomic thin-shelled gonggong extraction, (2) Genomic thick-shelled gonggong extraction. (M) Protein molecular weight marker

Thin-shelled gonggong is known as male gonggong (Cob *et al.* 2009a). According to Cob *et al.* (2008) that sea snails gonggong (*Strombus canarium*) are molluscs (gastropods) included male and female sex which can be distinguished by their shell size. The female gonggong has longer shell (male of 54.67±3.76 mm; female 55.56±3.72 mm), widher (male of 34.61±2.27 mm; female 35.38±1.99 mm) but lip thickness (male of 3.32±1.93 mm; female 2.83±0.87 mm) than the male gonggong, but this research of Figure 3 showed that the male gonggong had penis and the female had not penis. Physically comparison of between thick-shelled

Table 2. BLASTn of thin-shelled and thick-shelled gonggong

| Species | Homology (%) | No accession |
|---|---|---|
| *Strombus epidromis* gene partial histone H3 | 99 | DQ525268.1 |
| *Strombus vittatus* gene partial histone H3 | 98 | DQ525269.1 |
| *Strombus canarium* gene partial histone H3 | 98 | DQ525245.1 |
| *Strombus wilsoni* gene partial histone H3 | 97 | DQ525249.1 |

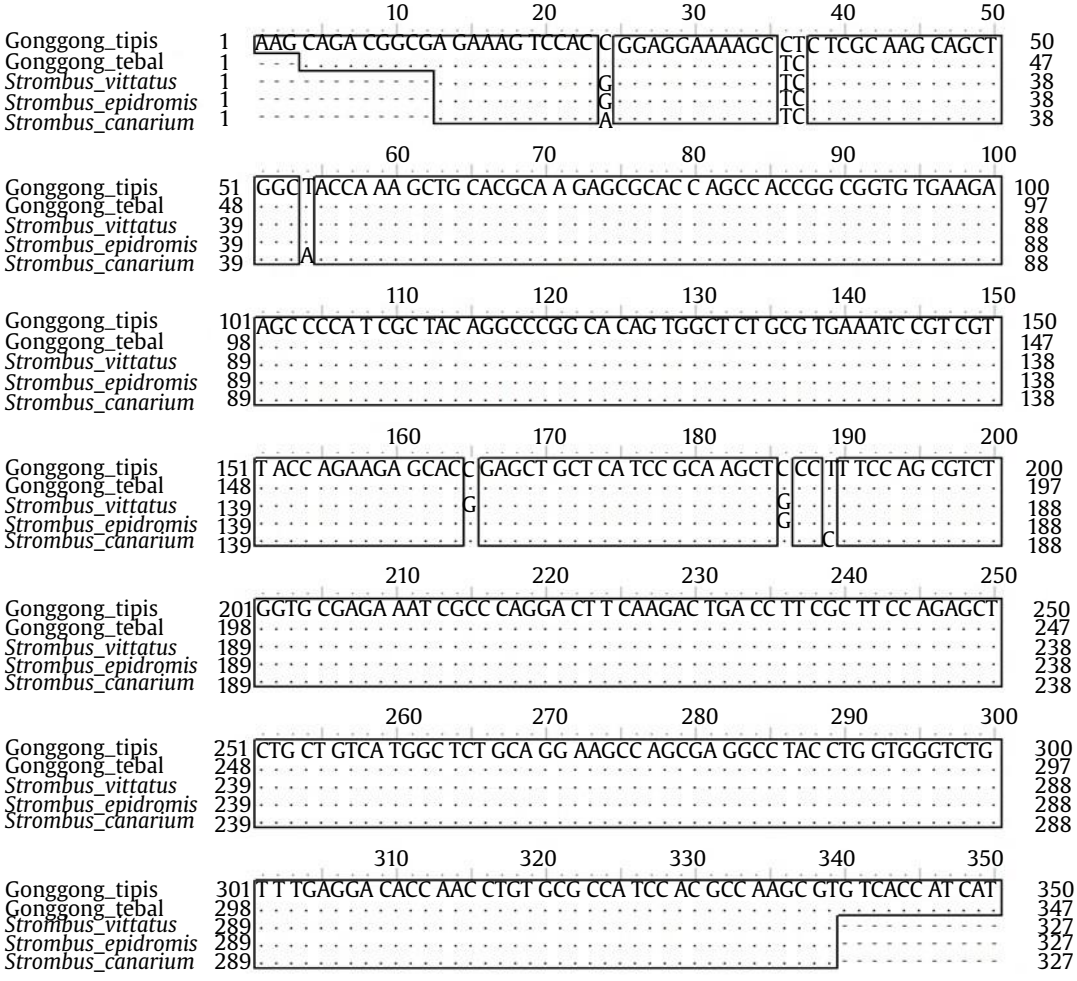


Figure 7. Nucleotide and amino acid sequences of gonggong. (1) Nucleotide and amino acid sequences of thick-shelled gonggong (gonggong tebal). (2) Nucleotide and amino acid sequences of thin-shelled gonggong (gonggong tipis)

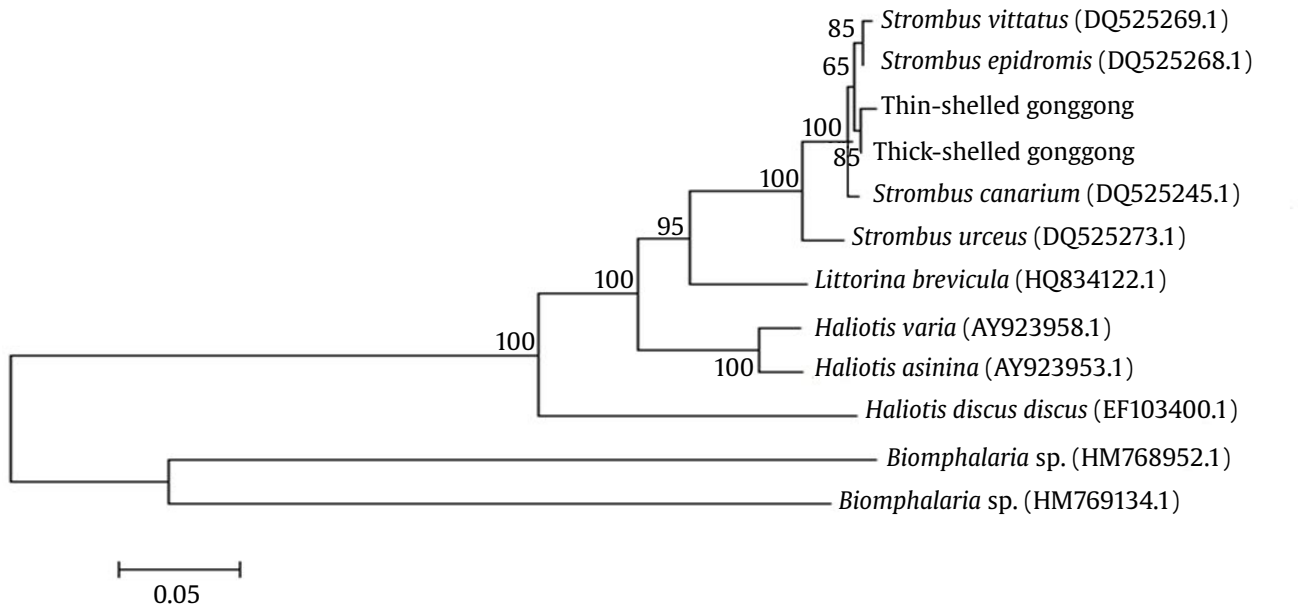

Figure 8. Phylogenetic tree of gonggong with other species from gastropoda

gonggong and thin-shelled gonggong were similar although shell phenotypes were different. In this fact, species gonggong has not been identified morphology directly, because characterization of morphological gonggong is very complex. Characteristics of gonggong with high morphological variations made they are difficult to identification. Thus, it has not been able to ensure thick-shelled and thin-shelled gonggong are one species. The identification of gonggong species based on morphologycal is complicated, so they will research for gonggong molecular characterization.

Figures 6 and 7 revealed that both thick-shelled and thin-shelled gonggong have similar nucleotide bases except for sites 36 and 37. Based on the BLASTn analysis, gonggong is closely related to *Strombus epidromis* (99%) (Table 2). The analysis using MEGA 6.06 program revealed that thin-shelled gonggong was expected as genus *Strombus* of Indonesia origin. It has specific nucleotide base of 5 sites from nucleotide bases (having singleton on sites 38 and 39), on this site mutation of transition substitution (pyrimidine base "T-C" become a pyrimidine base "C-T"). The opposite, thick-shelled gonggong was no mutation but also has 5 singleton sites. This condition indicated that Bintan gonggong snails were different from *Strombus epidromis*, *Strombus canarium,* and *Strombus vitatus*. Based on the analysis of the phylogenetic trees (Figure 8), both thick-shelled and thin-shelled gonggong were *Strombus turturella* (*Leavistrombus turturella*) species and were in one species as having a genetic distance of 1%. If a genetic distance of 1% or less than 3% so in one species (Hebert *et al.* 2003). In the phylogenetic tree can also be predicted that Bintan gonggong snail was ancestors of *Strombus epidromis* and *Strombus vitatus*. DNA sequences of Bintan gonggong have been registered in Gen-Bank with registration numbers MH348131 (thin-shelled gonggong) and MH348132 (thick-shelled gonggong).

## 5. Conclusion

Morphology identification of spesies Bintan gonggong snails indicated that thick-shelled and thin-shelled gonggong had shell width, lip thickness, and total weight significantly different ($p<0.05$). The thin-shelled and thick shelled gonggong were in one species with a genetic distance of 1% which correspond to *Strombus turturella* (*Leavistrombus turturella*) species. Gonggong Bintan was predicted to be the ancestor of both *Strombus epidromis* and *Strombus vitatus*.

## Acknowledgements

The authors are grateful to the Ministry of Research, Technology and Higher Education of The Republic of Indonesia, Director of the Southeast Asian Food and Agricultural Science and Technology (SEAFAST) Center-Bogor Agricultural University, Bogor-Indonesia and Maritime Raja Ali Haji University, Tanjungpinang-KEPRI-Indonesia.

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