Phenetic Relationship Study of Gold Ring Cowry, *Cypraea Annulus* (Gastropods: Cypraeidae) in Mollucas Islands Based on Shell Morphological

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Abstract

This study aims to construct taxonomic character of *Cypraea annulus* based on shell morphological; analyzed the developmental stages of the snail shell and investigated the similarities and phenotypic distances of snails with numerical taxonomic approaches. This research lasted four years on island of Larat and Ambon. The sample used was 2926. Construction of morphological taxonomic characters using binary data types with 296 test characters and ordinal types with 173 test characters; and 32 specimens of operational taxonomic units. The data is processed and analyzed on Lasboratory of Maritime and Marine Study Centre, University of Pattimura. The results of this study indicate that the development stages of *C. annulus* shell: juvenile stage (11.00-14.99 mm), sub adult (15.00-18.99 mm), adult (19.00-22.99 mm) and post adult (>23.00 mm). The distribution of similarity coefficient and phenetic distance values between 32 OTU *C. annulus* has a morphological phenetic similarity in the range of values above 95% and the phenetic distance in the range of less than 1.0. *C. annulus* numerical taxonomic based on morphological taxonomic character with 296 characters of test at binary data type, obtained two taxa phenotypes at a taxonomic phenon similarity level of 94%; Whereas with 173 character test of ordinal data type, obtained three taxa phenotype at taxonomic correlation level 0.970.

Keywords: Numerical taxonomy; *Cypraea annulus*; Taxonomic character; Phenotypic; Phenogram

Introduction

The diversity of marine life is very high and is estimated to amount to 2.2 million species and only about 0.8 million species have been identified [1]. A very large and varied number is certainly very interesting, but also difficult to learn. World marine biodiversity recorder sites or WoRMS reported that only 240,016 species are registered, 224,719 species have been verified and 441,796 are named synonyms [2]. The limited number of taxonomists is a major obstacle to identifying the large number of marine biota [3-5].

The taxonomic and classification of organisms is fundamental and important in human life and science [6,7]. Biologists have studied anatomical features and forms and used it for the analysis of organism forms for centuries [8-10], and classified the organism primarily by its forms. Davis et al. [11-13] state that the goal of taxonomy is to provide an easy method for identification and communication, to detect the evolutionary process and interpret the results and to provide a classification system for expressing kinship relationships among organisms. Without knowledge of taxonomy and classification, scientists and practitioners will find it difficult to identify and compare one organism with another in marriage or hybridisation.

Essentially, the taxonomy of marine biota is the process of analyzing the characters that exist in the individual, connecting the available characters [14-16] creating similarities and inequalities between a large number of individuals. Agombar et al. [17] state that it is not easy to find and measure similarities or resemblance in great diversity. However, in any biota with such great diversity, there must be some similarity in character and property albeit small and little. This similarity or uniformity is used taxonomists as the basis for classification. The creation of clear taxon boundaries becomes especially important when faced with a high diversity of marine biota, not only in quantity and species but also in ecosystem and climatic conditions, as in the tropics.

The factor of the lack of taxonomic character material being examined and the extent of the distribution of biota, also result in not all variations of taxonomic characters can be recorded from existing samples, so that many species produced will be reduced again in the future [18,19]. Without a background knowledge of the characteristics of marine biota, it is difficult to obtain critical and sufficient knowledge in limitation of taxon, its distribution area, its variability and consequence of its name and its synonym. Another factor that is also involved is the factor of the subjectivity of researchers in the selection of characters that are considered important and the element of appreciation of a person towards characters also influences the work of taxonomy [20,21]. If the restriction of the species is submitted to the researchers, the results will vary greatly according to the researcher's taste. Therefore, Avise [22] proposes that in suppressing researchers subjectivity should be anticipated by the use of all sources of taxonomic characters information that exist either morphology, anatomy, physiology, behavior, ecology, geographical distrubusi, biochemical, microscopic, and even molecular [10,23] through quantitative or numerical measures such as meristics, morphometrics and geometric morformometrics.

A qualitative morphological character tendency is used in conventional taxonomic work, due to the morphological approach providing the fastest way of obtaining biodiversity biota information and can be used as a general reference system for expressing data from

other fields [24,25]. In addition, morphological data can be viewed easily and quickly compared to data from other sources such as physiological, biochemical, biomolecular, DNA barcoding and amino acids. Many species have invaluable taxonomic constraints due to the phenotypic bias, especially the general structure of their morphology, so the use of this character should only support only taxonomic characters [26]. Descriptive or qualitative conventional taxonomies are very limited [27,28] because they are based on the morphology of the body and they use few characters, high skills are required and they are so subjective that bias occurs when repetition by other taxonomists is made. Sokal et al. [29] view the morphological approach as very subjective because different taxonomists can make different classifications for the same living creature. Dunn et al. [26,28,30] conclude that to avoid or at least reduce the nature of this subjectivity, particularly in grouping organisms can be done by: using as many taxonomic traits as possible; descriptions and measurements of the characters should be as clear and precise as possible; comparing the taxonomic character as precisely as possible, as well as quantitative or numerical. This approach has led to a phenetic or numerical approach based on the concept that the kinship relationship between living things is based on the number of degrees of equations that exist.

Numerical taxonomy is a method of evaluating the taxonomic character of an organism numerically or quantitatively on the basis of similarity and dissimilarity between units of taxonomic characters so that it can be classified according to the taxon correctly [29]. According to Sokal et al. [26,29,31] the advantages and benefits of numerical taxonomy can unify more character data expressed by organisms which are previously difficult to employ conventional taxonomy. In additional, that data from hundreds or even thousands of characters can integrated with a more effective data processing system and can be repeated by the ready without any meaningful deviation, since the processing is done in standardized and automated manner with the help of computer software. The use of many standardized characters and methods in processing data and easily evaluating the results, numerical taxonomy can reduce the bias of the researcher's subjectivity and increase the objectivity in the biota taxonomy [26,29].

The snail ring, *C. annulus*, known as the gold ring cowrie, is a small Cypraea type and belongs to the Cypraeidae family [32,33]. *C. annulus* belongs to a true cowrie. According to Lorenz [34,36] there are two types of cowries, the true cowries of the Cypraeidae family and the allied cowries of the ovulidae family. *C. annulus* shells are in great demand and much hunted by coastal communities for use as a medium of exchange or currency, forecasts and ornaments in Africa and Asia region since about the 14th century BC. That's why *C. annulus* is very popularly also called as money cowrie and named *Monetaria annulus* [34,36].

Research on *C. annulus* has been done a lot: the physical description of size, shape, color [32-34,37]. Morphology of the shell and body [35-37,39] Aspects of anatomy [32,33,38] Aspects of physiology [32,33,39,40]. Aspects of ecology [32,33,38,41]; Aspects of behavior (Natarajan et al. [32,33,38,42,43]; Growth and development Hughes et al. [33,44]); Reproductive aspects [33,35,43-46,38] Aspects of population dynamics [33,34,47,48] Aspects of conservation [33,34,36,45] However, from all such information or publications, there are still limitations and disadvantages of non-metric or qualitative approaches; The specimen's taxonomy still comes from mature, intact individuals and very little information, especially information from Indonesian waters. On the other hand, there has been no information or publication about the taxonomic character of

morphology in detail and thorough way. Therefore, the construction of the taxonomic character of gastropod especially *C. annulus* based on morphological characters will give important and great contribution to the development of taxonomy of *C. annulus*, in particular and gastropod in general.

This research aims to construct the taxonomic character of gold ring cowry, *C. annulus*, based on shell morphology; analyze the size and stages of the development of gold ring cowrys and investigate the similarities and phenotypic distances of gold ring cowrys in Ambon and Larat Island with numerical taxonomic approaches. This research is useful in separating and comparing populations and sub-populations, as well as monitoring and evaluating in detail and thoroughly *C. annulus* Gold ring cowry to establish taxon that will be used as a key identification in the classification according to taxon correctly, and can explain the actual *C. annulus* taxonomic position.

Materials and Methods

Specimen collection

The research of gold ring cowry numerical taxonomy, *C. annulus*, based on morphological characters was conducted for four years or sampling period which was 2013, 2014, 2015 and 2016. *C. annulus* shell specimens were collected from waters of Larat Island, West Southeast Maluku and Ambon Island waters, Ambon City (Figure 1). The *C. annulus* specimen used as many as 2926 shells consisted of 2160 from Larat Island and 766 from Ambon island. Observations and measurements of morphological characters were performed at the Bio-Image Center of Marine and Marine Affairs of Pattimura University.
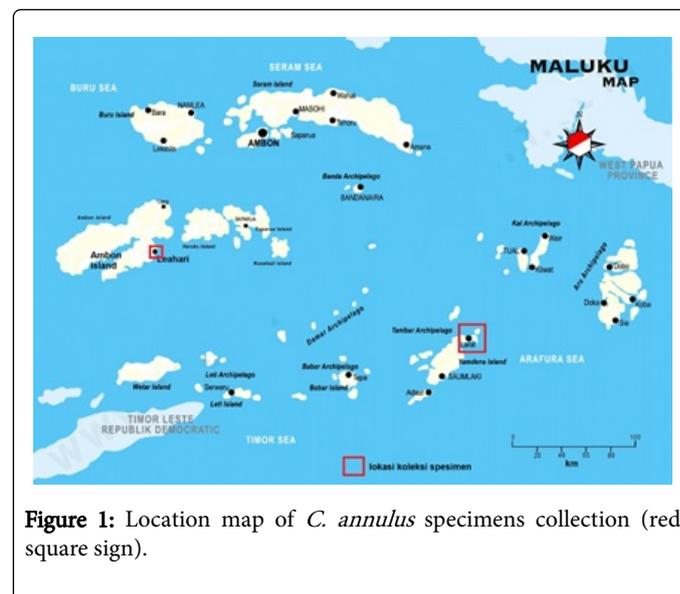

Figure 1: Location map of *C. annulus* specimens collection (red square sign).

This research used a total of 32 OTUs (Appendix 1) derived from intra-species or population or type of *C. annulus* strain based on: geographical location (Larat and Ambon Island), harvest periods or seasons for four seasons (2013, 2014, 2015 and 2016) and shell development stages such as: juvenile stage, sub adult, adult and post adult. The geographical location of the specimens (Ambon and Larat Island) is determined with the consideration that the number of samples is available in large quantities, available throughout the year, easy to obtain samples as well as long distances so it is assumed that there are distinct population characteristics or *C. annulus* strains and

not directly related to the environment and lineage. Determination of juvenile, adult, sub-adult and post adult shell development stages is based on two approaches: development stage of shell callus [49] and morphological stage of shell [32] Shown in Figures 2 and 3.
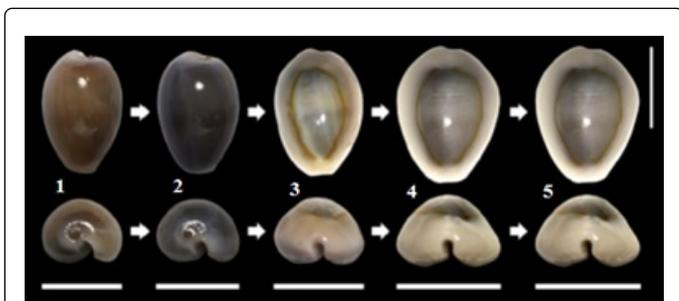

Figure 2: Types of developmental stages of the shell of *Monetaria annulus* based on callus development (Irie and Morimoto, 2016). Top: dorsal views; Down: anterior view. Horizontal bar line: shell width (W). 1. Metamorphosis of the shell. 2. Juvenile. 3. Sub adult. 4. Adult (callus growth ends); 5. Post-adult (cell growth stalled). Vertical bar line: 10 mm.
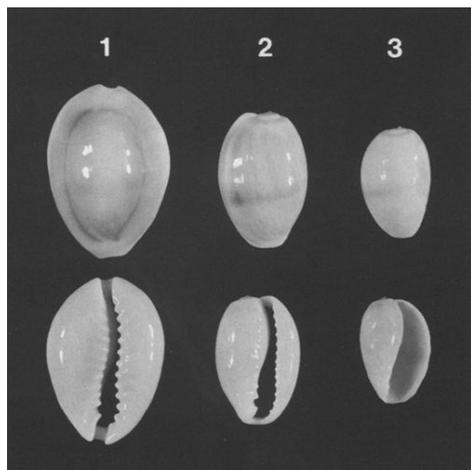
Figure 3: Stages of morphological development of the *C. annulus* shell (Katoh, 1989). Top: dorsal view, bottom: ventral view. 1: adult with ring on dorsal surface; 2: sub adult with primordial teeth; 3: juvenile with a large aperture.

Data Collection

Measurement of the size dimensions of the shell is done manually or directly using digital scales to measure shell or SM weight, whereas digital callipers are used to measure shell length or SL, shell width or SW, and shell height or SH with reference to schematics as shown in Figure 4. The shell length is measured from the anterior end to the posterior end of the shell; the width of the shell is measured from side to side on the widest whorl body; and the height of the shell is measured from the basal or ventral to the widest dorsal end.
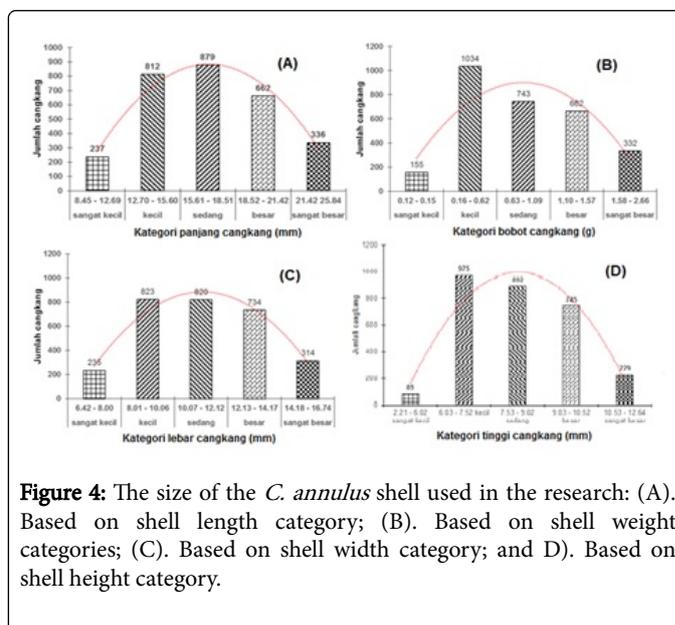
Figure 4: The size of the *C. annulus* shell used in the research: (A). Based on shell length category; (B). Based on shell weight categories; (C). Based on shell width category; and D). Based on shell height category.

The observed morphological taxonomic character refers to the overall structure and shape of characters that are the properties or features found in the *C. annulus* shell and/or compared with the structure and shape of characters in the gastropod class and the shell of the Cypraeidae family. The constructed shell morphology taxonomic characters are sourced from taxonomic books which have been used as reference for key identification of gastropods [50-57]. The structure and shape of shell morphology found and observed in this research consisted of the presence or absence of features, type and condition of characters owned by *C. annulus* shells, as compared with the overall morphology of the gastropod shells. Observations were made directly with the naked eye or eye touch and or using image processing software or Adobe Photoshop image ver. CS3 and Image J ver. 1.51i. The type and number of shell morphological test characters and their mention or character states constructed as taxonomic characters, are presented in appendices 2 and 3.

The coding of morphologic taxonomic characters was done by a binary or two state system for characters consisting of only two statements that were coded 1 or positive (+) and none was coded 0 or negative (-). Taxonomic morphological characters with more than two statements were coded or labeled 1, 2, 3, and so on. Each label containing a character was coded 1 while the other was coded 0. The use of "1" and "0" notations in n x t as an indication of the presence and absence of a property in a strain are used for the purposes of analysis using computer programs, since the computer program cannot recognize "+" and "-" notations. The construction of taxonomic morphological characters uses two approaches of the construction method based on the type of data they have: binary data type (two state characters) and ordinal or discrete data types (multi states characters).

Data Analysis

Standardization or weighting of characters with ordinal data types (multi-state) using formulas proposed by Sokal et al. [26,29].

where: SD is the standard deviation; Xij is character i for OTU j; is the mean character to i.

All character data and taxonomic units or OTUs are distanced in a hypothetical table of data sets in the form of next matrices, where n is the character and t is OTU to be calculated coefficients of similarity and dissimilarity between OTU pairs involving all test character data. Calculating coefficients of similarity of binary data types using Jaccard or SJ coefficients [26,29].

where: SJ is the Jaccard similarity coefficient; a is the number of positive, positive characters or "+ +"; b is the number of positive, negative characters or "+ -" and c is the number of negative-positive characters or "- +". Counting the coefficients of similarity for multistate and ordinal data or discrete data using spearman correlation coefficients [29].

where: ρxy is the spearman rank correlation coefficient; d is the difference between the shells of two variables; and N is the number of pair of observations.

The calculation of taxonomic character distance between specimens used Euclidean distance [58] with formula:

The coefficients of similarity and disimilarity which are resulted next then created a similarity matrix to show the similarity values of each OTU to one another. Furthermore, based on the similarity matrix, OTU grouping is done by the method of angglomerative hierarchical clustering (bottom-up). The clustering algorithm used in this research is UPGMA method and Ward method [59] where the union between OTU is based on the average value so that OTU fusion will occur. The identified OTU fusion results in a simple hierarchy in the form of phenon groups were displayed in phenogram form.

The OTU fusion results become phenon or taxa data in the form of phenogram, then the line at the level of the similarity coefficient and phenetic distance is drawn to obtain the number of taxon or taxa groups represented by OTU or specimens selected based on the similarity and taxonomic distance of all the characters tested. The taxa may be defined as form or sub strain, strain or subspecies, species, or genus and so on depending on the criterion of the selected and pre-determined specimen.

The similarity-level data on the phenogram is ardistanced in the phenogram evaluation matrix to perform the cophenetic correlation analysis with the initial similarity matrix. The result of matrix evaluation of phenogram and initial similarity matrix is used to determine Pearson correlation coefficient [60] with formula (2):

where: R is the value of the correlation coefficient; X is the initial similarity coefficient; And Y is the phenogram evaluation coefficient. The correlation coefficient value of R count is considered acceptable and accountable as the classification of taxa if R count ≥ 0.60 or 60% [29].

The data obtained in the research were processed and analyzed using multi-character or multivariate analysis based on the procedures proposed by Sokal et al. [26,29] such as factor analysis, PCA, clustering analysis and MDS. The assumption test is performed to determine whether all data have been met and feasible for multivariate analysis done with normality test; test of data homogeneity; test of sufficiency factor of Kaiser Meyer Oikin or KMO; freedom test between variables; and multicollinearity test. Meanwhile, to eliminate the influence of unit measurement of characters data transformation was performed in accordance with the needs of analysis and software used. Multi-character data analysis applied Microsoft Excell software ver. 2013, Xlstat release 2014, MVSP ver. 3.1 and Minitab ver.17.

Results

The size and stages of *C. annulus* shell development

The number of specimen used in this research were 2926 shells consisting of 2160 from Larat Island and 766 from Ambon island The results of the research for four -season periods found that *C. annulus* specimens in Ambon and Larat Island can be categorized into shell lengths: very small size (11.67-12.64 mm), small (12.65-15.57 mm), medium size (15.58-18.50 mm), large size (18.51-21.43 mm), and largest size (21.44-25.84 mm). Category of shell weight size of *C. annulus* specimens in Ambon and Larat Island is: very small size (0.14-0.16 g); small size (0.17-0.63 g); medium size (0.64-1.10 g); large size (1.11-1.57 g); and very large size (1.58-2.66 g). Shell width categories in Ambon and Larat Island consist of: very small size (4.22-7.97 mm); small size (7.98-10.04 mm); medium size (10.05-12.11 mm); large size (12.12-14.18 mm); and very large size (14.19-16.74 mm). The shell height size categories of the *C. annulus* specimen found in Ambon and Larat Island are composed of: very small size (2.21-6.02 mm); small size (6.03-7.52 mm); medium size (7.53-9.02 mm); large size (9.03-10.52 mm); and very large size (10.53-12.64 mm). In detail, the number of shells based on the weight, length, width and height of the shell presented in Figure 4.

Morphological observation of shell development stages based on criteria proposed by [32,50] obtained four stages of development namely juvenile, sub adult, adult and post adult. The length of the shell of *C. annulus* in Mollucas island based on developmental stages is categorized into four stages: juvenile stage (11.00 mm- 14.99 mm), sub adult (15.00 mm- 17.99 mm), adult (18.00- 22, 99 mm) and post adult (>23.00 mm). The results showed that the shell size of the different developmental stage in Ambon and Larat Island (Figure 5). Figure 5 shows that on the island of Larat have a shell size for each stage of development greater than the island of Ambon.

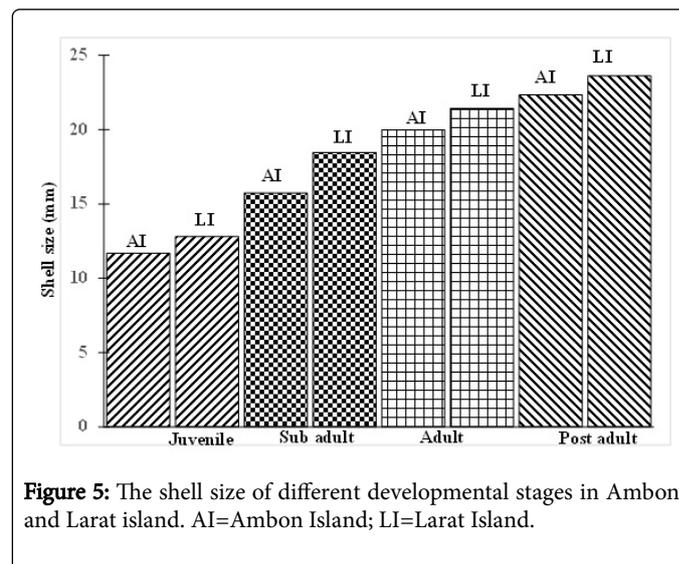**Figure 5:** The shell size of different developmental stages in Ambon and Larat island. AI=Ambon Island; LI=Larat Island.

Distribution of coefficient similarity value and phenetic distance between OTU

Figure 6 shows the distribution of similarity coefficient value and phenetic distances from the counting results of 32 *C. annulus* OTU pairs based on morphological characters of the shell with binary data

types and 296 test characters. In Figure 6A shows that coefficient similarity or resemblance value using jaccard coefficient obtained two distribution values that are in the distance 0.90 – 0.94 and 0.95-1.00 dominated by value of distribution 0.95-1.00; while the distribution of phenetic distance values between 32 OTU pairs based on Eucledian distances also obtained two distribution values that are in the distance of 0.00-1.00 and 4.00-4.99 dominated by the value of the distribution of 0.00 to 1.00 (Figure 6B). These results indicate that overall the similarity level of 32 OTU pairs for 296 morphological test characters is very high. However, there are still morphological phenetic distance on OTU pairs that are OTU pairs of 1, 2, 3 and 4 pair and OTU 5-32. This is interesting because with conventional taxonomic approach, the visual way is still difficult to distinguish or be considered the same, but with numerical taxonomic approach, this phenetic distance difference between OTUs can be shown.
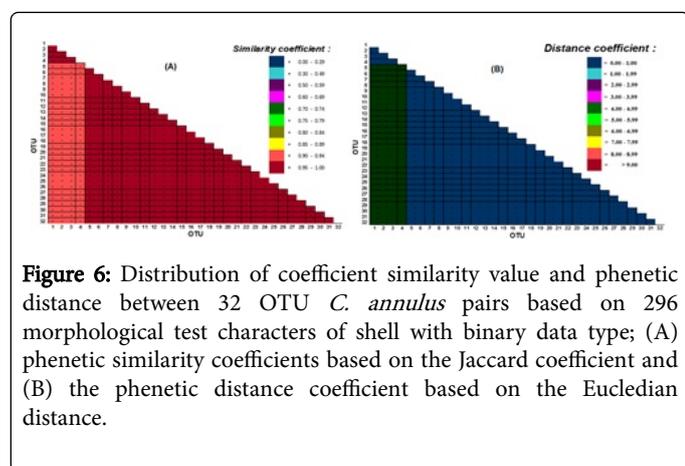
Figure 6: Distribution of coefficient similarity value and phenetic distance between 32 OTU *C. annulus* pairs based on 296 morphological test characters of shell with binary data type; (A) phenetic similarity coefficients based on the Jaccard coefficient and (B) the phenetic distance coefficient based on the Eucledian distance.

The distribution of coefficient similarity value and phenetic distance from the counting results to 32 OTU pairs based on the morphological character of the shell with ordinal data type and 173 test characters are shown in Figure 7A. Figure 7A shows that the distribution of coefficient similarity or resemblance value using Spearman correlation coefficient or ρ obtained only one distribution value that was in the distance of 0.95 to 1.00 dominated by the distribution value of 0.95 to 1.00. While the distribution of phenetic distance values between 32 OTU pairs based on Eucledian distances shows the distance of varied values dominated by the distribution value of 0.00 to 1.00 (Figure 7B).
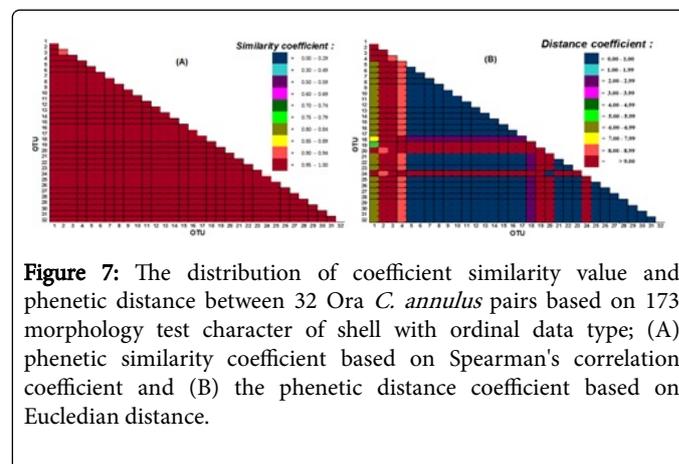
Figure 7: The distribution of coefficient similarity value and phenetic distance between 32 Ora *C. annulus* pairs based on 173 morphology test character of shell with ordinal data type; (A) phenetic similarity coefficient based on Spearman's correlation coefficient and (B) the phenetic distance coefficient based on Eucledian distance.

Phenogram similarity and taxonomic distance between OTU pairs

Binary data type (two state characters): Phenogram of similarity and taxonomic distance between 32 OTU pairs of *C. annulus* based on 296 characters of shell morphology, binary data types are presented in Figure 8. Figure 8A shows that the phenon level of similarity is 93%; All OTUs are incorporated in one taxa or cluster, while at phenon level which is 94%, the *C. annulus* phenotype is divided into two clusters or taxa whose first taxa group consisting of four OTUs or populations which is juvenile populations from 2013 to 2016 from Ambon island, while the second group consisting of 28 OTU or similar populations as presented in Table 1.

Phenon level from Figure 8						Phenon level from Figure 9			
Simmilarity 93%[a]	Simmilarity 94%[a]	distance 5,0[b]	distance 4,0[b]	correlation 0,970[a]	correlation 0,980[a]	correlation 0,990[a]	distance 10,0[b]	distance 5,0[b]	distance 2,0[b]
A3_ju	A3_ju	A3_ju	A3_ju	A3ju	A3ju	A3ju	A3ju	A3ju	A3ju
A4_ju	A4_ju	A4_ju	A4_ju	A6ju	A6ju	A4ju	A5ju	M5ju	M5ju
A5_ju	A5_ju	A5_ju	A5_ju	A3sa	A3sa	A5ju	A6ju	A4ju	A4ju
A6_ju	A6_ju	A6_ju	A6_ju	A4sa	A4sa	A6ju	A3sa	M6ju	A5ju
A3_sa	A3_sa	A3_sa	A3_sa	A5sa	A5sa	A3sa	A4sa	M6sa	A6ju
A4_sa	A4_sa	A4_sa	A4_sa	A6sa	A6sa	A4sa	A5sa	A5ju	A3sa
A5_sa	A5_sa	A5_sa	A5_sa	A3ad	A3ad	A5sa	A6sa	A6ju	A4sa
A6_sa	A6_sa	A6_sa	A6_sa	A4ad	A4ad	A6sa	A3ad	A3sa	A5sa
A3_ad	A3_ad	A3_ad	A3_ad	A5ad	A5ad	A3ad	A4ad	A4sa	A6sa
A4_ad	A4_ad	A4_ad	A4_ad	A6ad	A6ad	A4ad	A5ad	A5sa	A3ad
A5_ad	A5_ad	A5_ad	A5_ad	A3pa	A3pa	A5ad	A6ad	A6sa	A4ad

A6_ad	A6_ad	A6_ad	A6_ad	A4pa	A4pa	A6ad	A3pa	A3ad	A5ad
A3_pa	A3_pa	A3_pa	A3_pa	A5pa	A5pa	A3pa	A4pa	A4ad	A6ad
A4_pa	A4_pa	A4_pa	A4_pa	A6pa	A6pa	A4pa	A5pa	A5ad	A3pa
A5_pa	A5_pa	A5_pa	A5_pa	M3ju	M3ju	A5pa	A6pa	A6ad	A4pa
A6_pa	A6_pa	A6_pa	A6_pa	M4ju	M4ju	A6pa	M3ju	A3pa	A5pa
M3_ju	M3_ju	M3_ju	M3_ju	M5ju	M3sa	M3ju	M4ju	A4pa	A6pa
M4_ju	M4_ju	M4_ju	M4_ju	M3sa	M4sa	M4ju	M5ju	A5pa	M3ju
M5_ju	M5_ju	M5_ju	M5_ju	M4sa	M5sa	M3sa	M3sa	A6pa	M4ju
M6_ju	M6_ju	M6_ju	M6_ju	M5sa	M3ad	M4sa	M4sa	M3ju	M3sa
M3_sa	M3_sa	M3_sa	M3_sa	M3ad	M4ad	M5sa	M5sa	M4ju	M4sa
M4_sa	M4_sa	M4_sa	M4_sa	M4ad	M5ad	M3ad	M3ad	M3sa	M5sa
M5_sa	M5_sa	M5_sa	M5_sa	M5ad	M6ad	M4ad	M4ad	M4sa	M3ad
M6_sa	M6_sa	M6_sa	M6_sa	M6ad	M3pa	M5ad	M5ad	M5sa	M4ad
M3_ad	M3_ad	M3_ad	M3_ad	M3pa	M4pa	M6ad	M6ad	M3ad	M5ad
M4_ad	M4_ad	M4_ad	M4_ad	M4pa	M5pa	M3pa	M3pa	M4ad	M6ad
M5_ad	M5_ad	M5_ad	M5_ad	M5pa	M6pa	M4pa	M4pa	M5ad	M3pa
M6_ad	M6_ad	M6_ad	M6_ad	M6pa	A4ju	M5pa	M5pa	M6ad	M4pa
M3_pa	M3_pa	M3_pa	M3_pa	A4ju	M6ju	M6pa	M6pa	M3pa	M5pa
M4_pa	M4_pa	M4_pa	M4_pa	M6ju	M6sa	M5ju	A4ju	M4pa	M6pa
M5_pa	M5_pa	M5_pa	M5_pa	M6sa	A5ju	M6ju	M6ju	M5pa	M6ju
M6_pa	M6_pa	M6_pa	M6_pa	A5ju	M5ju	M6sa	M6sa	M6pa	M6sa
Notes: a=binary data type; b=ordinal data type									

Table 1: The taxa cluster formed at the phenon level in the similarity and distance phenogram.
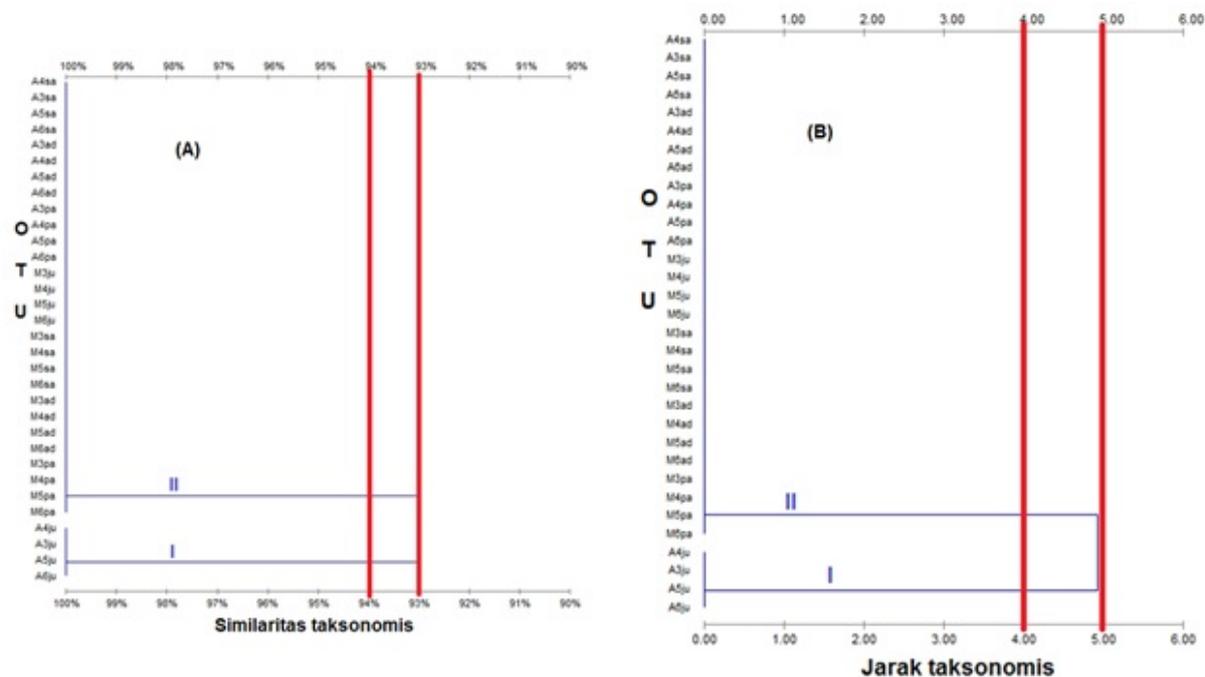
Figure 8: Phenogram of taxonomic similarity between 32 OTU *C. annulus* based on 296 morphological test characteristic of binary data type shell; (A) clustering with UPGMA method using Jaccard coefficient (Sj); (B) clustering with UPGMA method using Eucledian distance (Ed).

Figure 8A shows that the morphological characteristics of the *C. annulus* population in Ambon and Larat Island, have a degree of similarity of more than 90% which means still in one species. However, at the level of similarity above 94% in stages, the development of juvenile characteristic shells in Ambon Island in 2013, 2014, 2015 and 2016 have a phenotype that is somewhat different from other developmental stages from within Ambon island and among Ambon islands. The phenon level in similarity of 92% of all OTU specimens has the same phenotopic character. It can be argued that based on 296 morphological characters compiled and tested against 32 OTU specimens observed, the *C. annulus* phenotype of Ambon Island has a very high similarity to Larat Island, even as 100%.

The phenogram of the taxonomic distance between 32 OTUs based on 296 morphological characters of the shell tested with binary data type is still the same as the taxonomic similarity phenogram (Figure 8B). At the phenon level taxonomic distance of 5.0 also forms a cluster or phenotype group of *C. annulus* geographically both in Ambon and Larat Island, as well as by periods of years and stages of development of life. At the phenon level distance of 4,0, two phenotypes of *C. annulus* are formed; the first group with four forms or taxa and the second group with 28 forms or taxa, as presented in Table 1. As with the phenogram similarity, based on 296 morphological characters constructed and tested against 32 OTU specimens observed, the *C. annulus* phenotype of the island of Ambon is very close to Larat Island. The result of cophenetic correlation analysis of 98.3% shows r value which is greater than 60% [29] this means that the calculation results show valid or reliable results.

Cluster analysis of 296 morphological test characters of binary data type obtained three character classes owned by *C. annulus* shells both from Ambon and Larat Island: the first character class consists of 40 characters, the second class consists of 253 characters and the third class consists of three characters. The existence of the three classes of morphological characters of the shell is in each of the OTUs (Table 2). Table 2 shows that the first character class is all owned by first group or taxa, the third character class is owned by the second group or taxa, while the second character class is not owned by the *C. annulus* of the first or second group. All OTU specimens are included in the second character class, while the second and third character classes are only owned by some OTUs belonging to the juvenile and sub adult stages. The OTU specimens included in the second character class are the juvenile specimens from Ambon Island in 2014, the juvenile specimens from Larat Island in 2014, the juvenile specimens from Larat Island in 2016, and sub-adult specimens from Larat Island in 2016; whereas OTU specimens belonging to the third character class are juvenile specimens from Ambon Island in 2013, juvenile specimens from Ambon island in 2015, juvenile specimen from Larat Island in 2016 and sub-adult specimens from Larat Island in 2016. This indicates that the formation of the second and third character class is assumed because the morphological characters OTU-owned is still in the stage of growth and development which is not yet complete, while others have been completed. The OTU specimens included in the third morphological character classes are two specimens: the juvenile specimens of Larat Island in 2016 and sub-adult specimen from Larat Island in 2016.

OTU specimen	Morphological character class		
	I (40)	II (253)	III (3)
A3ju	-	+	+
A4ju	+	+	-

A5ju	-	+	+
A6ju	-	+	-
A3sa	-	+	-
A4sa	-	+	-
A5sa	-	+	-
A6sa	-	+	-
A3ad	-	+	-
A4ad	-	+	-
A5ad	-	+	-
A6ad	-	+	-
A3pa	-	+	-
A4pa	-	+	-
A5pa	-	+	-
A6pa	-	+	-
M3ju	-	+	-
M4ju	+	+	-
M5ju	-	+	-
M6ju	+	+	+
M3sa	-	+	-
M4sa	-	+	-
M5sa	-	+	-
M6sa	+	+	+
M3ad	-	+	-
M4ad	-	+	-
M5ad	-	+	-
M6ad	-	+	-
M3pa	-	+	-
M4pa	-	+	-
M5pa	-	+	-
M6pa	-	+	-

Notes: The value of "+" means included in the character class and "-" means not included in it; The number in brackets is the number of characters.

Table 2: The existence of a morphological character class on each OTU specimen based on binary data type.

The OTU specimen configuration map based on the morphological character class shows that 32 OTU specimens are at four points or positions (Figure 9). The juvenile specimens from Ambon island in 2013 are located closed to each other and are in the same quadrant as the juvenile specimens from Ambon island in 2015 so the phenotypes of them both can be said to be very similar. Similarly, the juvenile specimens from Larat Island in 2016 and sub-adult specimens from Larat Island in 2016 can be said to be very similar because they are located very close together even at one point and are in the same quadrant. Juvenile specimens from Ambon island in 2015 and juvenile specimens from Ambon island in 2013 are located far away and in different quadrants with juvenile specimens from Ambon island in 2014 and juvenile specimens from Larat Island in 2014 so it can be said that based on the character class, those two are slightly different when compared with juvenile specimens from Larat Island in 2016 and sub-adult specimens from Larat Island in 2016. Figure 8 also shows that morphologically there are six specimens located a bit far away from each other and in separate quadrants with other OTU groups such as juvenile specimens from Ambon island in 2013, juvenile specimens from Ambon island in 2015, juvenile specimens from Larat Island in 2016, sub-adult specimens from Larat Island in 2016, juvenile specimens from Ambon island in 2014 and juvenile specimens from Larat Island in 2014 so it can be said that these six specimens are quite different from other specimens.
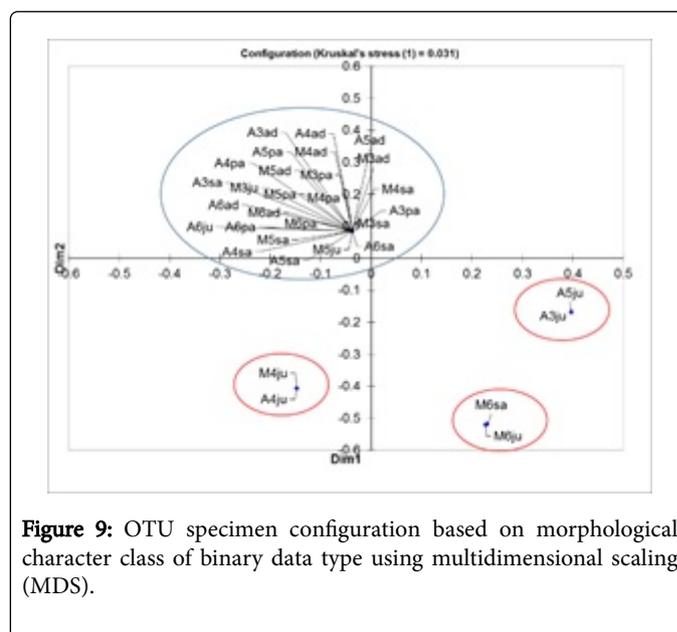
Figure 9: OTU specimen configuration based on morphological character class of binary data type using multidimensional scaling (MDS).

Ordinal data type (multi state characters): Phenogram of taxonomic correlations between 32 OTUs based on 173 shell morphologic characters with ordinal data type was presented in Figure 10A. The results show that phenon level of 0.970 correlation formed three groups or phenotype taxa where the first phenotype group consisted of 28 OTU, the second phenotype group consisted of three OTUs and the third phenotype group consisted of one OTU. The phenon level in the taxonomic correlation of 0.980 formed four *C. annulus* taxa; the first taxa consisting of 27 OTUs, the second taxa consisting of three OTUs, and the third and fourth clusters consisting of one OTU respectively. The phenon level of 0.990 correlation consists of seven clusters; first, second, third, fourth and sixth clusters consisting of one OTU respectably, fifth cluster with 25 OTU and seventh cluster consisting of two OTUs, as presented in Table 3. These results show that geographically, the shell's developmental stage and time period, the morphology of the *C. annulus* population in Ambon and Larat Island still has a degree of similarity of more than 96% which means that it is still in one taxa. However, for the level of similarity above 97% in stages, the development of juvenile characteristic shells in Ambon island in 2013, 2014, 2015 and 2016 have a phenotype that is somewhat

different from other developmental stages both within Ambon island and among Ambon islands. Based on 296 morphological characters compiled and tested against 32 OTU specimens observed, the *C. annulus* phenotype Ambon Island has a very high similarity to Larat Island.

Phenon level		
distance 10.0	distance 5.0	distance 2.0
A3ju	A3ju	A3ju
A5ju	M5ju	M5ju
A6ju	A4ju	A4ju
A3sa	M6ju	A5ju
A4sa	M6sa	A6ju
A5sa	A5ju	A3sa
A6sa	A6ju	A4sa
A3ad	A3sa	A5sa
A4ad	A4sa	A6sa
A5ad	A5sa	A3ad
A6ad	A6sa	A4ad
A3pa	A3ad	A5ad
A4pa	A4ad	A6ad
A5pa	A5ad	A3pa
A6pa	A6ad	A4pa

M3ju	A3pa	A5pa
M4ju	A4pa	A6pa
M5ju	A5pa	M3ju
M3sa	A6pa	M4ju
M4sa	M3ju	M3sa
M5sa	M4ju	M4sa
M3ad	M3sa	M5sa
M4ad	M4sa	M3ad
M5ad	M5sa	M4ad
M6ad	M3ad	M5ad
M3pa	M4ad	M6ad
M4pa	M5ad	M3pa
M5pa	M6ad	M4pa
M6pa	M3pa	M5pa
A4ju	M4pa	M6pa
M6ju	M5pa	M6ju
M6sa	M6pa	M6sa

Table 3: The cluster formed at the phenon level in the taxonomic distance phenogram of Figure 9B.

Figure 10: Phenogram correlation and taxonomic distance between 32 OTU *C. annulus* based on 173 morphological test of shell ordinal data type; (A) clustering with UPGMA method using Spearman correlation (ρ); (B) cluster with Ward method using Eucledian distance (Ed).

The phenogram of the taxonomic distance between 32 OTUs based on 173 morphological characters of the ordinal data type shell is shown in Figure 10B. At the phenon level taxonomic distance of 10.0 two clusters or phenotype group of *C. annulus* are formed where the first cluster consists of 29 OTU and the second cluster consists of three OTUs. At the phenon level distance of 5.0 four *C. annulus* phenotypic clusters were formed; the first cluster consisting of two OTUs, the second cluster having three OTUs, the third cluster consisting of two OTUs and the fourth cluster consisting of 25 OTUs. At phenon level distance of 2.0 there are five clusters or taxa; the first cluster consisting of two OTUs, the second cluster having one OTU, the third cluster having two OTUs, the fourth cluster consisting of 25 OTUs and the fifth cluster with two OTUs. In detail, the number and type of OTU at each phenon distance is presented in Table 4.

OTU specimen	Morphological character class		
	I (3)	II (169)	III (1)
A3ju	-	+	+
A4ju	+	+	-
A5ju	-	+	+
A6ju	-	+	-
A3sa	-	+	-
A4sa	-	+	-
A5sa	-	+	-
A6sa	-	+	-
A3ad	-	+	-
A4ad	-	+	-
A5ad	-	+	-
A6ad	-	+	-
A3pa	-	+	-
A4pa	-	+	-
A5pa	-	+	-
A6pa	-	+	-
M3ju	-	+	-
M4ju	+	+	-
M5ju	-	+	-
M6ju	+	+	+
M3sa	-	+	-
M4sa	-	+	-
M5sa	-	+	-
M6sa	+	+	+
M3ad	-	+	-
M4ad	-	+	-
M5ad	-	+	-
M6ad	-	+	-
M3pa	-	+	-
M4pa	-	+	-

M5pa	-	+	-
M6pa	-	+	-
Notes: The value of "+" means included in the character class and "-" means not included in it; The number in brackets is the number of characters.			

Table 4: The existence of the morphological character class on each OTU specimen based on the ordinal data type.

The results of the analysis of 173 test characters also still obtained three classes of characters owned by *C. annulus* shell either from Ambon and Larat Island that is the first class consisting of three characters, the second character class consisting of 169 characters and the third character class with one character . The existence of the morphological character class on each of the OTU specimens is presented in Table 5. Based on Table 5, it appears that all OTU specimens are included in the second character class, while the first and third character classes are only owned by some OTUs belonging to the juvenile and sub adult stages. The OTU specimens included in the second character class are the juvenile specimens from Ambon island in 2014, the juvenile specimens from Larat Island in 2014, the juvenile specimens from Larat Island in 2016, and sub-adult specimens from Larat Island in 2016; while OTU specimens belonging to the third character class are juvenile specimens from Ambon island in 2013, juvenile specimens from Ambon island in 2015, juvenile specimens from Larat Island in 2016 and sub-adult specimens from Larat Island in 2016. This indicates that the formation of second and third character classes is assumed because the morphological character of OTU is still in incomplete growth and development stage, while others are complete. The OTU specimens included in the three morphological character classes have two specimens: the juvenile specimens from Larat Island in 2016 and sub-adult specimen from Larat Island in 2016.

Phenon level		
correlation 0.970	correlation 0.980	correlation 0.990
A3ju	A3ju	A3ju
A6ju	A6ju	A4ju
A3sa	A3sa	A5ju
A4sa	A4sa	A6ju
A5sa	A5sa	A3sa
A6sa	A6sa	A4sa
A3ad	A3ad	A5sa
A4ad	A4ad	A6sa
A5ad	A5ad	A3ad
A6ad	A6ad	A4ad
A3pa	A3pa	A5ad
A4pa	A4pa	A6ad
A5pa	A5pa	A3pa
A6pa	A6pa	A4pa
M3ju	M3ju	A5pa

M4ju	M4ju	A6pa
M5ju	M3sa	M3ju
M3sa	M4sa	M4ju
M4sa	M5sa	M3sa
M5sa	M3ad	M4sa
M3ad	M4ad	M5sa
M4ad	M5ad	M3ad
M5ad	M6ad	M4ad
M6ad	M3pa	M5ad
M3pa	M4pa	M6ad
M4pa	M5pa	M3pa
M5pa	M6pa	M4pa
M6pa	A4ju	M5pa
A4ju	M6ju	M6pa
M6ju	M6sa	M5ju
M6sa	A5ju	M6ju
A5ju	M5ju	M6sa

Table 5: The taxa cluster formed at the phenon level in the taxonomic correlation phenogram of Figure 9A.

The OTU specimen configuration map based on the morphological character class, ordinal data type, shows 32 OTU specimens at four points or positions (Figure 11). Figure 11 shows that the juvenile specimens from Ambon island in 2013 are located closest together and are in the same quadrant as the juvenile specimens from Ambon island in 2015 so the phenotypes are both very similar. Similarly, the juvenile specimens from Larat Island in 2016 and sub-adult specimens from Larat Island in 2016 can be said to be very similar because they are located very close together even at one point and are in the same quadrant. The juvenile specimens from Ambon island in 2015 and juvenile specimens from Ambon island in 2013 are located far away and in different quadrants with juvenile specimens from Ambon island in 2014 and juvenile specimens from Larat Island in 2014 so that it can be said that based on the second character class, it is slightly different when compared to Juvenile specimens from Larat Island in 2016 and sub-adult specimens from Larat Island in 2016. Figure 11 also shows that morphologically there are six specimens located far away and in separate quadrants with other OTU groups such as juvenile specimens from Ambon island in 2013, juvenile specimens from Ambon island in 2015, juvenile specimens from island in 2016, sub adult specimens from island year 2016, juvenile specimens from Ambon island in 2014

and juvenile specimens from island in 2014 so it can be said that these six specimens are quite different from other specimens. The pattern of this configuration is still the same as the pattern in the morphological character class with binary data types, what distinguish them is the position of specimens in different quadrants.
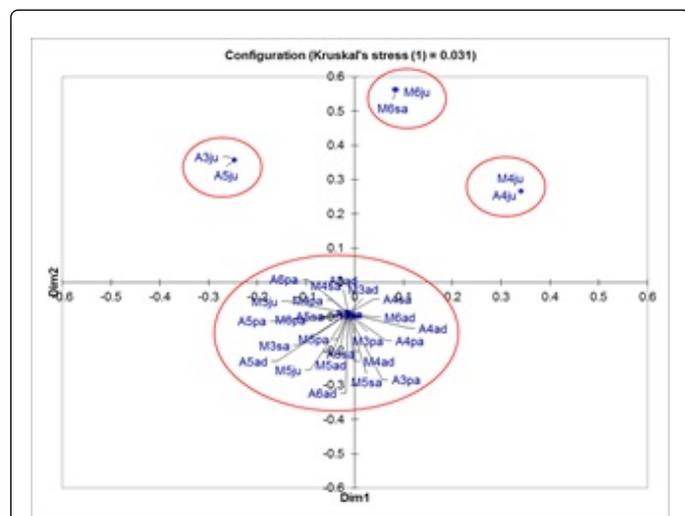

Figure 11: OTU specimen configuration based on morphological character class of ordinal data type using multidimensional scaling (MDS).

Discussion

This study obtained largest lenght of *C. annulus* between 21.44 and 25.84 mm. Meanwhile, [32] research obtained the largest length of *C. annulus* between 21.30 and 23.60. Zahida [47] reported that the size of *C. annulus* in the coastal waters of Sepanjang beach Yogyakarta reached less than 23 mm. Previous research has provided length information; it has not revealed the weight, width and height of the shell; a small number of specimens and less than one study time period means that this research provides detailed and comprehensive information.

The shell size of *C. annulus* in Mollucas island based on developmental stages is categorized into four stages: juvenile stage (11.00-14.99 mm), sub adult (15.00-17.99 mm), adult (18.00-22, 99 mm) and post adult (>23.00 mm). These results are no different from those obtained by [32,50] This research also showed that the shell size of the different developmental stage in Ambon and Larat Island. On the island of Larat have a shell size for each stage of development greater than the island of Ambon.According to Irie et al. [50] the existence of shell variations is strongly influenced by the location and geographical conditions in which these gold ring cowrys are located.

These results show that overall the similarity level of 32 OTU pairs for 173 morphological test characters is also very high. However, there is still a morphological phenetic distance in some OTU pairs; this is different from the binary data type. The difference is assumed that the binary data type contains only two statements for each test character that are zero and one while the ordinal type has more than two character statements so the result of the distance calculation is quite different. In addition, the variation in the number of statements between test characters is also suspected to be the cause of variation in distance between these OTU pairs even if it has been standardized to

173 test characters. The results of this research indicate the coefficient of similarity between OTU pairs; the phenetic distance is also not always the same. This is consistent with the concept that phenetic similarity coefficients are more emphasized on the degree of association and affinity between OTUs to the character being tested, while the phenetic distance emphasizes the magnitude of the numbers indicating the distance.

In general, it can be explained that the distribution of coefficient similarity and phenetic distances between 32 OTU *C. annulus* based on morphological test character indicates that *C. annulus* strain of Ambon island and Larat Island have a very high morphological phenetic similarity level that is >95% even some reach 100% and a very small phenetic distance is <1.0 distance. Not all OTU pairs have a 100% similarity because there is still a difference in morphological character expression (phenotypic) that is still at the stage of development and growth of the shell, especially the juvenile and sub-adult stages. In addition, the phenotypic character of the morphology of the gold ring cowry shell is greatly influenced by the environmental factors in which they live [36]. However, the results of the construction of the test character are numerous and combine the higher character levels of the Cypraea genus to the gastropod class from providing consistent taxonomic results, preferably using the stages of development of juvenile shells to post adult.

The construction of the numerical taxonomy of *C. annulus* is fundamental and important because it has two benefits: practical and theoretical benefits. Practically, the numerical taxonomy of *C. annulus* is useful for evaluating and determining the level of taxon, biological status and conditions, and placing it at the proper level of classification in organism systematics. Meanwhile, theoretically, it can express the phylogenetic relationship of intraspecies and interspesies, as well as the possibility of changes in the quality of biota and its environment. During this time, *C. annulus* taxonomy is still based on the character of shell phenotype and qualitative body anatomy. According to Irie et al. [61] that these characteristics are of course very limited in describing and explaining phylogenetic relationships between species, but quite useful in explaining the various phenotypic characters between individuals within a species. This is due to the variety of phenotype characters which are strongly influenced by genetic material or genotype and environment or ecotype. The consequence is that the species taxonomy is not absolute and will change according to the new method involved. Numerical taxonomy, which can evaluate all characters simultaneously, is one of the most popular methods used in the taxonomy of many species because it is more objective than traditional taxonomic methods. Identification of species and taxonomy depends greatly on different shapes, sizes, physiological characters, DNA characters, and the environment, and so on from the sample. These characters can be divided into two types, qualitative characters and quantitative characters.

There are various concepts in taxonomy of marine biota. The first concept is a taxo-species concept that is the concept of biota classification based on similarities that reaches 70% or more. The second concept, the geno-species concept is the concept of classification based on genotype biota. The third concept is the genomic-species concept that a biota or individual is said to be a species if it has DNA relatedness of more than or equal to 70%, while the fourth concept is the nomen-species concept, an individual is said to be a species if it refers to the same type of strain. Based on these various concepts, the classification or taxonomy includes the classification of monothetic, polithetic classification, chemotaxonomic

classification, and molecular classification. Monothetic classification is based on the number of characters owned by an individual connected with an individual or a phylogenic classification. Polithetic taxonomy is based on the type of character that can be measured called phenetic taxonomy. This research constructs taxonomy of marine life, especially *C. annulus*, based on numerical taxonomic approach with taxo-species concept. Numerical or polithetic taxonomy is one way to get an objective classification result based on as many characters as possible. This taxonomy has five *adansonian* concepts [62] based on as many characters, each character is given equal and even value in constructing taxon, OTU is a function of similarity proportion of shared properties, the taxa is formed on the basis of similarity of nature, and similarity is phenetic.

Based on 296 characters morphologically constructed with binary data types or two state observed by the naked eye or by using software obtained two groups or taxa of the *C. annulus* phenotype in Ambon and Larat Island. The results of this research indicate that the morphology of *C. annulus* from Ambon island is very similar to *C. annulus* from Larat Island both in the development stage and time period, even when the level of similarity is still below 100%. The similarities are smaller than 100%, more at different stages of development especially in the juvenile stage. This is understandable because at the juvenile stage the gold ring cowrys are still immature and still experience the healing and development of the shell. This is in accordance with the statement put forward by [32,50].

The advantage of numerical taxonomy is that it can unite data from a wide variety of character sources and in large quantities, which was previously difficult to do with conventional taxonomy. In addition, character data, through computers, can be integrated with existing data processing systems and their work is more efficient, accurate and effective because it is done automatically. This is in accordance with the opinion of Sneath et al. [62,63] that numerical taxonomy can also reduce subjective bias and increase objectivity because by using many characters, the method is standardized and the results can be evaluated quickly. The prediction of similarity is a very important and fundamental procedure in numerical taxonomy. This is done by collecting test character information on the taxonomic groups studied. Numerical taxonomic studies are performed on many characters or variables and based on the analysis of the similarity of these characters the grouping of taxa among groups of organisms will be resulted. Numerical taxonomy uses many variables, so the analysis is multivariable or multivariate analysis [64-68].

This research indicates that there are similarities and differences of clustering results between binary, ordinal and numeric data types. In clustering based on morphological test characters on binary and ordinal data types bases, there similarity that is indicated by OTU or C. annulus strains which have already united at a very high phenon level above 90% according to the taxo species concept that requires a similarity level above 70%. This means that the binary and ordinal type approach method in the morphology class has been able to show 32 OTUs in one species, yet the order of combination between Ambon islands and Larat Island still shows a difference in the taxonomic similarity level of 94% and the taxonomic distance of 4,0 either at the development stage or time period. This is quite interesting because conventional taxonomic approaches have not been able to produce detailed results compared to the numerical taxonomic approach. Approach based on morphological characters of ordinal data types shows quite different things from binary data types. There are two main differences: the very high taxonomic correlation value between

32 OTU which is the correlation phenon level of 0.960 that has incorporated 32 OTU specimens into one phenotype group which means that the strain similarity Ambon and Larat Island is very high. The second difference is that the ordinal data type approach can set the taxonomic correlation phenon level up to 0.990 to separate the OTU into seven taxa phenotypes, which are useful for detecting and evaluating the character of the biota accurately and in detail by involving numerous characters [69,70]. The numerical taxonomy of the two test character classes shows fairly consistent results generating in the *C. annulus* phenotype taxa group at taxonomic similarity levels above 70% and taxonomic correlations above 0.700. *C. annulus* numerical taxonomy based on morphological test characters with binary data types or two states obtained two Phenotype taxa at taxonomic similarity phenon level of 94% and ordinal obtained three phenotypic taxa at the taxonomic correlation level of 0.970.

Conclusion

The size category of gold ring cowry shell in Ambon and Larat Islands consists of: length size with very small size (11.67-12.64 mm), small (12.65-15.57 mm), medium size (15.58-18.50 mm), large size (18.51-21.43 mm), and largest size (21.44-25.84 mm). Category of shell weight size of *C. annulus* specimens in Ambon and Larat Island is: very small size (0.14-0.16 g); small size (0.17-0.63 g); medium size (0.64-1.10 g); large size (1.11-1.57 g); and very large size (1.58-2.66 g). Shell width categories in Ambon and Larat Island consist of: very small size (4.22-7.97 mm); small size (7.98-10.04 mm); medium size (10.05-12.11 mm); large size (12.12-14.18 mm); and very large size (14.19-16.74 mm). The shell height size categories of the *C. annulus* found in Ambon and Larat Island are composed of: very small size (2.21-6.02 mm); small size (6.03-7.52 mm); medium size (7.53-9.02 mm); large size (9.03-10.52 mm); and very large size (10.53-12.64 mm).

The developmental stages of *C. annulus* based on the length size in Ambon and Larat Island: juvenile stage (11.00-14.99 mm), sub adult (15.00-18.99 mm), adult (19.00-22, 99 mm) and post adult (>23.00 mm).

The distribution of coefficient similarity value and phenetic distance between 32 OTU *C. annulus* based on character of morphological test indicate that C. annulus strain from Ambon island and Larat Island have a very high morphological phenetic level that is about 95% or some reach 100% and very small phenetic distance is of <1.0.

C. annulus numerical taxonomy based on morphological test character with binary data type or two states obtained two phenotypic taxa at taxonomic similiarity phenon level of 94% and ordinal obtained three taxa phenotype at taxonomic correlation level of 0,970.

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