

# Genetic variation and population structure of brachiopods, *Lingula anatina* Lamarck, 1801 in the Northern Aceh shore, Indonesia

CHITRA OCTAVINA<sup>1,2,\*</sup>, MUTIA RAMADHANIATY<sup>1,3</sup>, RAHMA EFFENDIE DAULAY<sup>1</sup>, IRMA DEWIYANTI<sup>1,2</sup>, MARIA ULFAH<sup>1,2</sup>

<sup>1</sup>Department of Marine Science, Faculty of Marine Science and Fisheries, Universitas Syiah Kuala. Jl. Teuku Nyak Arief No. 44, Banda Aceh 23111, Aceh, Indonesia. \*email: chitraoctavina@usk.ac.id

<sup>2</sup>Laboratory of Marine Biology, Faculty of Marine Science and Fisheries, Universitas Syiah Kuala. Jl. Teuku Nyak Arief No. 44, Banda Aceh 23111, Aceh, Indonesia

<sup>3</sup>Laboratory of Genetics and Aquatic Biodiversity, Faculty of Marine and Fisheries Universitas Syiah Kuala. Jl. Teuku Nyak Arief No. 44, Banda Aceh 23111, Aceh, Indonesia

Manuscript received: 30 June 2023. Revision accepted: 19 July 2023.

**Abstract.** Octavina C, Ramadhaniaty M, Daulay RE, Dewiyanti I, Ulfah M. 2023. Genetic variation and population structure of brachiopods, *Lingula anatina* Lamarck, 1801 in the Northern Aceh shore. Biodiversitas 24: 3951-3959. *Lingula anatina* is one of the brachiopods recognized as one of the most primitive groups with a fossil record from the early Cambrian period. Although *L. anatina* has been targeted as an object of biological studies, the information about the genetic variation within species is still limited. The purpose of this research is to identify the genetic variation and population structure of the species *L. anatina* found in the Aceh northern shore based on the mtDNA Cytochrome Oxidase I (COI) gene sequence. In this study, *L. anatina* specimens collected from five populations on Aceh's northern shore, namely Krueng Raya, Syiah Kuala, Ulee Lheue, and Ujong Pancu, with complete mitochondrial genome sequences were analyzed and compared with genebank sequences. The total nucleotide base of *L. anatina* is 657 bp, with 18 haplotypes. Based on the results of a comparison of the distribution of *L. anatina* in Asia shows that the Japanese population has the highest population structure value compared to the other five populations. The Fst value obtained from this study is 0.7, so it is classified as high and structured. Phylogenetic analysis showed that the correlation between geographic location and geographic distance affects the genetic distance between populations.

**Keywords:** Geographic, Lingulidae, marine invertebrate, mitochondrial genome, phylogenetic

## INTRODUCTION

Aceh waters have a unique current pattern because of its territorial boundaries. The northern part of the Aceh region faces the Andaman Sea; meanwhile, the eastern part faces the Malacca Strait, and the western region of Aceh province relates to the Indian Ocean (Ilhamsyah et al. 2018; Setiawan et al. 2020). The distribution of marine animal diversity is inseparable from the role of various forms of currents that occur in Indonesian waters. One of them is lamp shell (common name) or baree/biree (local name), or *Lingula anatina* (scientific name) that have been discovered in Southern Aceh (Octavina et al. 2021), Alue Naga coastal area (Agustina et al. 2019), and Aceh Tamiang (Darmarini et al. 2017). This species is not only from Aceh but also distributed in Probolinggo, East Java (Ambarwati et al. 2021) and on the coast of Madura Island (Rakmawati dan Ambarwati 2020).

Even though this species is found on several coastlines in Indonesia, *L. anatina* has a sporadic distribution characteristic that makes this species only found on some coasts (Christian 2016; Fujii et al. 2019; Karagozlu et al. 2021). Even so, the geographical area where this species is discovered has a specific characteristic. It can be seen at the coast of Aceh Tamiang with a sandy and loamy area (Darmarini et al. 2017) at shallow depths of 20 meters

(Samanta 2014; Samanta 2015). The same characteristic was discovered on the northern shore of Aceh, where the area contains muddy sand (Octavina et al. 2021). Meanwhile, if observed at the bottom topography of Probolinggo waters located in the Madura strait, it has a 26 m depth, and the semi-enclosed bay characteristic, so these coastal areas are affected by tides (Hasanudin et al. 2016).

*Lingula anatina* is one of the Linguliformea subphylum species recognized as one of the most primitive groups of brachiopods (Luo et al. 2015; Goto et al. 2022). Considering the fossil record that has been found from the early Cambrian period, many scientists recognize *L. anatina* as a "Living fossil" (Maddison et al. 2021). The high morphological variation value in the previous studies of *L. anatina* shows that many of them are morphologically different. *L. anatina* found on the northern coast of Aceh, has a square anterior valve with a whitish-green color (Agustina et al. 2019). Meanwhile, the species discovered in the southern area of Aceh have an oval anterior valve with a dark brown shell (Octavina et al. 2021). The color of the shell from Lubuk Damar, Aceh Tamiang, tends to be brighter and has a brown-greenish color (Darmarini et al. 2017). However, the studies done in Indonesia confirmed that the *Lingula* species is the *L. anatina* species.

Therefore detailed study of molecular genetics analysis is needed to confirm *Lingula* to the species stage and to

observe their intraspecies taxonomy (Maddison et al. 2021). Molecular DNA is an identification tool to elaborate the population structure of marine organisms (Madduppa et al. 2014). Mitochondrial DNA analysis is often used to study population genetic diversity and phylogenetic relationships through maternal inheritance patterns (Karagozlu et al. 2017; Gerdol et al. 2018). Moreover, genetic variation is a response to various environmental conditions which will cause species with different ecologies to have their patterns of genetic variation (Manel et al. 2013; Munday et al. 2013). Therefore, species' genetic variation can be evaluated using a molecular marker that permits precise genetic diversity estimation (Pino-Querid et al. 2015; Dias et al. 2018). The purpose of this study was to analyze the genetic variation and population structure of the *L. anatina* species found in the northern waters of Aceh based on the Cytochrome Oxidase Subunit I (COI) mtDNA gene sequence.

## MATERIALS AND METHODS

### Study area

This research was carried out in March 2022 at Northern Aceh shore, Indonesia. The specimens were taken in Syiah Kuala (95°19'37.20"E, 5°35'34.85"N); Ujong Pancu (95°14'32.25"E, 5°32'57.38"N); Ulee Lheue (95°18'23.58"E, 5°35'29.87"N); and Krueng Raya (95°30'44.96"E, 5°35'42.70"N) coastal areas (Figure 1).

### Procedures

#### Specimen sampling

The specimen (20 specimens) was carried out at the Syiah Kuala, Ujong Pancu, Ulee Lheue, and Krueng Raya

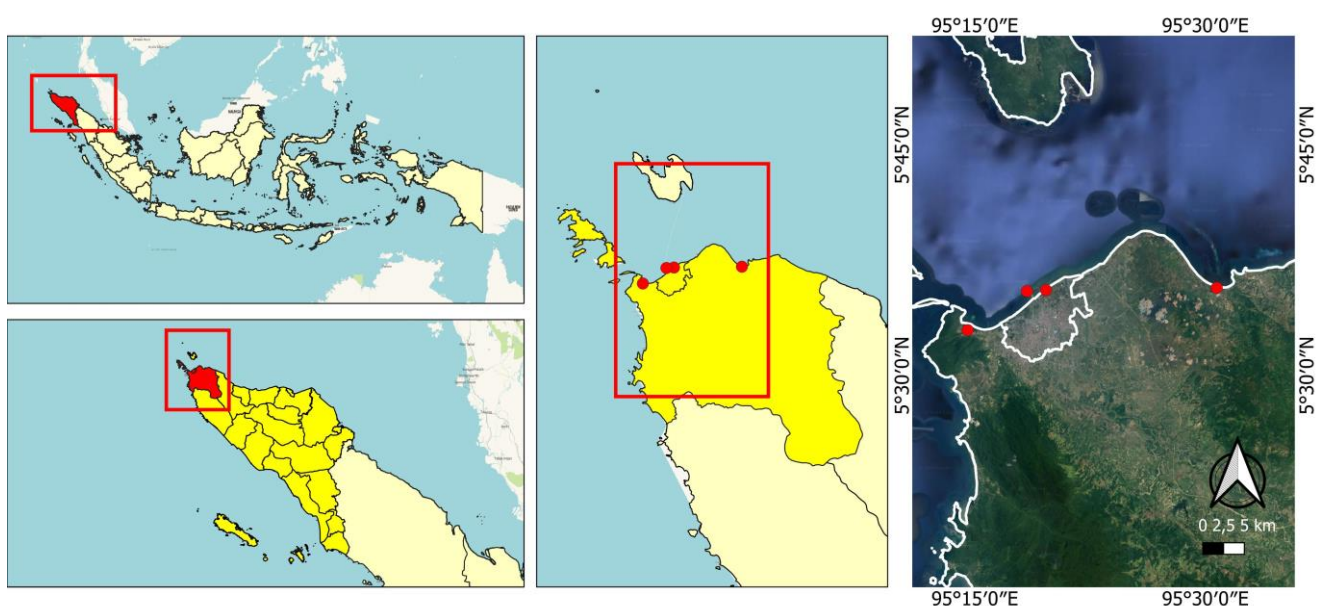
coasts using machetes as tools. Next, the samples were taken to the Laboratory of Genetics and Aquatic Biodiversity, Faculty of Maritime Affairs and Fisheries, to undergo the process of preserving the sample with 96% ethanol to keep the sample in good condition during analysis.

#### Sequence retrieval from GenBank

*Lingula anatina* sequencing data were taken from GenBank to compare the genetic variation and population structure (Table 1).



**Figure 2.** Some specimens of *Lingula anatina*



**Figure 1.** Location of specimen sampling in the Northern Aceh shore, Indonesia, i.e. Syiah Kuala, Ujong Pancu Ulee Lheue, Krueng Raya

**Table 1.** Sequence data from GenBank

Species	Location	GenBank code	References
<i>Lingula anatina</i>	East Java	MW454800	(Ambarwati et al. 2021)
		MW454813	
		MW454814	
		MW454815	
	South Korea	KY091123	(Kim et al. 2017)
		KY091124	
		KY091125	
	China	GU056041	(Wu 2010)
		GU056040	
	Japan	KP881498	(Saito et al. 2000; Endo et al. 2001; Luo et al. 2015)
AB026520			
AB056460			
<i>Pinctothis picta</i>		AB026506	

#### DNA extraction of *L. anatina*

DNA extraction is the first step in studying specific DNA sequences in complex DNA populations and analyzing genomic structure. DNA extraction aims to separate the DNA from all components of a cell. In *L. anatina*, most of the DNA is localized in the nucleus, an organelle separate from the cytoplasm. The DNA extraction method used in this study is Cetyl Trimethyl Ammonium Bromide (CTAB) (Quang et al. 2018). This extraction method contains three important stages, such as lysis and the destruction of the cell wall, which need to avoid strong forces that can damage or cut the DNA. Afterward, protein and RNA are removed to focus more on DNA concentration, purity, and quantity (Kalendar et al. 2021) obtained from the lophophore pedicle and *L. anatina* tentacle sample from the sampling areas.

#### DNA amplification of *Lingula anatina*

The extracted samples were amplified using the Polymerase Chain Reaction (PCR) technique in vitro using a pair of forward and reverse primers from the Cytochrome Oxidase subunit 1 (COI) gene. DNA components used in the amplification process were 2 µl with the addition 8,5 µl DdH<sub>2</sub>O, 1 µl forward primer LCO1490 5'-GGT-CAA-ATC-ATA-AAG-ATA-TTG-6-3', 1 µl reverse primer HCO2198 5'-TAA-ACT-TCA-GGG-TGA-CCA-AAA-AAT-CA-3', and 12,5 µl red master mix. The amplification process was carried out using a Sensoquest Labcycler machine (SensoQuest GmbH Hannah-Vogt-Str.1) with 40 cycles. Each cycle consists of Pre-denaturation at 94°C for 1 minute, Denaturation at 94°C for 45 seconds, Annealing at 45°C for 45 seconds, extension at 72°C for 1 minute 30 seconds, and Final extension at 72°C for 10 minutes (Ambarwati et al. 2021).

#### Electrophoresis

After the sample was amplified, they were visualized through an electrophoresis process. This process is needed to separate chemical compounds by using the momentum motion of the molecules in an electric current. The electrophoresis result is observed using the UV transilluminator UVITEC Fire-Reader V10-Plus. A

positive sample will be marked by the appearance of a clear and bright DNA band with the size of 400-600 base pair (Banerjee 2014). Next, the sequencing process was carried out by sending the positive sample to First Base Laboratories, Malaysia, using the Sanger method (Walker et al. 2013) to acquire the nucleotide base sequence.

#### Sea current mapping

The current distribution was visualized using ArcMap 10.3 software to analyze the current pattern in the year the research was conducted. The data used in the current distribution map is the Near Real Time Geostrophic Current Dataset from CoastWatch Caribb-NOAA/AOML.

#### Data analysis

The result of the COI (Forward) gene sequence were aligned using the ClustalW algorithm in the Molecular Evolutionary Genetic Analysis (MEGA X) software to confirm species similarity with the sequence data from GenBank (Tamura et al. 2013). The genetic distances were analyzed by reconstructing the phylogenetic tree using mega x with the maximum likelihood (ML) method with 1000 times bootstrap replication. Then, the saturation tests were examined using Data Analysis and Molecular Biology and Evolution (DAMBE) software for substitution and evaluated the suitability of the order for phylogenetic analysis. Haplotype distribution was observed using DnaSP 5.10 programs (Bilandžija et al. 2013), and the connectivity between geographical populations can be observed using Network software. Eventually, haplotype diversity (Hd), nucleotide diversity ( $\pi$ ), number of haplotypes (Hn), and Fst values are examined using ARLEQUIN software (Rozas et al. 2017).

## RESULTS AND DISCUSSION

#### Phylogenetics tree reconstruction

The specimen of *L. anatina* that were successfully amplified in the amplification processes were 20 samples in total with 657 base pairs of DNA band lengths from the four area populations, namely Ujong Pancu, Syiah Kuala, Ulee Lheue, and Krueng Raya coasts. The *L. anatina* kinship relationship can be observed through the phylogenetic tree in (Figure 3) where the populations of the Aceh northern shore, Japan, and East Java are in the first clade with a similarity value of up to 95%. Meanwhile, Japanese, China, and South Korean populations are in the second clade with a similarity value of 99%.

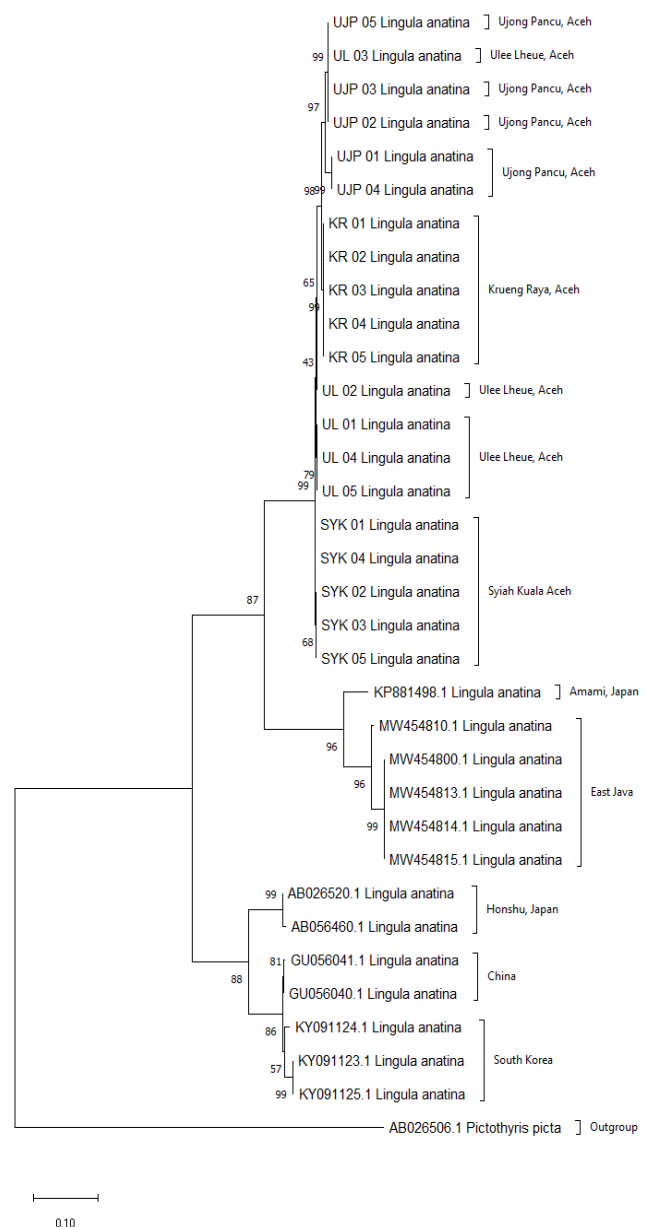
Phylogenetic analysis reveals the evolutionary ancestry of species that can be deduced into a phylogenetic tree from the alignment of protein or gene sequences assuming proximity along the total length of nucleotides sample between homologous sequences (Temereva et al. 2015) to reveal phylogenetic relationships between species (Schreiber et al. 2013). The amplified COI gene in this study has a 657 bp DNA length with a ladder as a comparison. The optimum temperature for attaching the primer is 45°C. A phylogenetic relationship between *L. anatina* species was observed through the distance between

the other *L. anatina* inferred from the phylogenetic tree where the population of Aceh's northern shore, Japan, and East Java were in the same clade with a similarity reaching 95%. Meanwhile, the population of Japan, China, and South Korea is in the second clade with a 99% similarity. The geographical distance between populations can impact the low gene flow amount and is considered sporadic (Christian 2016; Fujii et al. 2019; Karagozlu et al. 2021). That also can be seen in the population of Aceh's northern shores, where the four populations are grouped into one clade. The phylogenetic tree (Figure 2) shows the Japanese population in two clades. In this case, Japan has two different sampling locations. In the first clade, the sample was taken from Amami Island, located in the Southern region of Japan, and had a similar current pattern direction as Northern Aceh. While in the second clade, the sample was taken from Mutsu Bay, located in northern Honshu, Japan, facing the South Korean population. It has similar characteristics to the other two populations in the second clade. This also happened in Yang et al. (2013), where the clade is still separated in the entire phylogenetic tree of the same species. However, mitochondrial DNA data and geographic location can support taxonomy in explaining phylogenetic trees.

### Genetic variation

The average nucleotide composition of Adenine (A) = 26.18%, Cytosine (C) = 16.91%, Thymine (T) = 37.69%, Guanine (G) = 19.22% (Table 2; Figure 4). Based on the nucleotide composition, the COI gene showed that the nucleotide base composition of AT was higher than the GC, with the number of AT at 63.87% and the GC at 36.13%.

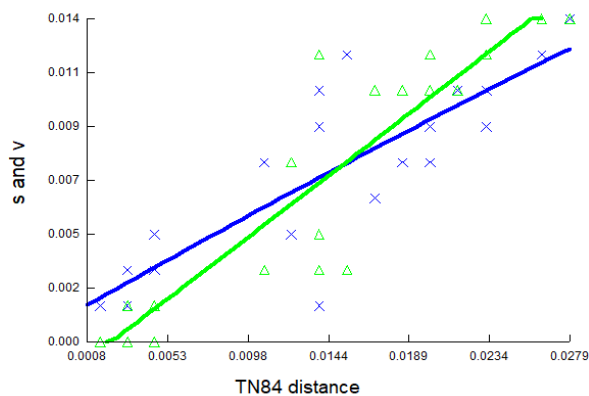
Sequence analysis using the COI locus of mitochondrial DNA showed that the nucleotide base length of *L. anatina* was 657 bp (base pair) with an average nucleotide composition of 26.18% Adenine (A), 16.91% Cytosine (C), 37.69% Thymine (T), and 19.22% Guanine (G) so that it can be seen that the average nucleotide base of GC is 36.13%, it shows that the AT content is higher, namely 63.87% (Table 2). This happens because the GC content has decreased from the order of the third codon position (Li et al. 2016). The nucleotide composition obtained in this study was inversely proportional to that of Nugroho et al. (2017), where there are fewer GC nucleotides than AT nucleotides. Hence, the protein translation results show no pseudogenes in the amino acid sequence. However, the GC nucleotide results in this study were similar to those of Sloan et al. (2017), where the GC value in mollusk was 37%. Then it can be seen that the A and T values distributed on the two DNA strands (Sloan et al. 2017) increase, especially in the T value, which has a maximum value of 37.90% (Table 2). DNA repair genes affect the number of mutations that cause an increase in AT so that base mutations are shown to be more inclined towards AT nucleotides (Li et al. 2016). In addition, mutations that occur in mtDNA cause high genetic variation (Allio et al. 2017).



**Figure 3.** The phylogenetic tree of 34 *Lingula anatina* sequences uses the Maximum Likelihood method with 1000 bootstrap

**Table 2.** Statistical summary table of the nucleotide frequency distribution of COI sequences of *Lingula anatina* samples collected in the Aceh northern shore

Percentage nitrogen base (%)	Value		
	Average	Minimum	Maximum
T %	37.69	37.44	37.90s
C %	16.91	16.74	17.35
A %	26.18	25.57	26.79
G %	19.22	18.42	19.79
GC %	36.13	35.77	36.53
GC % Codon Pos 1	24.73	24.20	25.57
GC % Codon Pos 2	44.86	43.84	45.21
GC % Codon Pos 3	38.79	37.90	39.73



**Figure 4.** Transition and Transversion Rates plotted against genetic differences. The blue line represents the transversion (s), and the green line represents the transition (v), calculated using TN84 (Tajima and Nei, 1984)

Genetic diversity is variation in form in a population that occurs because there is diversity between individuals who are members of a population (Kusuma et al. 2016). High and low genetic variation can be indicated by the number and diversity of haplotypes (Hd) and nucleotides ( $\pi$ ) (Li et al. 2013). Based on (Table 4) genetic diversity in the population of Aceh's northern shore has a moderate level of haplotype diversity, which ranges from 0-0.7. The lowest haplotype diversity was found in the Krueng Raya population, with a value of 0. In contrast, the highest haplotype diversity was found in Japanese, South Korean, and China populations, each of which had a value of 1. Haplotype composition showed the characteristics of a species in each habitat (Fernández-Pérez et al. 2017). Nucleotide diversity ( $\pi$ ) in this study ranged from 0-0.524, with the lowest value in the Krueng Raya population and the highest in the Japanese population (Table 4). Genetic variation values ranging from 0.8 to 1 are included in the

high category, while values of 0.5-0.7 are classified as medium, and 0.1-0.4 are included in the low category (Li et al. 2013). High genetic variation indicates a large population size, so a decrease in population size results in a decrease in genetic diversity.

### Population structure

Genetic distance between populations (interspecies) and within populations of the same species (intraspecies) can be seen after observing the population structure. Aceh northern shore has the highest genetic distance, especially in Ujong Pancu and Ulee Lheue, with a value reaching 0.01 (Table 6); the highest genetic distance in the five populations reaches 0.21 in the Japanese population (Table 7). Meanwhile, in the northern part of Aceh, the Ulee Lheue region has the highest nucleotide biodiversity (Table 7).

Natural selection, species adaptation, and genetic drift depend heavily on a species' past and present population structure. The population's genetic structure can be explained through the Fst value, which relates the diversity in the population to the total genetic diversity (Meirmans et al. 2018). The value of genetic distance characterizes genetic variation between populations (Li et al. 2013) because genetic distance shows the value of kinship between populations (Nugroho et al. 2017). The smaller the value of the genetic distance, the smaller the diversity between the populations. Hence the value of the genetic distance is small; there will be more similarities between the two populations. Meanwhile, if the genetic distance between populations is higher, the population will have a high heterosis effect when crossed. The variation of a population can be observed from the number of haplotypes in one population and the diversity of the haplotypes in that population. Meanwhile, populations with low genetic distance indicate that these populations have close kinship between individuals (Silitonga et al. 2018).

**Table 3.** Nucleotide Substitution Saturation Test (DAMBE) on the COI mtDNA dataset in Aceh's northern shores

Num OTU	Iss	Iss. cSym	T	DF	P	Iss. cAsym	T	DB	P
4	0.01	0.76	131.7	656	0	0.674	115.654	656	0

Description: NumOTU: Number of operational taxonomic units. Iss: Substitution saturation index. Iss.C Asym: Critical Value for symmetric tree topology. Iss.C Asym: Critical Value for asymmetric tree topology. Q: Value of T. DB: Degrees of freedom. Q: Probability that Iss is significantly different from the critical value (Iss.cSym or Iss.cAsym). Test using both sides with a result of 10,000 replications

**Table 4.** Genetic diversity of *Lingula anatina* in Aceh's northern shore

Species	Population	N	Hn	Hd	$\pi$
<i>Lingula anatina</i>	Krueng Raya	5	1	0	0
	Syiah Kuala	5	2	0.6	0.02
	Ujong Pancu	5	2	0.6	0.24
	Ulee Lheue	5	3	0.7	0.25

Description: Hd: Haplotype diversity,  $\pi$ : Nucleotide diversity, Hn: Number of haplotypes, N: Total number of samples per population, Fst: Pairwise distance value



Ujong Pancu and Ulee Lheue have a genetic distance value of 0.01, so it can be concluded that it is higher than the coasts of Krueng Raya and Syiah Kuala (Table 6). However, the genetic distance between the Syiah Kuala and Ujong Pancu populations has the same value, namely 0.02, with the Ujong Pancu and Ulee Lheue populations. Therefore, if observed in Figure 3, Ujong Pancu, Ulee Lheue, and Syiah Kuala populations are on the same coastline facing the Andaman Sea. Meanwhile, the population of Krueng Raya, which is in the form of a bay, tends to face the Malacca Strait. Overall, the North Aceh population has a genetic distance value of 0.013 within the population (Table 7). Then, it can be seen that the value of genetic distance within the population and between populations on Aceh's northern shore is categorized as low (Banerjee 2014). Even so, the highest genetic distance value in the five populations was in the Japanese population, with a value of 0.21, and the lowest genetic distance value in the population, with a value of 0.0034, was in the China population (Table 5). This happens because Japan has current characteristics that cause a period of larval distribution (Ohshimo et al. 2017). Meanwhile, the location where the *L. anatina* species were found in the China population is an enclosed bay (Wu 2010).

Paired distance test analysis (Fst) was analyzed to make it easier to test the existence of a hierarchical population structure where individuals are grouped into populations (sample locations) and populations are grouped into higher group levels (Meirmans 2012). The Fst value of the *L. anatina* species shows a value of 0.75 which belongs to the high category, namely 0.6-1.00 (Ramadhaniaty et al. 2018). Fst values that are in the high category tend to have structured characteristics. Overall, the genetic relationship in these five populations can be observed from the phylogenetic tree (Figure 4), which is composed of 2 large clades with high bootstrap values so that the phylogenetic tree that is constructed is better (Saleky and Dailami 2021). The four populations originating from the Aceh northern shore are in 1 cluster allegedly because they have the closest genetic distance values. The populations of Northern Aceh and East Java, which are in 1 clade, show a genetic distance value that is not far away, which is 0.01 (Table 6). This is evidenced (Table 7) that the genetic distance value is 0.24 in Northern Aceh and East Java populations.

Connectivity between populations on the north coast of Aceh, East Java, Japan, China, and South Korea were analyzed using sequence data to view pattern data from the network and their haplotype maps (Figure 3, Figure 4, and Figure 5). The five populations produced 18 haplotypes

from 34 sequences of *L. anatina*. Haplotypes 1,2,3,4,5,6 and 7 came from the Northern Aceh population, while haplotypes 8 and 9 came from the East Java population, haplotypes 10,14,15 and 18 came from the Japanese population, haplotypes 11,12, 13 were from South Korea, and haplotypes 16 and 17 are from the Chinese population (Table 7).

Analysis of Molecular Variance (AMOVA) shows the diversity relationship within the population (intraspecies) with genetic variation with a relatively high Fst value of 0.75 (Table 8). The correlation between geographic differences and genetic distance indicates that geographic distance can be used as the basis for the genetic distance between two populations (Cruz 2013). As described by Luo et al. (2015), phylogenetic studies show that specimens from *L. anatina* that are geographically closer are also observed as species that are closer to the phylogenetic tree. This can be observed from the 5<sup>th</sup> haplotype found in 2 populations, namely Ujong Pancu and Ulee Lheue. Geographically, the Ujong Pancu and Ulee Lheue populations are located on the north coast of Aceh, and both face the Andaman Sea, in contrast to the Krueng Raya population, which faces the Malacca Straits (Figure 5; 6; 7). Then, it can be concluded that the current types adapted to the Ujong Pancu and Ulee Lheue populations have similarities.

**Table 5.** Genetic diversity of *Lingula anatina* in five populations of Indonesian, Japanese, China, and Korean coastal

Species	Population	N	Hn	Hd	$\pi$
<i>Lingula anatina</i>	Krueng Raya	5	1	0	0
	Syiah Kuala	5	2	0.6	0.00161
	Ujong Pancu	5	2	0.6	0.01448
	Ulee Lheue	5	3	0.7	0.01501
	East Java	5	2	0.4	0.01716
	Japan	4	4	1	0.52413
	South Korea	3	3	1	0.02324
	China	2	2	1	0.00536

**Table 6.** Genetic distance within the population (bold) and between populations of *Lingula anatina* in the mitochondrial COI gene in the Aceh northern shore

Population	Krueng Raya	Syiah Kuala	Ujong Pancu	Ulee Lheue
Krueng Raya	<b>0.00</b>			
Syiah Kuala	0.01	<b>0.00</b>		
Ujong Pancu	0.01	0.02	<b>0.01</b>	
Ulee Lheue	0.01	0.01	0.02	<b>0.01</b>

**Table 7.** Genetic distance within the population (bold) and between populations of *Lingula anatina* in the mitochondrial COI gene

Population	Aceh northern shore	East Java	Japan	South Korea	China
Aceh northern shore	<b>0.013</b>				
East Java	0.2436	<b>0.01</b>			
Japan	0.2637	0.2327	<b>0.2149</b>		
South Korea	0.2869	0.3208	0.1894	<b>0.0134</b>	
China	0.2888	0.3193	0.18	0.0165	<b>0.0031</b>

**Table 8.** Fst values between and within populations

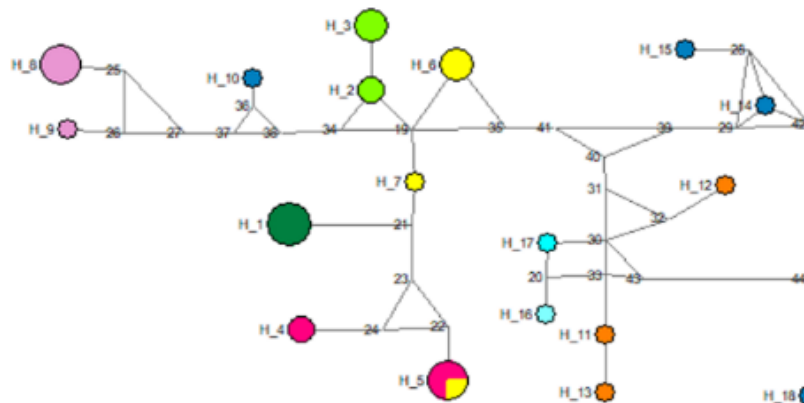
Species	Source of variation	d.f	Variation (%)	Fst
<i>Lingula anatina</i>	Between Populations	7	75.71%	0.75712
	Within population	26	24.29%	

Description: d.f: Degrees of freedom, Fst: Fixation Indeks



Description :

Krueng Raya Syiah Kuala Ujong Pancu Ulee Lheue

**Figure 5.** *Lingula anatina* haplotype connectivity from the Aceh northern shore

Description:

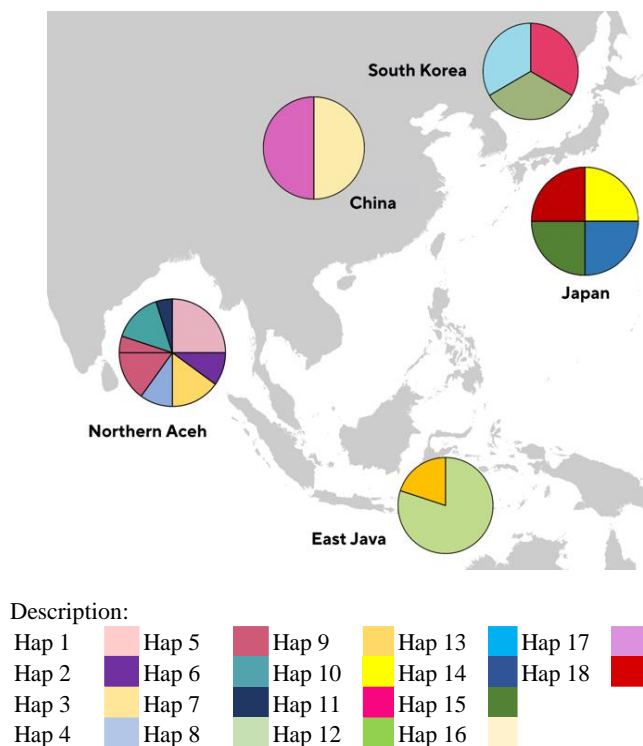
Krueng Raya Syiah Kuala Ujong Pancu Ulee Lheue  
East Java Japan South Korea China

**Figure 6.** *Lingula anatina* haplotype connectivity from five Asian populations

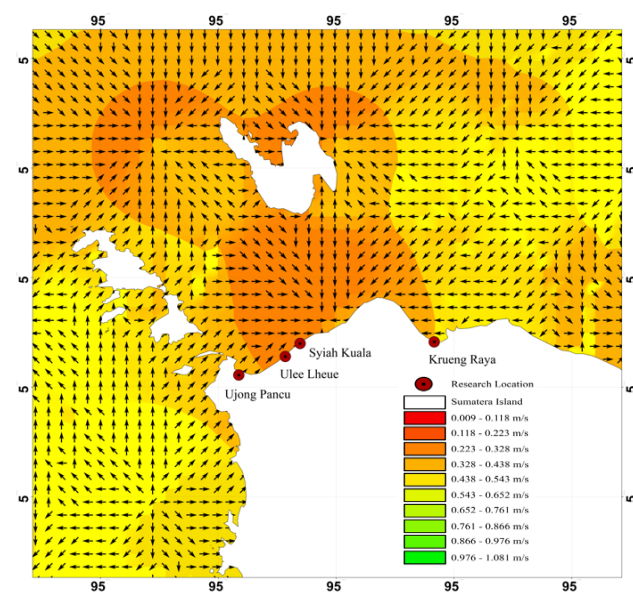
Ocean currents are important in the distribution of marine life because the biota relies on currents to move them to suitable breeding, feeding, and water areas (Lal et al. 2017). The direction of surface currents has a close relationship with the wind. Cold currents from the North Pacific and warm currents from the South Pacific confluence meet in the Northwest Pacific Ocean (Masujima et al. 2014). Larvae from the southern part of the Japanese archipelago and the Sea of Japan are carried to the southwestern coastal areas of Japan by the warm Kuroshio and Tsushima currents (Ohshimo et al. 2017). Whereas in East Java waters it has the shape of a semi-enclosed bay so that the tides affect the physical process, namely the

seawater being thrown onto the beach due to waves so that the water mass rises into the estuaries, lagoons, and bays (Hasanudin et al. 2016). So that the currents flow towards the northern shore of Aceh (Surinati and Wijaya 2017).

The direction of current patterns affects current circulation, so it can potentially increase biological variability, especially diversity, in identifying aquatic organisms (Chan et al. 2022). In Figure 8, it can be seen that the direction of the ocean currents from Ujong Pancu is towards Ulee Lheue, with an average speed of sea surface currents in Ujong Pancu and Ulee Lheue is 1.3305 m/s. This allows *L. anatina* larvae from Ujong Pancu to be carried by currents to the Ulee Lheue area.



**Figure 7.** The Asian haplotype distribution map



**Figure 8.** Current pattern direction in the West Pacific

Therefore, it can be concluded that genetic variation in a population can be observed from the number of haplotypes and the diversity of haplotypes in one population. Hence, it can be seen that there are 18 haplotypes, where the fifth haplotype consists of two populations. The Japanese population structure has the highest value among the five populations. In contrast, in the northern waters of Aceh, namely, Ujong Pancu and Ulee Lheue, the population structure is higher than the other two populations. In addition, the value of the paired distance test ( $F_{st}$ ) obtained in this study was high.

## ACKNOWLEDGEMENTS

This research is supported by a local grant with a number of contracts: 172/UN11/SPK/PNBP/2021.

## REFERENCES

- Agustina S, Octavina C, Sarong A, Nurhaliza A, Dewiyanti I, Iqbal T. 2019. The density and distribution of *Lingula* sp. in Aceh Northern Shore. Proc 2nd ICFAES 2019 Intl Conf Fish Aquat Environ Sci 2019. Conjunctions with The 6th ASI 2019 Annual Conference of The Asian Society of Ichthyologist 2019, 19-20 June 2019.
- Ambarwati R, Rahayu DA, Rachmadiarti F, Khaleyla F. 2021. DNA barcoding of lamp shells (Brachiopoda: *Lingula anatina*) from Probolinggo, East Java, Indonesia. Biodiversitas 22: 1764-1774. DOI: 10.13057/biodiv/d220421.
- Allio R, Donega S, Galtier N, Nabholz B. 2017. Large variation in the ratio of mitochondrial to nuclear mutation rate across animals: implications for genetic diversity and the use of mitochondrial DNA as a molecular marker. Mol Biol Evol 34: 2762-72. DOI: 10.1093/molbev/msx197.
- Banerjee S, Bright SA, Smith JA, Burgeat J, Martinez-Calvo M, Williams DC, Kelly JM, Gunnlaugsson T. 2014. Supramolecular approach to enantioselective DNA recognition using enantiomerically resolved cationic 4-amino-1, 8-naphthalimide-based tröger's bases. J Org Chem 79: 9272-9283. DOI: 10.1021/jo501711g.
- Bilandžija H, Morton B, Podnar M, Četković H. 2013. Evolutionary history of relict Congeria (Bivalvia: Dreissenidae): unearthing the subterranean biodiversity of the Dinaric Karst. Front Zool 10: 1-8. DOI: 10.1186/1742-9994-10-5.
- Chan BK, Tsao YF, Wangkulangkul K, Amjud K, Sukparangsi W. 2022. Biogeography and biodiversity of the intertidal barnacle *Tetracita* species in the Gulf of Thailand and Andaman Sea-influences of oceanographic currents and Pleistocene glaciations. Front Mar Sci 8: 2127. DOI: 10.3389/fmars.2021.774041.
- Christian B, Skovsted BP, Topper TP, Marissa JB, Guoxiang L, Glenn AB. 2016. The operculum and mode of life of the lower Cambrian hyolith Cupithecra from South Australia and North China. Palaeogeogr Palaeoclimatol Palaeoecol 443. DOI: 10.1016/j.palaeo.2015.11.042.
- Cruz CD. 2013. Genes: a software package for analysis in experimental statistics and quantitative genetics. Acta Scientiarum. Agronomy 35: 271-276. DOI: 10.4025/actasciagron.v35i3.21251.
- Darmarini AS, Wardiatno Y, Prartono T, Soewardi K. 2017. New record of a primitive brachiopod, *Lingula* sp. in Lubuk Damar, Indonesia. Biodiversitas 18: 1438-1444. DOI: 10.13057/biodiv/d180419.
- Dias PJ, Gilg MR, Lukehurst SS, Kennington WJ, Huhn M, Madduppa HH, McKirdy SJ, De Lestang P, Teo SL, Lee SS, McDonald JI. 2018. Genetic diversity of a hitchhiker and prized food source in the Anthropocene: the Asian green mussel *Perna viridis* (Mollusca, Mytilidae). Biol Invasions 20: 1749-1770. DOI: 10.1007/s10530-018-1659-6.
- Endo K, Ozawa T, Kojima S. 2001. Nuclear and mitochondrial gene sequences reveal unexpected genetic heterogeneity among northern Pacific populations of the brachiopod *Lingula anatina*. Mar Biol 139: 105-112. DOI: 10.1007/s002270100555.
- Fernández-Pérez J, Froufe E, Nantón A, Gaspar MB, Méndez J. 2017. Genetic diversity and population genetic analysis of *Donax vittatus* (Mollusca: Bivalvia) and phylogeny of the genus with mitochondrial and nuclear markers. Estuar Coast Shelf Sci 197: 126-135. DOI: 10.1016/j.ecss.2017.08.032.
- Fujii R, Ueno R, Yamamoto T. 2019. Breeding season and life history of *Lingula anatina* after settlement in Amami-Oshima Island, Kagoshima, Japan. Plankton Benthos Res 14: 45-51. DOI: 10.3800/pbr.14.45.
- Gerdol M, Luo YJ, Satoh N, Pallavicini A. 2018. Genetic and molecular basis of the immune system in the brachiopod *Lingula anatina*. Dev Comp Immunol 82: 7-30. DOI: 10.1016/j.dci.2017.12.021.
- Goto R, Takano T, Seike K, Yamashita M, Paulay G, Ku'ulei SR, Hunter CL, Tongkerd P, Sato SI, Hong JS, Endo K. 2022. Stasis and diversity in living fossils: species delimitation and evolution of



- lingulid brachiopods. *Mol Phylogenet Evol* 175: 107460. DOI: 10.1016/j.ympev.2022.107460.
- Hasanudin M, Kusmanto E, Budisetyawan W. 2016. Amplifikasi pasang surut dan dampaknya terhadap perairan pesisir Probolinggo. *OLDI (Oseanologi dan Limnologi di Indonesia)* 1: 69-80. DOI: 10.14203/oldi.2016.v1i3.72. [Indonesian]
- Ilhamsyah Y, Koesmaryono Y, Hidayat R, Nurjaya IW, Atmadipoera AS, Rizal S. 2018. Characteristics of hydro-oceanography in the Aceh waters, Indonesia: expedition by R/V Baruna Jaya IV. *Adv Environ Sci* 10: 200-208.
- Karagozlu MZ, Kim SG, Dhin Thinh D, Kim CB. 2017. Complete mitochondrial genome analysis of *Lingula anatina* from Korea (Brachiopoda, Lingulida, Lingulidae). *Mitochondrial DNA Part B* 2: 829-830. DOI: 10.1080/23802359.2017.1407711.
- Karagozlu MZ, Do TD, Kim JI, Choi TJ, Kim SG, Kim CB. 2021. An Investigation of the variations in complete mitochondrial genomes of *Lingula anatina* in the Western Pacific Region. *Biology* 10: 367. DOI: 10.3390/biology10050367.
- Kalendar R, Boronnikova S, Seppänen M. 2021. Isolation and purification of DNA from complicated biological samples. *Mol Plant Taxon: Methods Protoc* 2021: 57-67. DOI: 10.1007/978-1-0716-0997-2\_3.
- Kim SG, Karagozlu MZ, Kim CB. 2017. Phylogenetic investigations of *Lingula anatina* among some northwestern Pacific populations, based on mitochondrial DNA cytochrome c oxidase subunit I gene. *J Asia-Pac Biodivers* 10 (2): 162-166. DOI: 10.1016/j.japb.2017.04.007.
- Kusuma P, Brucato N, Cox MP, Pierron D, Razafindrazaka H, Adelaar A, Sudoyo H, Letellier T, Ricaut FX. 2016. Membandingkan asal-usul linguistik dan genetik dari populasi sumber Malagasi di Asia. *Laporan Ilmiah* 6: 26066.
- Lal MM, Southgate PC, Jerry DR, Bosserelle C, Zenger KR. 2017. Swept away: ocean currents and seascape features influence genetic structure across the 18,000 km Indo-Pacific distribution of a marine invertebrate, the black-lip pearl oyster *Pinctada margaritifera*. *BMC Genomics* 18: 1-21. DOI: 10.1186/s12864-016-3410-y.
- Li FW, Kuo LY, Pryer KM, Rothfels CJ. 2016. Genes translocated into the plastid inverted repeat show decelerated substitution rates and elevated GC content. *Genome Biol Evol* 8: 2452-2458. DOI: 10.1093/gbe/evw167.
- Li J, Ye Y, Wu C, Qi P, Guo B, Chen Y. 2013. Genetic variation of *Mytilus coruscus* Gould (Bivalvia: Mytilidae) populations in the East China Sea inferred from mtDNA COI gene Sequence. *Biochem Syst Ecol* 50: 30-38. DOI: 10.1016/j.bse.2013.03.033.
- Luo YJ, Satoh N, Endo K. 2015. Mitochondrial gene order variation in the brachiopod *Lingula anatina* and its implications for mitochondrial evolution in lophotrochozoans. *Mar Genomics* 24: 31-40. DOI: 10.1016/j.margen.2015.08.005.
- Madduppa HH, Timm J, Kochzius M. 2014. Interspecific, spatial and temporal variability of self-recruitment in anemonefishes. *PloS ONE* 9: e90648. DOI: 10.1371/journal.pone.0090648.
- Madison AA, Kuzmina TV, Temereva EN. 2021. Analysis of the juvenile shell of *Lingula anatina* (Brachiopoda: Linguliformea) provides insight into the evolution of life cycles of fossil brachiopods. *Paleobiology* 47: 134-148. DOI: 10.1017/pab.2020.51.
- Manel S, Holderegger R. 2013. Ten years of landscape genetics. *Trends Ecol Evol* 28: 614-21. DOI: 10.1016/j.tree.2013.05.012.
- Masujima M, Kato Y, Segawa KH. 2014. Numerical studies focusing on the early life stage of Pacific bluefin tuna (*Thunnus orientalis*). *Bull Fish Res Agency* 38: 51-55.
- Meirmans PG, Liu S, van Tienderen PH. 2018. The analysis of polyploid genetic data. *J Hered* 21: 283-296. DOI: 10.1093/jhered/esy006.
- Meirmans PG. 2012. AMOVA-based clustering of population genetic data. *J Hered* 103 (5): 744-750. DOI: 10.1093/jhered/ess047.
- Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ. 2013. Predicting evolutionary responses to climate change in the sea. *Ecol Lett* 16: 1488-1500. DOI: 10.1111/ele.12185.
- Nugroho ED, Nawir D, Amin M, Lestari U. 2017. DNA barcoding ikan nomei (Synodontidae: Harpadon sp.) di Pulau Tarakan, Indonesia. *Akuakultur, Akuakultur, Konservasi & Perundang-undangan*, 10: 1466-1474.
- Octavina C, Agustina S, Sarong M, Sari P, Sahidin A, Razi NM, Agustiari M, Sakinah R, Fazillah M. 2021. Length-weight relationship of *Lingula* sp. in Aceh Southern Shore. *IOP Conf Ser: Earth Environ Sci* 674. DOI: 10.1088/1755-1315/674/1/012021.
- Ohshimo S, Atsushi T, Tomoko O, Satoru N, Taiki I, Mikio W, Keisuke S, Toshiyuki T, Osamu A. 2017. Horizontal distribution and habitat of Pacific bluefin tuna, *Thunnus orientalis*, larvae in the waters around Japan. *Bul Mar Sci* 93 (3): 769-787. DOI: 10.5343/bms.2016.1094.
- Pino-Querido A, Álvarez-Castro JM, Vera M, Pardo BG, Fuentes J, Martínez P. 2015. A molecular tool for parentage analysis in the Mediterranean mussel (*Mytilus galloprovincialis*). *Aquac Res* 46 (7): 1721-1735. DOI: 10.1111/are.12329.
- Quang Le H, Suffredini E, Tien Pham D, Kim To A, De Medici D. 2018. Development of a method for direct extraction of viral RNA from bivalve molluscs. *Lett Appl Microbiol* 67: 426-434. DOI: 10.1111/lam.13065.
- Rakmawati R, Ambarwati R. 2020. Komunitas bivalvia yang berasosiasi dengan Kerang Lentera (Brachiopoda: Lingulata) di zona intertidal Selat Madura. *Jurnal Riset Biologi dan Aplikasinya* 2: 36-41. DOI: 10.26740/jrba.v2n1.p36-41. [Indonesian]
- Ramadhaniaty M, Setyobudiandi I, Madduppa HH. 2018. Morphogenetic and population structure of two species marine bivalve (Ostreidae: *Saccostrea cucullata* and *Crassostrea iredalei*) in Aceh, Indonesia. *Biodiversitas* 19 (3): 978-988. DOI: 10.13057/biodiv/d190329.
- Rozas J, Ferrer-Mata A, Sánchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sánchez-Gracia A. 2017. DnaSP 6: DNA sequence polymorphism analysis of large data sets. *Mol Biol Evol* 34: 3299-3302. DOI: 10.1093/molbev/msx248.
- Saito M, Kojima S, Endo K. 2000. Mitochondrial COI sequences of brachiopods: genetic code shared with protostomes and limits of utility for phylogenetic reconstruction. *Mol Phylogenet Evol* 15 (3): 331-344. DOI: 10.1006/mpev.2000.0773.
- Saleky D, Dailami M. 2021. Konservasi genetik ikan kakap putih (*Lates calcarifer*, Bloch, 1790) melalui pendekatan DNA barcoding dan analisis filogenetik di sungai Kumbe Merauke Papua. *Jurnal Kelautan Tropis* 24 (2): 141-150. DOI: 10.14710/jkt.v24i2.10760. [Indonesian]
- Samanta SCA. 2014. New record of a primitive brachiopod benthic fauna from the North-East coast of India. *Intl J Curr Res Acad Rev* 2: 70-73.
- Samanta SCA. 2015. Eco-biology of a precambrian intertidal benthic brachiopod, *Lingula anatina* from the confluence of Subarnarekha estuary with Bay of Bengal, India. *J Mar Biol Assoc India* 57: 41-46. DOI: 10.6024/jmbai.2015.57.1.1836-06.
- Schreiber HA, Bitner MA, Carlson SJ. 2013. Morphological analysis of phylogenetic relationships among extant rhynchonellide brachiopods. *J Paleontol* 8: 550-569. DOI: 10.1666/12-115.
- Setiawan IC, Haditir A Y, Ikhwan MU, Nufus ZA, Syukri MU, Ismail NA, Rizal SY. 2020. Modeling of M2-TIDE in the western waters of Aceh, Indonesia. *J Sustain Sci Manag* 15: 122-135. DOI: 10.46754/jssm.2020.12.011.
- Silitonga AS, Masjuki HH, Ong HC, Sebayang AH, Dharma S, Kusumo F, Siswanto J, Milano J, Daud, K, Mahlia TMI, Chen WH. 2018. Evaluation of the engine performance and exhaust emissions of biodiesel-bioethanol-diesel blends using kernel-based extreme learning machine. *Energy*, 159: 1075-1087.
- Sloan DB, Havird JC, Sharbrough J. 2017. The on-again, off-again relationship between mitochondrial genomes and species boundaries. *Mol Ecol* 26: 2212-2236. DOI: 10.1111/mec.13959.
- Surinati D, Wijaya JHM. 2017. Arus Selatan Jawa. *Oseana* 42: 1-8. DOI: 10.14203/oseana.2017.Vol.42No.3.78. [Indonesian]
- Tajima F, Nei M. 1984. Estimasi jarak evolusi antara sekuen nukleotida. *Biologi molekuler dan evolusi*, 1: 269-285.
- Tamura K, Stecher D, Peterson A, Filipski, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol Biol Evol* 30: 2725-2729. DOI: 10.1093/molbev/mst197.
- Temereva EN, Gebruk AA, Malakhov VV. 2015. Demonstration of the preoral coelom in the brachiopod *Lingula anatina* with consideration of its phylogenetic significance. *Zoologischer Anzeiger-A J Comp Zool* 256: 22-27. DOI: 10.1016/j.jcz.2015.03.002.
- Walker SE, Lorsch J. Sanger dideoxy sequencing of DNA. *Methods in Enzymology* 2. Academic Press.
- Wu L, Sheng G, Lai X, Hou X, Yuan. 2010. Geographical pattern analysis of the brachiopod *Lingula anatina* based on mitochondrial COI gene sequences. *Geol Sci Technol Inform* 29 (1): 17-22.
- Yang S, Lai X, Sheng G, Wang S. 2013. Deep genetic divergence within a "living fossil" brachiopod *Lingula anatina*. *J Paleontol* 87: 902-908. DOI: 10.1666/12-127.