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Morphological variation in three populations of the pill ark cockle, *Anadara pilula* (Mollusca: Bivalve) of Java, Indonesia

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Abstract. *Anadara pilula* is a species in the family Arcidae, which is important economically and used widely as a food source. The cockles have a wide distribution, especially on the north coast of Java Island, Indonesia, and can be found off the coasts of Labuan, Cirebon and Gresik. Differences in the characteristics of the coastal habitat in northern Java Island has led to morphological differences in *A. pilula*. This study aimed to assess differences in morphological characteristics of *A. pilula* from three locations on the northern coast of Java Island. Shell dimensions of 252 individuals from the three regions were measured. Simple regression analysis of shell length versus shell depth showed a difference in slope between the Gresik and Cirebon populations. One-way ANOVA and discriminant analysis were performed to compare and describe morphological differences among locations. The results showed that three morphometric characteristic significantly differed among the three populations (umbo height of right valve, symmetry of right valve and symmetry of left valve). *A. pilula* from the Gresik coast differred the most in morphometric characters.
Key Words: *Anadara pilula,* Cirebon coast, Gresik coast, Labuan coast, morphometric variation.

Introduction. Indo-Pacific region is a region with the world's highest diversity of bivalves. Southeast Asia, including Indonesia is one of the main parts of the Indo-Pacific region (Hutomo & Moosa 2005). Based on recent data, there are 38 known species of the subfamily Anadarinae in this region (Lutaenko 2011), one of them is *Anadara pilula*. Distribution of this species had been recorded in Indo-Pacific regions such as Philippines (Lozouet & Plaziat 2008; Dolorosa & Dangan-Galon 2014), Thailand (Sanpanich 2011), New Hebrids Island, (Solem 1959), Australia (Li & Morrison 2011), Hongkong (Cheung & Wang 2008), Vietnam (Evseev & Lutaenko 1998), China (Feng et al 2011) and Indonesia (Mudjiono & Kastoro 1997; Satrioajie et al 2013; Akhrianti et al 2014). *A. pilula* or pill ark cockle has fairly extensive distribution in Indonesia, it was found in Tegal coast (Satrioajie et al 2013), Panimbang (Mudjiono & Kastoro 1997), as well as in the coast of East Belitung (Akhrianti et al 2014). In their habitat, *A. pilula* is often found in association with other cockles, such as blood cockle (*A. granosa*), *A. indica* and *A. inequivalvis*. *A. pilula* plays an important role as a deposit and filter feeder (Mudjiono & Kastoro 1997) controling the growth of phytoplankton. Additionally, *Anadara* cockles also act as a natural prey of gastropods *Natica maculosa*, and *Thais carinifera* (Broom 1982), *Thais forbesi*, shorebirds, and stingrays (Okera 1976).

A. pilula is one species of the family Arcidae which has an important economic value in Indonesia, as a protein source. Though it has a great potential and benefits, the study of *A. pilula* is still very limited, in Indonesia as well as in the world. Several studies have been conducted on *A. pilula*, for examples its distribution and abundance in Gulf Miskam Panimbang, Indonesia (Mudjiono & Kastoro 1997), DNA barcoding (Feng et al 2011), morphometric characteristics and growth (Satrioajie et al 2013), heavy metal accumulation (Cheung & Wang 2008) and is found in the study of bivalves distribution conducted in East Belitung, Indonesia (Akhrianti et al 2014), Illawarra lagoon, Australia

(Li & Morrison 2011), Iwahig River-Estuary, Philippines (Dolorosa & Dangan-Galon 2014), Vietnam (Evseev & Lutaenko 1998), Gulf of Thailand (Sanpanich 2011) and New Hebrides Island (Solem 1959).

Morphometrics variation is determined by biotic (Levitan 1988; Preston & Roberts 2007) and abiotic environment variability (Laudien et al 2003; Tarnowska et al 2009; Mariani et al 2002; Soares et al 1999), genetic factors (Kawecki & Ebert 2004), and also interaction of environment and genetic factors (Pigllucci 1996; Soares et al 1999). Cockles experience meroplankton in the larval stages, thus allowing exchange of genotypes between regions due to ocean currents. In addition, cockles will adapt to the environment in which they lived. Several previous studies indicated a difference in morphometric characters in bivalve from several different areas, such as the research conducted by Luttikhuizen et al (2003) and Tarnowska et al (2009). Luttikhuizen et al (2003) suggested variations of morphological characters of *Malcoma balthica* shells in the Wadden Sea and the North Sea were caused by the presence of selective predation. Meanwhile, Tarnowska et al (2009) who conducted a study of cockles *Cerastoderma glaucum* in some regions of Europe stated the smallest size shells of *C. glaucum* found in the region with the lowest salinity due to osmotic stress.

Differences in habitat characters along north coastal of Java Island suspected to lead adaptation of *A. pilula* through mechanisms of physiology, morphology, and behavior. Genetic differences and morphological adaptation lead to difference in shell morphological characters among cockles originating from different regions in the north coast of Java Island. This study aim at assessing shell morphological characters of *A. pilula* population in three locations in north coast of Java Island.

Material and Method

Sampling location. *A. pilula* individuals were sampled using rake in intertidal areas at Labuan, Cirebon and Gresik (Indonesia) in the 2014 and 2015 (Figure 1). Shells from 252 individuals originating from these regions were measured. Nine morphometric characteristics were examined, including shell length, shell depth, ligament length, height of right and left valves, umbo height of right and left valves, and symmetry of right and left valves (Figure 2). Shell dimensions were measured using digital calipers to the nearest 0.01 mm.

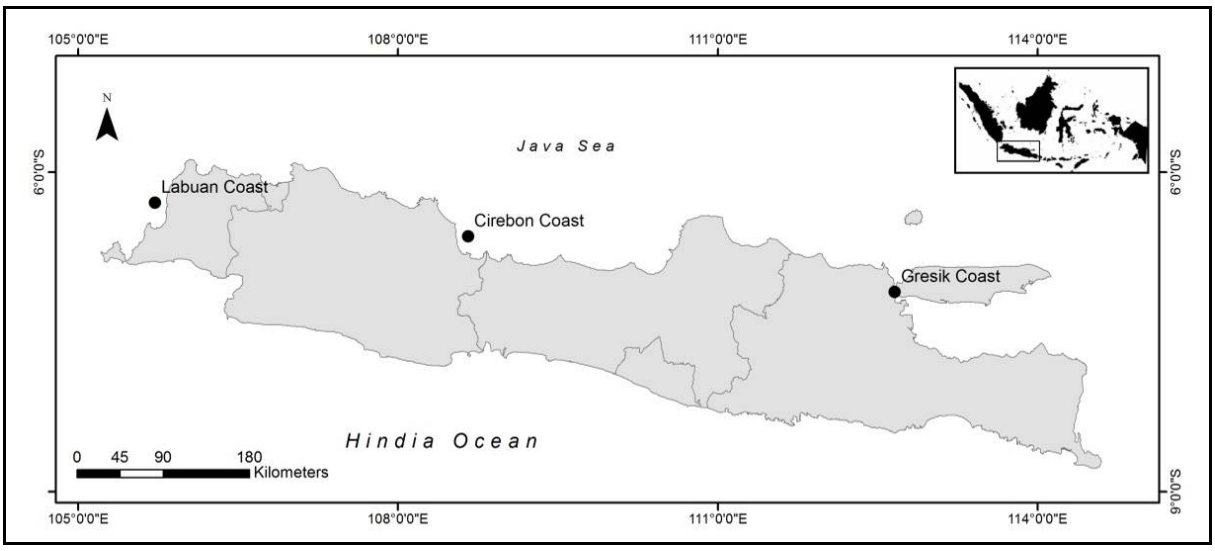

Figure 1. Sampling locations of *Anadara pilula* on the north coast of Java Island (Map source: Badan Informasi Geografis 2014).

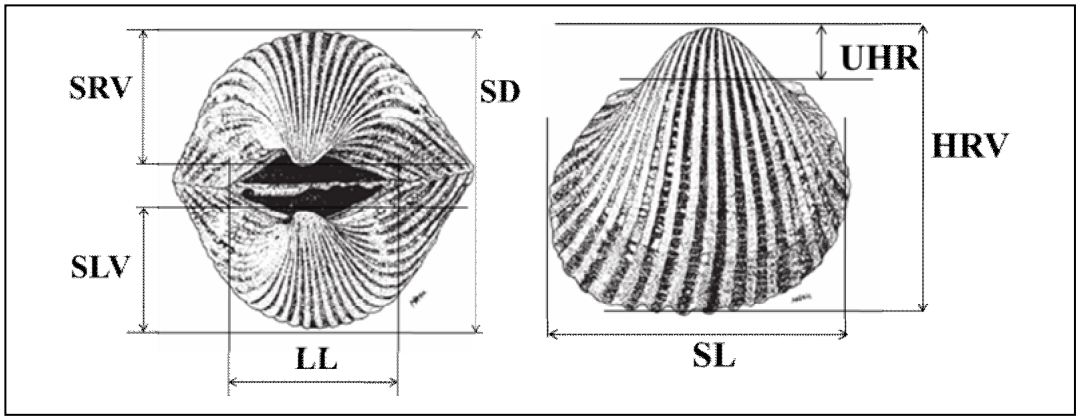

Figure 2. Measurements of morphological characteristics of *Anadara pilula* (SL: shell length, SD: shell depth, LL: ligament length, HRV: height of right valve, UHR: umbo height of right valve, SRV: symmetry of right valve, SLV: symmetry of left valve) (Picture source: Poutiers 1998).

Statistical analysis. All morphology data were corrected for length to make comparisons among sites. Morphological data among sites were compared using one-way ANOVA (analysis of variance). The post hoc Games-Howel test was used to determine statistical differences between two sites.

Simple regression analysis was performed between shell length and shell depth and between shell length and shell height for each cockle population. The slope and intercept between two regressions were identified by analysis of covariance (ANCOVA) to determine the difference between two regressions.

Discriminant analysis was performed to display separation among the three populations of *A. pilula.* The classification functions within discriminant analysis were used to assess how accurately individual cockles grouped into different populations. Cluster analysis was used to display a dendrogram of populations based on the similarity of morphological characteristics.

Results and Discussion

Morphological inter-site comparisons. Results of morphometric ratio comparisons of *A. pilula* from the three regions (Labuan coast, Cirebon coast, Gresik coast) using one-way ANOVA showed that three characteristics were significantly different among the sites ($p < 0.05$): umbo height of the right valve, symmetry of the right valve and symmetry of the left valve (Table 1). The post hoc Games-Howell test was conducted to determine the region with significantly different morphometric characteristics.

Based on the post hoc Games-Howell test, there was significant difference in ratio of the umbo height of the right valve to shell length between *A. pilula* populations from Labuan and Cirebon. The ratios of symmetry of the right and left valves to shell length showed similar results, with significant differences among all three populations of *A. pilula* ($p < 0.05$).

Results from the simple regression analysis of morphometric characteristics are shown in Table 2. Simple regression analysis conducted on shell length versus shell depth showed a significant difference in slope between Cirebon and Gresik *A. pilula* populations ($p < 0.05$) (Figure 3a). The intersection point between the regression lines for the Cirebon and Gresik populations occurred at a shell length of 24.4 mm and shell depth of 20.2 mm (24.4, 20.2). *A. pilula* from Gresik with a length shorter than 24.4 mm have greater shell depth than that of *A. pilula* from Cirebon of the same length, but with shell lengths longer than 24.4 mm, individuals from Gresik are flatter than those from Cirebon of the same length. In contrast, *A. pilula* populations from Cirebon and Labuan show no differences in slope and intercept ($p > 0.05$), indicating that shell depth does not significantly differ between individuals of the same length. Comparison between Labuan and Gresik populations showed significant differences in intercept values, with Gresik individuals having greater shell depth than Labuan individuals of the same length ($p < 0.05$).

Regression analysis of shell length versus shell height showed no difference in slope among populations, but there was a difference between population intercepts between Cirebon and Gresik and between Labuan and Gresik populations. Shell height for Cirebon versus Gresik and Labuan versus Gresik populations differed significantly, and the Gresik population consistently had a greater shell height than the others ($p<0.05$) (Figure 3b). In contrast, the Cirebon-Labuan cockle population showed no difference in shell height ($p>0.05$).

Table 1

Morphometric characteristic comparison of *Anadara pilula* (mean±SE) among Cirebon, Labuan and Gresik populations

Characters	*Cirebon coast (n = 106)*	*Labuan coast (n = 101)*	*Gresik coast (n = 47)*
SD/SL	0.8343±0.0037	0.8336±0.0031	0.8464±0.0053
LL/SL	0.7367±0.0026	0.7354±0.0033	0.7367±0.0039
HLV/SL	0.9554±0.0028	0.9517±0.0029	0.9563±0.0040
HRV/SL	0.9145±0.0028	0.9110±0.0026	0.9222±0.0040
UHL/SL	0.1674±0.0015	0.1645±0.0015	0.1620±0.0022
UHR/SL	0.1606±0.0017^{a}	0.1529±0.0016^{b}	0.1595±0.0026^{ab}
SRV/SL	0.3055±0.0021^{a}	0.3203±0.0021^{b}	0.2854±0.0030^{c}
SLV/SL	0.2920±0.0021^{a}	0.3007±0.0019^{b}	0.2767±0.0030^{c}

^{a,b,c} within the same row, values with different superscript are significantly different ($p < 0.05$). SL: shell length, SD: shell depth, LL: ligament length, HLV: height of left valve, HRV: height of right valve, UHR: umbo height of right valve, UHL: umbo height of left valve, SRV: symmetry of right valve, SLV: symmetry of left valve.

Table 2

Results of simple regression analysis and ANCOVA of shell length versus shell depth and shell length versus shell height

Location	*y=a+bx*	R^2	*N*	*ANCOVA result*	
				Slope	*Intercept*
	shell length-shell depth				
Labuan Cirebon	SL = 1.47+0.76SD ($p<0.001$) SL = 0.81+0.79SD ($p<0.001$)	0.84 0.86	100 106	parallel ($p>0.05$)	$a_1 = a_2$
Labuan Gresik	SL = 1.47+0.76SD ($p<0.001$) SL = 4.33+0.65SD ($p<0.001$)	0.84 0.73	100 46	parallel ($p>0.05$)	$a_1 \neq a_2$
Cirebon Gresik	SL = 0.81+0.79SD ($p<0.001$) SL = 4.33+0.65SD ($p<0.001$)	0.86 0.73	106 46	non-parallel ($p<0.05$)	-
	shell length-shell height				
Labuan Cirebon	SL = 2.27+0.84SH ($p<0.001$) SL = 2.32+0.85SH ($p<0.001$)	0.89 0.93	100 106	parallel ($p>0.05$)	$a_1 = a_2$
Labuan Gresik	SL = 2.27+0.84SH ($p<0.001$) SL = 2.92+0.82SH ($p<0.001$)	0.89 0.87	100 46	parallel ($p>0.05$)	$a_1 \neq a_2$
Cirebon Gresik	SL = 2.32+0.85SH ($p<0.001$) SL = 2.92+0.82SH ($p<0.001$)	0.93 0.87	106 46	parallel ($p>0.05$)	$a_1 \neq a_2$

SL: shell length.

The three populations of *A. pilula* on the north coast of Java Island showed differences in morphometric characteristics among sites. Three morphometric characteristics (umbo height of the right valve, symmetry of the right valve and symmetry of the left valve) significantly differed among regions. Differences in morphometric characteristics in *A. pilula* populations may be due to genetic (Benton et al 1994) and environmental influences (Mariani et al 2002; Laudien et al 2003; Pigllucci 2005; Guarneri et al 2014). Several environmental factors that affect morphometric characteristics of bivalves include temperature (Laudien et al 2003), salinity (Eisma 1965), type of sediment (Newell & Hidu

1982), tide level (Griffiths 1981; De Montaudoin 1996) and presence of predators (Dalziel & Boulding 2005).

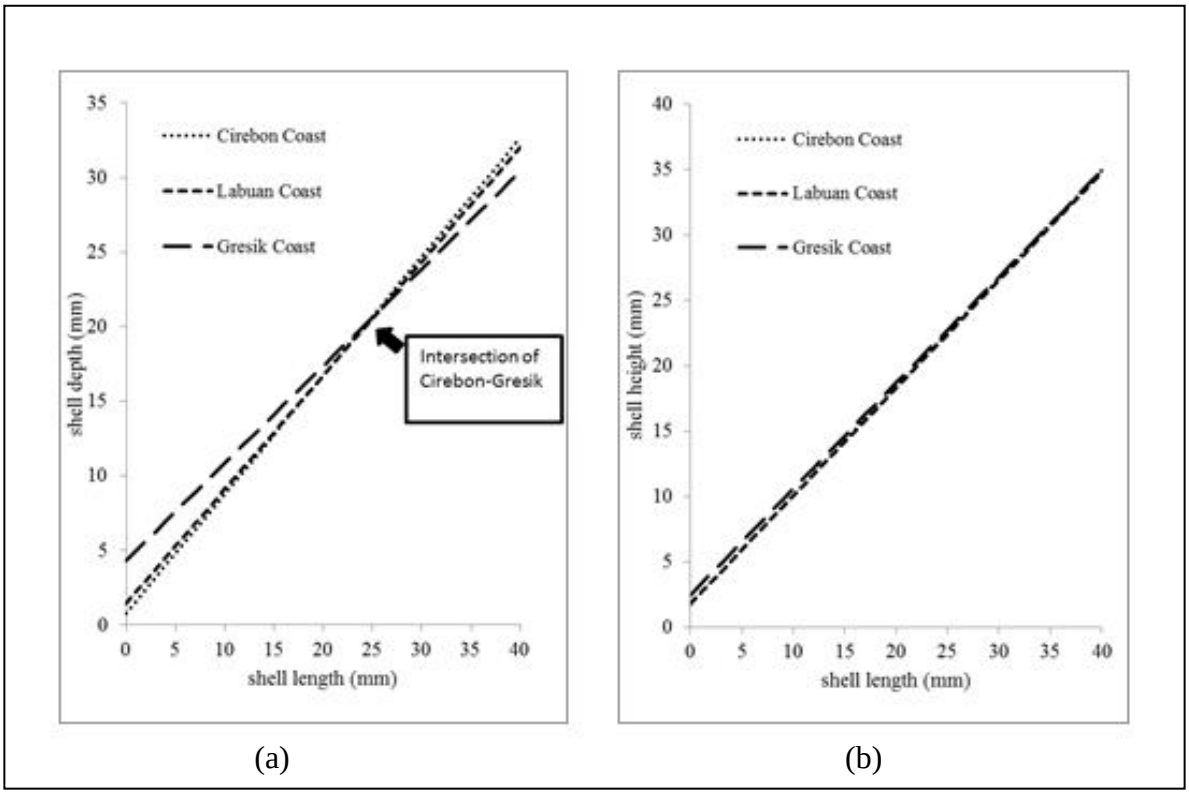

Figure 3. Regression analysis of *Anadara pilula* morphometric characteristics. (a) Shell length versus shell depth regression analysis with respect to region of origin, and (b) shell length versus shell height regression analysis with respect to region of origin.

Based on simple linear regression analysis of shell length versus shell depth and shell length versus shell height, we showed that shell length growth occurs at a faster rate than those of shell height and shell depth. ANCOVA showed that the regression slopes of shell length and shell depth between the Cirebon and Gresik populations were significantly different ($p < 0.05$). The intersection point of the two lines was 24.4 mm for shell length and 20.2 mm for shell depth, indicating that above this size, individuals of the Cirebon population will have a greater shell depth than those of the Gresik population of the same length. Cockles of the same length showed no differences in shell height and shell depth between Cirebon and Labuan ($p > 0.05$). Differences in growth rate and growth pattern between the different populations may be caused by various external factors. Some examples of these external factors are length of the immersion period due to tidal levels (Griffiths 1981), population density, which may lead to intraspecies competition (Jensen 1993) and parasitic disturbance (Miura & Chiba 2007). In addition, growth is also strongly influenced by internal factors (Paynter & Mallonee 1990; Meyer & Manahan 2010).

Discriminant analysis and morphometrics dendrogram. Discriminant analysis was performed to determine the overall morphometric differences among populations and to classify individuals based on morphometric shell characteristics. Discriminant functions from eight ratios of morphometric characteristics showed significant results ($p < 0.05$, with a classification success of 69% in distinguishing among the three populations of *A. pilula* (Figure 4). *A. pilula* individuals from Labuan were appropriately grouped at a rate of 79%, while those from Gresik and Cirebon were appropriately grouped at rates of 57% and 76%, respectively.

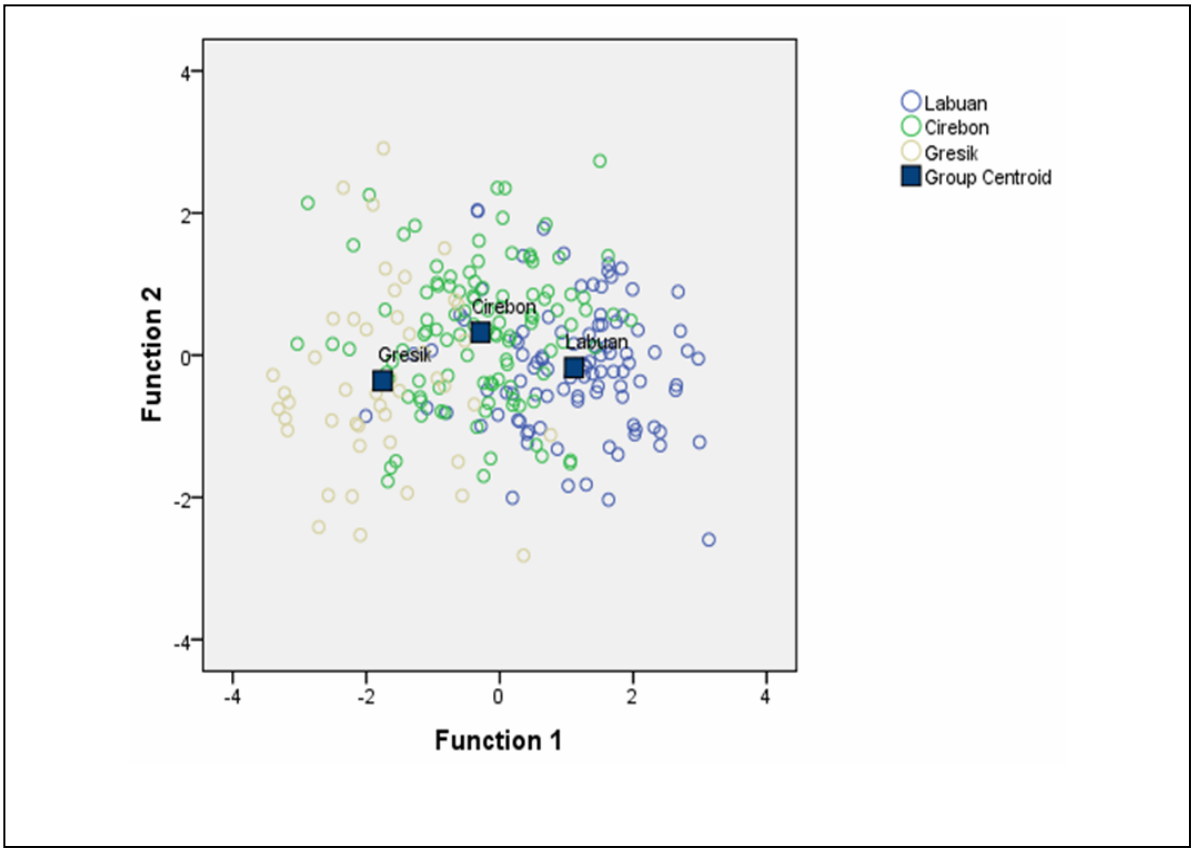

Figure 4. Distinction of morphological characteristics among *Anadara pilula* collected from three regions.

The dendrogram formed using the morphometric characteristics ratio data showed that *A. pilula* from Cirebon had the highest similarity to cockles from Labuan, while those from Gresik differed the most in morphometric characteristics (Figure 5). Cluster analysis revealed similar results to discriminant analysis.

A. pilula from Gresik differred the most in morphometric characteristics of the populations examined. This is based on the morphometric dendrogram and on discriminant analysis, in which the centroid of the Gresik population was the greatest distance from the others. Differences in morphometric characteristics of *A. pilula* populations may be due to differences in environment, such as temperature (Laudien et al 2003), salinity (Tarnowska et al 2009; Mariani et al 2002), tidal level (De Montaudoin 1996) and predation (Luttikhuizen et al 2003). Laudien et al (2003) showed differences in morphometric characteristics in the clam *Donax serra* with respect to temperature, in that clams in cooler waters had flatter shells compared with those from warmer waters. The research of De Montaudoin (1996), conducted in Arachon Bay, showed that the period of immersion due to tidal level was positively correlated with average shell length in the cockle population (*Cerastoderma edule*). In addition, the type of sediment also affects shell shape in benthic organisms (Newell & Hidu 1982).

Other external factors, such as density of individuals, can also affect the morphometric characteristics of marine animals. Irie (2006) found that juveniles of the gastropod *Cypraea annulus* living at high densities had smaller size and lower callus thickness. Eisma (1965) found that temperature and salinity can affect the shape and size of shells. This was demonstrated by the small shell size of a *C. glaucum* population living in the Baltic Sea, which has a low average salinity (Tarnowska et al 2009). Shell shape changes were also seen in a *C. edule* population from Scotland, which exhibited a more spherical shape due to low salinity (Mariani et al 2002).

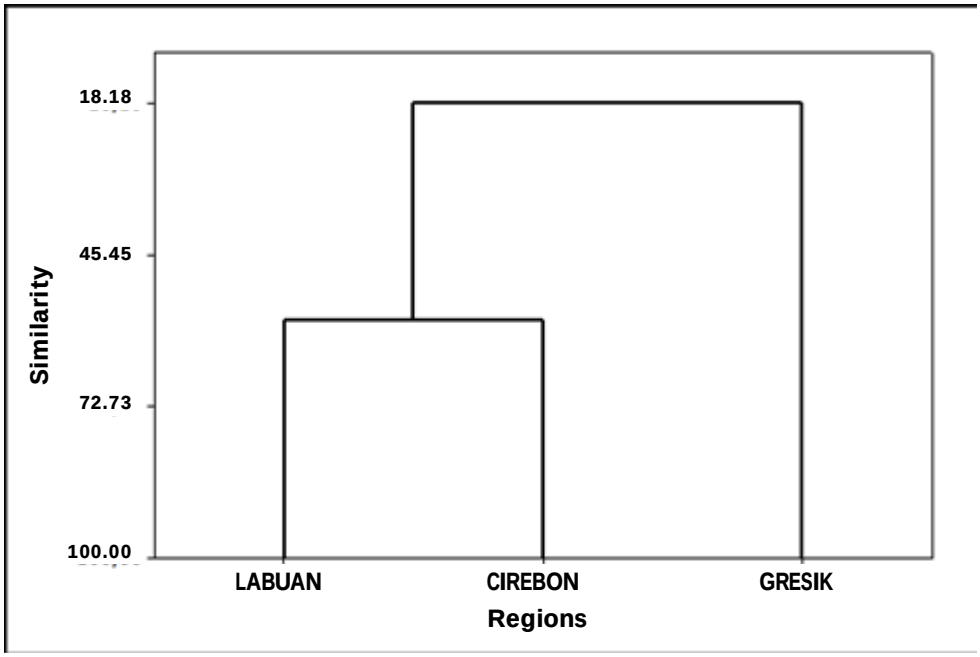

Figure 5. Dendrogram of morphometric characteristics of *Anadara pilula* populations from Labuan, Cirebon and Gresik.

Geographically, Gresik is the furthest distance from the other sites on the eastern side of Java Island, while Labuan and Cirebon are located in the west. Additionally, Gresik is located on a relatively narrow strait (Madura Strait) minimizing its connection with other regions. Therefore, genotypic differences may be a source of the differences in shell morphometric characteristics in addition to differences in habitat condition. How environmental factors affect morphometric characteristics among these three locations requires further investigation. Although much evidence suggests that shell morphometric characteristics are strongly influenced by local environmental conditions, the influence of genetic factors cannot be excluded and have not yet been assessed.

Conclusions. There are differences in morphometric characteristics among *A. pilula* from Labuan, Cirebon and Gresik as indicated by umbo height of the right valve, symmetry of the right valve and symmetry of the left valve, which significantly differed among the populations. *A. pilula* from Gresik differed the most in morphometric characteristics. Differences in environmental factors among sites are suspected to be the cause of variation in morphometric characteristics. Along with environmental factors, genetic factors may also affect shell shape in *A. pilula*.

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