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Morphometric Analysis and Molecular Identification of the Asian Moon Scallops (*Amusium pleuronectes*) in the Waters of Bone Bay and the Makassar Strait

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ABSTRACT

Identification of organism species can be done by morphometric analysis and molecular identification such as DNA sequencing. Morphometric analysis provide an overview of the habitat conditions of Asian moon scallops (*Amusium pleuronectes*) while molecular identification strengthen the morphometric analysis. This study provided the first report of molecular identification and morphometric analysis of Asian moon scallops in Bone Bay and the Makassar Strait waters. Genetic test samples were taken from adductor tissue which were then preserved using alcohol at the next stage. Morphometric analysis was carried out by analyzing the relationship between shell length (cm) and shell weight (g) using a regression test. DNA sequencing results were analyzed using MEGA 10 software, which included BLAST, Haplotype, genetic distance analysis, and phylogenetic tree. The results of morphometric analysis showed that *A. peluronectes* in the waters of Bone Bay and Makassar Bay had a negative allometric growth pattern (b<3) and a coefficient of determination (R) <60%, meaning that there was a close relationship between length and weight. The same growth pattern was influenced by habitat conditions such as temperature, substrate, and depth at both sampling locations which tended to be the same. Analysis of the genetic distance of Asian moon scallops obtained in the waters of Bone Bay and the Makassar Strait ranged from 0.001 to 0.006, indicating a low genetic distance. This finding concludes the phylogenetic tree analysis demonstrated that the genetic variations in Asian moon scallops were closely related.

INTRODUCTION

Pectinidae family are generally called scallops and has more than 400 species worlwide. Scallops are spread throughout the world's waters, from subtropical waters to tropical waters **(Shumway & Parsons, 2006)**. Asian moon scallop has wide distribution across the Indian Sea, South China Sea, Indo-China, Japan, the Philippines, Papua New Guinea, Indonesia, and Australia **(Poutiers, 1988 in Carpenter & Niem, 2002)**. *Amusium pleuronectes* belongs to the Pectinoidea superfamily which local people often call Simping scallops or red and white clams. The habitat of these clams can be found on various substrates from sand to sandy mud at a depth of 5-50m **(Widowati *et al.*, 2002)**.

South Sulawesi Province is a province located in the southern part of Sulawesi Island. This province borders Central Sulawesi to the north, to the east with Bone Bay and Southeast Sulawesi, to the west it borders the Makassar Strait and West Sulawesi. The waters of Bone Bay and the Makasar Strait are waters that have a great diversity of marine animals such as shellfish. This area needs to be preserved to maintain sources of biodiversity. Asian moon scallops are one of the varieties that must be protected in the waters of Bone Bay and the Makassar Strait because these shellfish have potential and economic value so many fishermen catch them. The increasing number of shellfish fishermen will threaten the the Asian moon scallops population and causes the decrease value of this commodity in the water **(Karyanti & Khairiyah, 2019)**.

Species identification is an effort to answer biological issues, including diversity, biosecurity, and species protection **(Dayrat, 2005; Frézal & Leblois, 2008; Ni *et al.*, 2012; Mamat *et al.*, 2021)**. Identification of organism species in an area can be done in several ways, one of which is by carrying out morphometric analysis. According to **Agboola and Anetkhai (2008)**, morphometric analysis such as the relationship between length and weight can provide information about mollusk biology which is related to the habitat conditions where the molluscs live. **Harding (2007)** and **Farias-Tafalla *et al.* (2015)** stated that the shape of mollusk shells depends on population density and the ratio of length and width of the mollusk, as well as environmental conditions such as substrate, temperature, and salinity. Thus, indirectly, knowledge related to morphometrics can also provide an overview of habitat conditions which is important information for future cultivation activities to maintain the sustainability of the species.

Aside from morphometric analysis, species identification can also be accomplished through molecular identification. Molecular identification is used to reinforce the results of morphometric and morphological analysis. In this case, the basis of taxonomic identification according to **Pathwardhan *et al.* (2014)** often provides inaccurate conclusions. Molecular identification has been widely used bsinceecause this method can identify species accurately and precisely **(Simbolon *et al.*, 2021)**. One of the latest molecular marker methods used for species identification is mitochondrial DNA cytochrome oxidase I (mtCOI) (DNA barcoding) because this marker is non-recombinant and does not depend on environmental conditions **(Palanisamy *et al.*, 2020)**, therefore DNA barcoding has become the standard in molecular identification **(Hebert *et al.*, 2003)**. DNA barcoding is widely used to confirm the taxonomy of marine animals, even to the molecular phylogeny of molluscs **(Barco *et al.*, 2016)**. Apart from that, this method is also used to prove the diversity of mollusk species in waters **(Sun *et al.*, 2016)**. The use of DNA barcoding in molecular species identification has been widely used in research **(Liu & Zhang, 2018)**.

Studies regarding molecular identification and morphometric analysis of Asian moon scallops have not yet been widely carried out, especially in Bone and Makassar Regencies. This is the first research to provide information regarding molecular identification and morphometric analysis of Asian moon scallops in Bone Bay and the Makassar Strait waters. Several parameters were observed such as shell length, shell width, meat weight, and shell thickness. To confirm these, the identification was carried out molecularly. The growth pattern and habitat condition were also analyzed.

MATERIALS AND METHODS

1. Sampling location

The sampling locations were at Bone Bay (04°39.512´S; 120°29.939´E; 04°39.634´S; 120°30.095´E; 04°39.990´S; 120°29.554´E) and Makassar Strait (04°46.38´S; 119°29´02.67"E: 04°45.25´S; 119°28´18.54"E; 04°46.990´S; 119°28´29.39"E). These sampling locations were selected based on catching scallops activities in these waters.

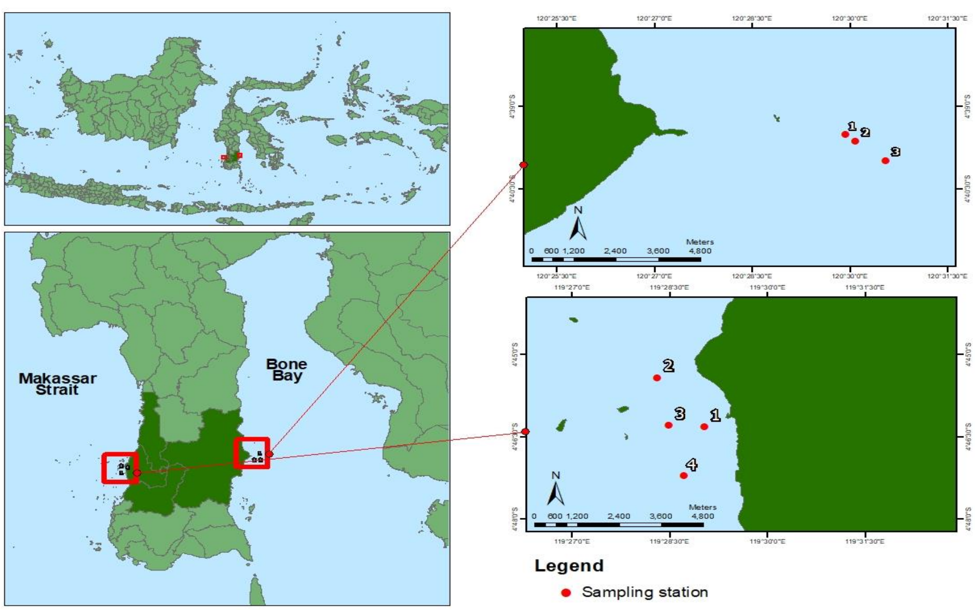

Fig. 1. Map of research locations and sampling stations

2. Sample collection

This sample was taken using a purposive sampling method. Sampling was carried out with the help of fishermen. The Asian moon scallop samples taken were dependent on the amount caught by the fishermen at that location. Sampling was carried out every month, but researchers did not necessarily get samples every month. Therefore, data were taken based on the number of samples at the two locations, namely in January, July, September, and October. Samples used for molecular analysis were taken from the adductor tissue and treated with 96% alcohol preservative. These samples were then molecularly tested at the Public Health Laboratory, Hasanuddin University.

3. Morphometric measurements

The shell length (SL), shell width (SW), and shell thickness (ST) of the specimens were measured with a digital caliper to an accuracy of 0.1mm. Next, the sample was weighed for total weight (W), and total weight (TW) (g). The length-weight relationship (LWR) was estimated using the equation:

$$W = a \text{ x } L \text{ x } b$$

Where, W: Weight (g); L: Length (mm)); “a” is the coefficient and “b” is the slope and exponent indicating isometric growth when b = 3. Conversely, b > 3 indicates positive allometric growth and b < 3 indicates negative allometric growth. The t-test was used to evaluate the type of growth and correlation test was used to determine relationship between the morphometric aspect measured.

4. DNA identification

A total of 30 samples were used for DNA identification consisting of the processes of DNA extraction, DNA amplification, electrophoresis, and DNA sequencing which were carried out following the Public Health Laboratory procedures at Hasanuddin University, Makassar. The primers used were Mt DNA *A. pleuronectes* F 5’ GCAAATGCTTCCATGGGCAA 3’ and Mt DNA *A. Pleuronectes* R 5’ GCAGGAGGAAGGACCCAAAA 3’. Confirmation of sequencing results and alignment of DNA sequences were carried out using the Clustal W program from the Molecular Evolutionary Genetic Analysis 6 (MEGA 6) software **(Tamura *et al.*, 2013)**. Species identification was carried out using Basic Local Alignment Search Tools (BLAST) to determine the match of the target DNA sequence with the DNA sequence in the database (GenBank). Identification was continued with phylogenetic tree analysis and genetic distance analysis based on Kimura-2-parameters using MEGA 10 software. Phylogenetic trees were built based on the UPGMA method of the Pdistance evolution model and 1000x bootstrap replication using Mega 10 software **(Tamura *et al.*, 2013).**

RESULTS AND DISCUSSION

The results of SL, ST, SW, and TW measurements varied every month. This indicates that the size of the shells during the four months of sampling varied, namely small, medium, and large sizes. In September, the average total weight of Asian moon scallops in the waters of Bone Bay and Makassar Bay tended to be higher than in other months. This is thought to be because in September most of the Asian moon scallops experienced the TKG VI period so the gonads in their bodies were large and this influenced the increase in body weight of the Asian moon scallops. According to **Nursalim *et al.* (2012)**, Asian moon scallops spawn in November, so their gonads mature in September.

Fig. 2. Dimension of morphometric measurements: **A.** shell length (SL), **B.** shell width (SW), **C.** shell thickness (ST) and total weight (TW)

Table 1. Morphometric measurements of Asian moon scallops in the Waters of Bone Bay in January, July, September, and October. SL (Shell length), ST (Shell thickness), SW (Shell width), TW (Total weight)

Morphometric	Month	Mean	Min	Max	SD
SL (cm)	January	59.63	52.00	69.00	04.02
ST (cm)		58.89	50.00	67.32	04.08
SW (cm)		11.04	08.00	16.00	01.28
TW (g)		14.33	08.21	21.87	03.12
SL (cm)	July	66.22	26.10	92.3	11.52
ST (cm)		64.87	24.90	89.35	11.22
SW (cm)		12.64	43.00	17.50	02.46
TW (g)		16.07	01.01	36.17	06.46
SL (cm)	September	64.40	29.00	92.50	20.51
ST (cm)		63.86	26.60	93.30	21.58
SW (cm)		10.01	00.10	20.60	07.00
TW (g)		26.02	2.07	53.17	17.44
SL (cm)	October	57.33	14,20	80.50	21.88
ST (cm)		52.98	13,00	83.70	22.96
SW (cm)		11.88	01,70	36.20	7.023
TW (g)		17.89	00,70	33.85	11.12

Based on the correlation test results for the relationship between shell length and total weight in the waters of the Makassar Strait, the following coefficients of determination (R^2) were obtained: $R^2 = 0.7611$, $R^2 = 0.6397$, $R^2 = 0.9782$, and $R^2 = 0.9675$, as shown in the ABCD image. The constant bbb obtained was b<3 (Fig. 3). The results of the t-test analysis indicated that Asian moon scallops in Bone waters exhibited negative allometric growth, meaning that length growth was faster than weight growth during each month of sampling. Similarly, in the waters of Makassar Bay, negative allometric growth was observed in samples from January, July, September, and October. Animals with negative allometric growth grow more slowly in weight than in size, causing them to appear thinner as they mature **(Farías-Tafolla *et al.*, 2015)**.

Table 2. Morphometric measurements of Asian moon scallops in Makassar Bay Waters in January, July, September, and October. SL (Shell length), ST (Shell thickness), SW (Shell width), TW (Total weight)

Morphometric	Month	Mean	Min	Max	SD
SL (cm)	January	65.95	05.81	85.95	10.15
ST (cm)		111.35	05.85	86.23	10.05
SW (cm)		11.89	04.22	17.11	02.03
TW (g)		21.34	04.22	50.21	08.80
SL (cm)	July	65.43	26.10	99.00	10.59
ST (cm)		63.88	24.90	90.50	10.03
SW (cm)		12.57	04.30	19.40	02.44
TW (g)		16.52	01.01	56.51	07.72
SL (cm)	September	66.03	31.40	85.95	09.43
ST (cm)		11.89	04.22	17.00	02.12
SW (cm)		63.36	05.85	86.00	11.81
TW (g)		21.21	04.98	50.21	09.10
SL (cm)	October	48.36	14.20	86.90	25.58
ST (cm)		47.80	13.00	88.90	26.93
SW (cm)		08.22	03.20	13.70	03.37
TW (g)		11.72	00.70	32.56	11.23

Based on the correlation results of the relationship between shell length and total weight in the waters of the Makassar Strait, the coefficients of determination were obtained successively in the ABCD image, namely R2 = 0.9554, R2 = 0.9271, R2 = 0.7429 and R2 = 0.9338 (Fig. 4). The growth of Asian moon scallops is influenced by several factors including habitat conditions, food available in these waters, and the substrate in the waters of Bone Bay and Makassar Bay. This is in line with research by **Frézal and Leblois (2008)** that shellfish growth is influenced by environmental conditions such as food availability, substrate, salinity, temperature, and current. **Preston and Roberts (2007)** and **Kovitvadhi (2008)** said that growth is influenced by biotic and abiotic factors. Morphometric characters are influenced by genetics, and environmental conditions, such as food supply and water temperature **(Pouvreau *et al.*, 2001; Abraham *et al.*, 2021; Silahi *et al.*, 2022)**.

Similar research results in 2005 in Brebes, Tegal, and Kendal waters found a positive allometric growth type **(Prasetya *et al.*, 2010)**. However, different research results from Kendal and Pekalongan waters in May 2001-2003 found a positive allometric growth type **(Prasetya *et al.*, 2010)**, while in Brebes waters in April 2009, May 3 and 24, 2008, the results obtained positive allometry, positive allometry, and negative allometry, respectively. The growth of scallops in April and early May showed that scallops experienced a faster total weight growth than the growth of their shell dimensions, but at the end of May, scallops experienced a faster shell dimension growth than the growth of their total weight. This is thought to be related to the reproductive cycle of scallops **(Suprijanto & Widowati, 2007)**. Morphometric characters are influenced by genetics, and environmental conditions, such as food supply and water temperature **(Pouvreau *et al.*, 2001; Abraham *et al.*, 2021; Silahi *et al.*, 2022)**. These environmental conditions will influence the increase in shell length and height which will be used to protect tissues and carry out movement. **Nursalim *et al.* (2012)** stated that bivalves are also included in the species *A. Pleuronectes* which has a faster growth in shell length because the shell formed acts as a reinforcement.

The results of observations of habitat conditions in the waters of Bone Bay and Makasar Bay tended to be the same. The results of temperature and substrate measurements were the same, namely 28-30°C with clay, clay loam, and silty clay substrates, pH ranged from 7.4-8.75 in the waters of Bone Bay and 7.2-8.70 in the waters of the Makassar Strait. Salinity in Bone and Makassar waters ranged from 29.0–30.10ppt and 29.5–32.04ppt, respectively. Asian moon scallops were found at depths of 15–25m in Bone and 16–17.2 m in Makassar. Table (3) shows that Asian moon scallops in Bone Bay were more dominantly found in deeper waters compared to those in Makassar Bay, indicating that the depth of their habitat can vary. **Widowati *et al*. (1999)** found that Asian moon scallops in Pekalongan were typically found at depths of 20–30m, while in Tegal, they were found at depths of 21–30 m **(Ernawati *et al.*, 2011)**. In general, **Hortle and Cropp (1987)** reported that Asian moon scallops are found at depths of 16–22m, with optimal growth occurring at depths of 16–20m.

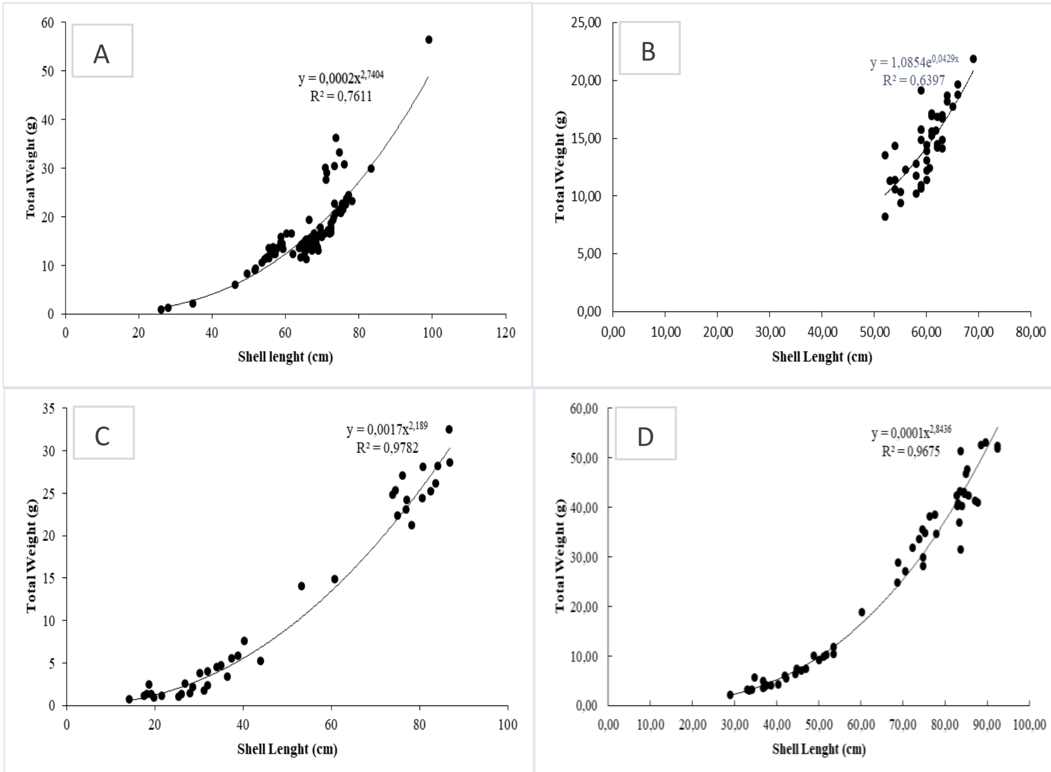

Fig. 3. Morphometry of *A. Pleuronectes* from Makassar Strait Waters. Relationship between shell length (SL) and total weight (TW) in **(A)** January, **(B)** July, **(C)** September and **(D)** October

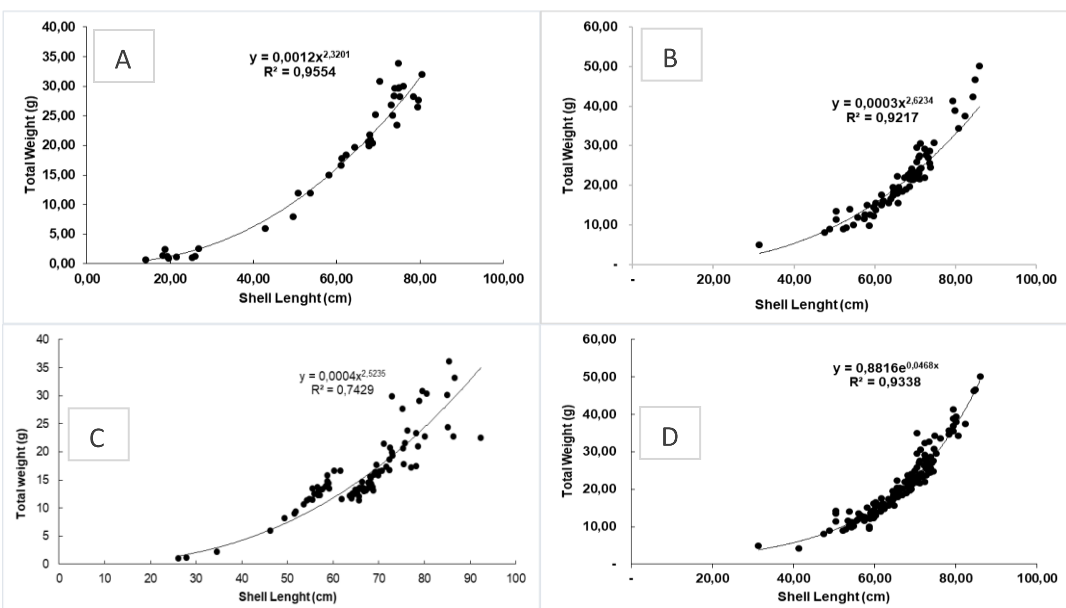

Fig. 4. Morphometry of *A. Pleuronectes* from Bone Bay Waters. Relationship between shell length (SL) and total weight (TW) in **(A)** January, **(B)** July, **(C)** September and **(D)** October

Table 3. Ecological conditions of Bone Bay and Makassar Bay Waters in January, July, September, and October

Parameter	Bone Bay Waters	Makassar Bay Waters
Temperature (°C)	28-30	28-30
pH	7.4-8.75	7.2-8.70
Salinity (ppt)	29.0-30.10	29.5-32.04
Dissolved Oxygen (mgL^{-1})	5.10-5.60	5.45-6.00
Depth (m)	15.0-25.0	16.0-17.2
Substrate	Clay, Clay Loam, Silty Clay	Clay, Clay Loam, Silty Clay

The depth of the water is related to the suitability of the temperature, where lower temperate occurred in the increases of depth. **Sahri *et al.* (2014)** stated that shellfish, including Asian moon scallops, tend to prefer lower temperatures (29-30°C). This revealed the suitability of the temperature parameters at sampling stations in Bone Bay and Makassar Bay to the temperature preferences of Asian moon scallops. Like other tropical species, Asian moon scallops will grow well in habitats with temperatures above 24°C **(Marine Cultured Center, Directorate General of Fisheries, 1994)**. **Dharmaraj *et al.* (2004)** stated that Asian moon scallops can grow optimally in a temperature range of 24.5-30°C. According to **Makmur *et al.* (2012)** and **Haryatik (2013)**, the normal temperature for growth of Asian moon scallops is around 28-34°C. While the optimum temperature range for gonad development of Asian moon scallops is around 20-30°C **(Shumway & Parsons, 2006)**.

Amusium sp. is known to like a variety of substrates, such as rocky, muddy, and even substrates consisting of a mixture of sand and shells **(Franklin *et al.*, 1980)**. At sampling stations in Bone Bay and Makassar Bay, *Amusium* was more commonly found in clayed substrates, clay loam, and silty clay, where at both locations the substrate conditions tended to be the same. Habitat conditions will greatly influence the distribution of an organism which is closely related to the animal's ability to adapt **(Sahri *et al.*, 2014)**. Normal water quality concentrations for shellfish growth are pH reaching 6.00-8.50, salinity reaching 25-35ppt, and dissolved oxygen reaching >5 mg/L **(Makmur *et al.*, 2012; Haryatik *et al.*, 2013)**.

Table 4. Results of molecular identification of *A. pleuronectes* from the Waters of Bone Bay and the Makassar Strait.

Code	Identity (%)	Accession Number	Species
Bone-1	99.57	NC_057147.1	*Amusium pleuronectes*
Bone-2	99.57	NC_057147.1	*Amusium pleuronectes*
Bone-3	99.47	NC_057147.1	*Amusium pleuronectes*
Bone-4	99.36	NC_057147.1	*Amusium pleuronectes*
Bone-5	99.37	NC_057147.1	*Amusium pleuronectes*
Bone-6	99.26	NC_057147.1	*Amusium pleuronectes*
Bone-7	99.15	NC_057147.1	*Amusium pleuronectes*
Bone-8	99.46	NC_057147.1	*Amusium pleuronectes*
Bone-9	99.50	NC_057147.1	*Amusium pleuronectes*
Bone-10	99.37	NC_057147.1	*Amusium pleuronectes*
Mks-1	99.04	NC_057147.1	*Amusium pleuronectes*
Mks-2	99.37	NC_057147.1	*Amusium pleuronectes*
Mks-3	99.57	NC_057147.1	*Amusium pleuronectes*
Mks-4	99.04	NC_057147.1	*Amusium pleuronectes*
Mks-5	99.26	NC_057147.1	*Amusium pleuronectes*
Mks-6	99.47	NC_057147.1	*Amusium pleuronectes*
Mks-7	99.15	NC_057147.1	*Amusium pleuronectes*
Mks-8	99.47	NC_057147.1	*Amusium pleuronectes*
Mks-9	99.26	NC_057147.1	*Amusium pleuronectes*
Mks-10	99.15	NC_057147.1	*Amusium pleuronectes*
Mks-11	99.04	NC_057147.1	*Amusium pleuronectes*
Mks-12	99.36	NC_057147.1	*Amusium pleuronectes*
Mks-13	99.26	NC_057147.1	*Amusium pleuronectes*

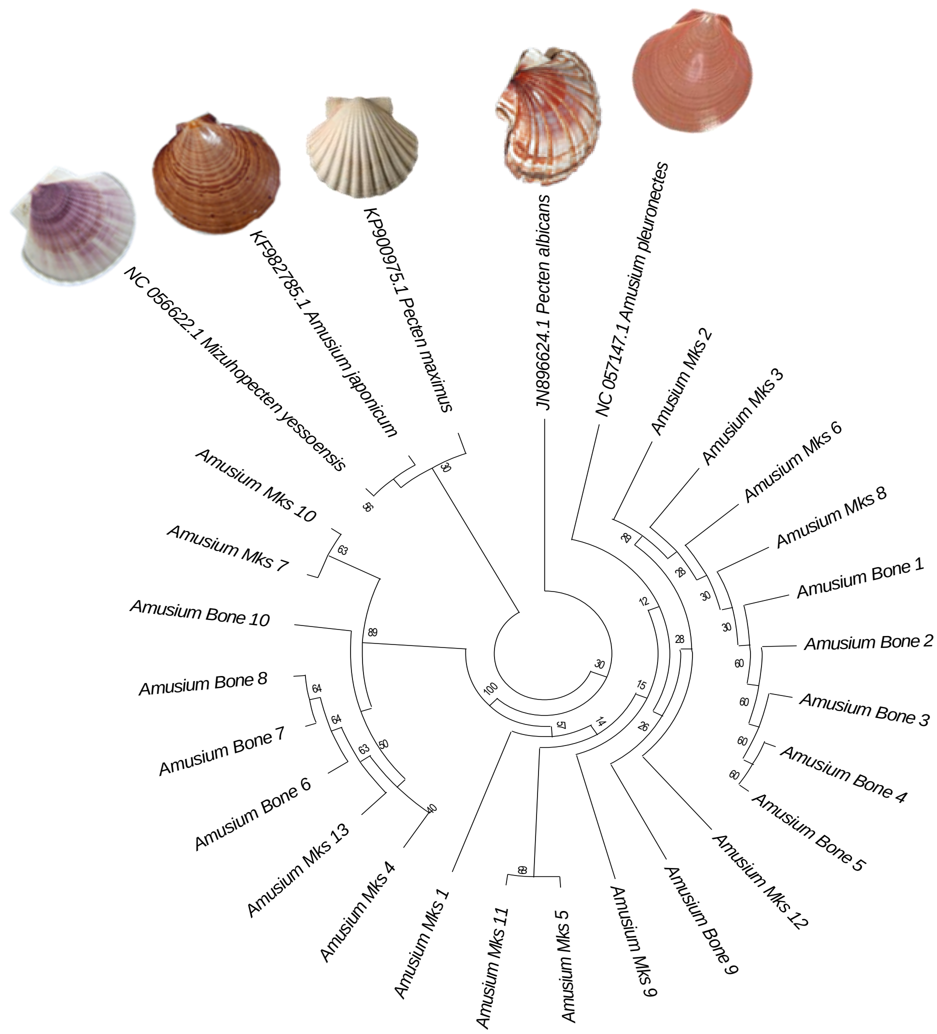

Fig. 5. Phylogenetic tree of Asian moon scallops taken from the waters of Bone Bay and the Makassar Strait

The results of scallops identification using the BLAST method in both Bone and Makassar waters resulted 99% indent percentage, showed that all samples belonged to the species *A. pleuronectes*. The average haplotype diversity result was 0.5714 and nucleotide diversity was 0.06684 in Bone Waters. Meanwhile, haplotype diversity in the waters of the Makassar Strait was 0.13333, and nucleotide diversity was 0.00034.

The results of calculating haplotype diversity and nucleotide diversity in Bone Bay waters tended to be higher compared to research by **Mahidol *et al.* (2007)**, where Asian Moon Scallops had a mean nucleotide diversity of 0.0006 and mean haplotype diversity of 0.237 observed in each population were considerably lower. However, in the waters of the Makassar Strait, these two parameters tended to be low **(Mahidol *et al.*, 2007)**. The results of the BLAST (Basic Local Alignment System Tool) analysis in Table (4) show that the species identified were indeed the *A. Pleuronectes* species. This proves that morphometric analysis and molecular identification showed the same identification results in all samples.

The genetic distance of Asian moon scallops samples obtained in the waters of Bone Bay and the Makassar Strait ranged from 0.001 to 0.006. This indicates low genetic distance. According to **Nei (1972)**, a genetic distance of 0.010-0.099 is included in the low category. The results of the same research on clam shellfish showed very low results, namely 0.0000-0.0103. Genetic distance shows the closeness of the relationship **(Triandiza *et al.*, 2022)**. The lower the value of the genetic distance obtained, the closer the relationship between the populations. This showed the same results as BLAST identification where all Asian moon scallops identified were *A. Pleuronectes* species, so the genetic distance was low or very related. According to **Hebert *et al*. (2003)**, organisms are declared to be different species if the genetic distance based on the COI gene sequence is more than 2%.

This phylogenetic tree was analyzed using the neighbor-joining and maximum-likelihood methods. These two trees function to strengthen the tree formed from this analysis **(Mamat *et al.*, 2021)**. Based on the results of phylogenetic tree analysis, the genetic variations in Asian moon scallops were closely related to each other. The phylogenetic results were divided into 5 groups (Fig. 5), namely the large group *A. Pleuronectes* with a genetic distance of 100, and 4 other groups of the *Pecten albicans*, *Pecten maximus*, *Mizuhopecten yessoensis*, and *Amusium japonicum* species. However, overall samples taken from the waters of Bone Bay and the Makassar Strait showed that *A. Pleuronectes* scallop type was closely related.

CONCLUSION

Morphometric analysis of Asian moon scallops in January, July, September and October in the waters of Bone Bay and Makasar Strait showed a negative allometric growth pattern. Factors that influenced the growth of Asian moon scallops were depth, temperature, substrate, salinity, pH and dissolved oxygen. The results of the analysis of habitat conditions in the waters of Bone Bay and the Makassar Strait tended to be the same. Morphometric analysis and molecular identification showed the same identification results in all samples. The results of the genetic distance analysis showed a low distance, which is in line with the results of the phylogenetic tree analysis. Thus, all samples taken in the two waters were closely related to each other.

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