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Close Genetic Relatedness of Chromis Fish (*Abudefduf* sp.) Between Indonesia and Korea Population

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Abstract

The purpose of our study was to look closely relationship of Chromis fish taken from Korean waters and Indonesia based on Deoxyribose Nukleid Acid (DNA) mitochondrial marker. Samples of data taken from each of the 21 identified samples of the *Abudefduf vaigiensis* species, *Abudefduf sexfasciatus* and *Abudefduf bengalensis*. The samples taken were then extracted, amplified, and sequenced based on the mitochondrial DNA marker from the CYTB locus. The DNA sequence result is processed in MEGA 6.06 software to analyze the phylogenetic tree for determine close relatedness. The result showed there were 4 main clades including one clade outgrup explaining the chromis fish close relatedness i.e. 1) *Abudefduf vaigiensis group*, *Abudefduf sexfasciatus* and *Abudefduf bengalensis*, 2) *Abudefduf Bengalensis* group and 3) *Abudefduf vaigiensis* group from Buton waters location. The closest relatedness between Chromis fish found in Korea and Indonesia in the *A. vaigiensis* Korea and *A. vaigiensis* species in Sorong waters, ie, by the genetic distance of 0.0008. This genetic distance value in pairs of 10000 pairs of nucleotide have 8 pairs of different nucleotide.

Keyword: Genetic; Chromis fish; *Abudefduf vaigiensis; Abudefduf sexfasciatus* dan *Abudefduf bengalensis;* Korea; Indonesia.

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1. Introduction

Family Demselfish or Pomacentridae, which is the species Abudefduf sp. It contains over 385 species diversity primarily inhabiting tropical climates and shallow waters, near the coast [10, 21, 27]. Abudefduf fish which is also often referred to as Chromis own fish first recorded in 1957 in the Mediterranean sea was found a young individual who was identified as Abudefduf saxatilis in Naples Bay, Italy [24]. The use of character identification based on morphological characters is a common step in the type identification process. The use of morphological character often raises doubts or errors, so another approach is needed to eliminate doubt in the identification process. A molecular approach is one method that can be used to minimize the chances of identification error. DNA barcoding is a method in molecular taxonomy that uses a standardized, standardized DNA sequence and agreed on its position in the genome to identify species [11]. DNA is the smallest unit in the cell that contains the descendants of a living being and can be found in the nucleus (core DNA) and organelles in the cytoplasm (mitochondrial DNA) [8]. The use of DNA as a feature of a species has several advantages, which are more thermostable than proteins, more sensitive, unaffected by the environment and growth factors and almost all tissue can be used as a source of genetic material [9]. A phylogenetic tree is a logical approach to showing the evolutionary relationship between organisms and showing genetic proximity due to gene flow between populations [6]. Phylogenetics can analyze the changes that occur in the evolution of different organisms. One important component of a population is genetic diversity, which is important information in monitoring changes in biodiversity that occur in nature [16, 25]. Information on genetic diversity is fundamental in the fish population and breeding management program to improve productivity and sustainability. Genetic diversity is important for the long-term sustainability of a species and may fluctuate and affect genetic structures between populations that are affected by changes in genotype fitness status in the recruitment and gap processes of the environment in nature. Genetic diversity is an important information in the short and long term for a population [1]. Meanwhile, genetic diversity is the lowest hierarchy in biodiversity level and is an important key for a species to survive, maintaining the sustainability of the population and increasing the productivity of a species [12]. The diversity of Chromis fish is known by looking at factors Which affect their presence in the oceans, such as habitat differences such as coral cover, availability, and type of food Loss of genetic diversity will reduce the ability of the species to adapt to environmental changes Information on genetic diversity can be obtained in several ways, including by analyzing mitochondrial DNA (mtDNA) In the field of fisheries, mtDNA has been widely used to determine the genetic diversity of a population, since the mtDNA analysis is more sensitive than that of the widely practiced protein analysis [26].

Based on the climate, Indonesian waters have a tropical climate, while Korean waters have a subtropical climate. However, Chromis fish can be found in both areas of the waters. With geographical conditions far enough then it can be compared the genetic diversity of Chromis fish geographically. Genetic distances with geographical distances among populations may indicate that geographic isolation plays an important role in the determination of genetic differences between populations. The high genetic diversity in a population reflects the size of the population, while the decrease in population size will reduce genetic diversity [2]. Genetic differences that occur between species due to mutation, transversion, deletion or inversion in the cell's genetic coding, which causes the process to occur because of adaptation to the world's climate change occurring or even the factors of population differences [22]. The purpose of this look closely relationship of Chromis fish taken

from Korean and Indonesia Waters based on Deoxyribose Nukleid Acid (DNA) mitochondrial marker.

2. Material and Methods

2.1. Sample Collection

Data collection in Indonesian waters was conducted in September 2016 until February 2017 i.e. in Pramuka Island waters, Buton Island waters, Palabuhanratu waters and Sorong waters. While data collection in Korean waters was conducted in November 2016 in the Southern waters of Jeju Island, South Korea. The samples obtained were 21 samples inserted into plastic samples that have been filled ethanol 96% and labeled according to each individual. Tubes that already contain samples and ethanol are kept neatly in the laboratory.

2.2. Material and Tools

The data collected in this research consisted of primary data of *Abudefduf sp*. of Indonesia and Korean waters, ethanol 96%, chelex 10%, ddH2O, buffer solution, dNTP, loading dye, low mass ladder, taq polymerase enzyme, MgCl, primer, agarose and EtBr. The tools used in the study are book and pencils, scissors, cutter, tube (2 ml, 0.6 ml, 0.3 ml, and 0.2 ml), petri dish, gloves, bunsen, vortexmicrosentrifuges, heating block, forceps, pippettemen (10, 20, 200 Ml), pippet tips, erlenmeyer tube, measuring cylinder, parafilm, microwave, electrophoresis machine, UV machine, computer, camera, BLAST, Mega 6.06 software.

2.3. DNA Extraction and Amplification

DNA extraction aims to destroy cells and take tissue on samples. The extraction method used was Chelex 10% (Walsh and his colleagues 1991). The process of DNA amplification using the method of *Polymerase Chain Reaction* (PCR) Hotstart is an enzymatic technique or methodology (replication) of DNA.

The main components in PCR is DNA template, dNTPs, PCR buffer, MgCl, primers, and enzimpolymerase, Primer which is used for this Chromis fish for amplification Cyt-B is using GludgL14724 primer: 5'-CCA TGA YCG CTT GAA GAA TTG-3 '[20] and CB2-H15149: 5'-AAA CCC CAG CTG ATA AGA TTT GTC CTC CTC A-3 '[30]. PCR process in this research is done 30 times cycle each cycle consist of three stages. PCR was performed by using a PCR machine (thermo cyler) consisting of several processes, namely the separation of the DNA of a double thrass (denaturation) at 94 ° C for 30 seconds, attaching at 49 ° C for 15 seconds and extending the DNA segment at the temperature 72 ^{o}C for 45 minutes.

2.4. Electrophoresis

Electrophoresis is a technique of separating a charged component or molecule based on its different migration rates in an electric field. Electrophoresis aims to determine the quality of DNA in PCR products. Compounds with their electron charge will migrate in one electric field proportional to their charge density rate. Different DNA fragments of DNA are visualized using specific fluorescent dyes for DNA, such as ethidium bromide. The type of gel used is agarose which can indicate band or size of base pair fragments that can be seen with ultraviolet.

2.5. DNA Sequencing

DNA sequencing is a method for determining the sequence of nucleotides present in DNA. The sequence of DNA is related to genetic information in the nucleus, plasmids, mitochondria, and chloroplasts that form the basis for the development of all living things. Samples that have been amplified by the PCR method, are further sequenced to obtain the nucleotide sequence. Sequence method used is Sanger method. DNA sequencing process is sent to Pukyong National University to be sequenced.

2.6. Data Analysis

Spesies identification analyze using BLAST (Basic Local Alignment Search Tool) process in the MEGA 6.06 (Molecular Evolutionary Genetic Analysis) [13]. Then, identification result is aligned using ClustalW which is already available in the software in the MEGA 6.06 program to look at the diversity of the nucleotide. In determining the results of the species, a comparison of DNA sequence databases of genomes linked to genomes (http: //blast.ncbi.nlm.nih.-gov) was performed.

The processing of genetic data is the most important process of all processes that have been done to obtain genetic information of living things. Results of DNA sequences that have been analyzed and known to be reprocessed by the MEGA 6.06 program to determine the results of their nucleotide composition [13] which has the function to explore, find and analyze DNA and protein sequences [3].

The basic principle of MEGA is the statistical calculation of the sequence data. Alignment (alignment) of a process to match homologous characters derived from a common ancestor [4].

The composition and difference nucleotide model of chromis fish species using *compute nucleotide composition model* tools in MEGA 6.06. Then, to construct the phylogenetic tree using Neighbour-Joining (NJ) clustering method with 1000 bootstrap and using model Kimura2-parameter model. According to [19], the Neighbour-Joining (NJ) framing method is the most suitable method for correctly predicting trees.

3. Result and Discussion

3.1. Genetic Structure of Chromis Fish

The genetic diversity of a population can be seen from the nucleotide composition of the population. The nucleotide component consists of one nitrogenous, one pentose sugar (deoxyribose), and one phosphate group. The composers of the nitrogen themselves are Adenine (A) and Guanine (G) called purines, cytosine (C) and thymine (T) called pyrimidines. One DNA molecule has two strands of nitrogen or double helical base, where A paired with T and G couples with C bound by hydrogen bonds.

The A-T base pair has two hydrogen bonds, and the base pair C-G has three hydrogen bonds. According to Chargaff rules the composition of the nucleotide between individuals varies from one to the other, with the composition of base A more or less equal to T, and the nucleotide composition of G approximately equal to C.

Table 1: Nucleotide compotition of Chromis fish in Indonesia dan Korea

ID Number	T(U)	C	A	G	Total
ITK.MKS.AV.02.CYTB_Abudefduf vaigiensis666*	29.3	31.0	23.9	15.8	652.0
ITK.MKS.AV.03.CYTB_Abudefduf vaigiensis666*	29.1	31.1	23.9	15.8	652.0
ITK.MKS.AV.05.CYTB_Abudefduf vaigiensis666*	29.1	31.1	23.9	15.8	652.0
ITK.PL.AS.01.CYTB_Abudefduf sexfasciatus666*	28.5	31.6	24.5	15.3	652.0
ITK.PL.AS.02.CYTB_Abudefduf sexfasciatus775*	28.5	31.6	24.4	15.5	652.0
ITK.PL.AV.01.CYTB_Abudefduf vaigiensis666*	28.5	31.6	24.5	15.3	652.0
ITK.PL.AV.02.CYTB_Abudefduf vaigiensis666*	28.7	31.4	24.2	15.6	652.0
ITK.PL.AV.03.CYTB_Abudefduf vaigiensis666*	28.5	31.6	24.5	15.3	652.0
ITK.PL.AV.04.CYTB_Abudefduf vaigiensis666*	28.5	31.6	24.4	15.5	652.0
ITK.PL.AV.05.CYTB_Abudefduf vaigiensis666*	28.5	31.6	24.4	15.5	652.0
ITK.PS.AS.01.CYTB_Abudefduf sexfasciatus797*	28.5	31.6	24.4	15.5	652.0
ITK.PS.AS.02.CYTB_Abudefduf sexfasciatus666*	28.5	31.6	24.4	15.5	652.0
ITK.SOR.AB.01.CYTB_Abudefduf bengalensis666*	26.8	32.7	24.8	15.6	652.0
ITK.SOR.AB.02.CYTB_Abudefduf bengalensis666*	28.5	31.4	24.2	15.8	652.0
ITK.SOR.AV.01.CYTB_Abudefduf vaigiensis666*	28.5	31.6	24.4	15.5	652.0
PKU7971_Abudefduf vaigiensis800^	28.5	31.6	24.4	15.5	652.0
PKU51814_Abudefduf vaigiensis765^	28.5	31.6	24.4	15.5	652.0
PKU51816_Abudefduf vaigiensis760^	28.5	31.6	24.4	15.5	652.0
PKU51818_Abudefduf vaigiensis768^	28.4	31.7	24.4	15.5	652.0
PKU52020_Abudefduf vaigiensis768^	28.5	31.6	24.1	15.8	652.0
PKU52022_Abudefduf vaigiensis770^	28.5	31.6	24.4	15.5	652.0
Abudefduf vaigiensis KU553878.1	28.5	31.6	24.2	15.6	652.0
Carcharhinus limbatus FJ519116.1	35.1	22.9	26.4	15.6	652.0
Avg.	28.8	31.2	24.4	15.6	652.0

Note : * Sample from Indonesia

^ Sample from Korea

The sequence of nucleotides in the alignment process results in a varied sequence of species *A. vaigiensis*, *A. sexfaciatus* and *A. bengalensis* from Indonesia and Korea. Based on Table 1, the percentage of nucleotide composition of each speciese that is *A. vaigiensis*, *A. sexfaciatus* and *A. bengalensis* from Indonesian waters and *A. vaigiensis* from Korean waters does not much different. Table 1 also explains that in each species, the most common nucleotide composition is C, (ranging from 31% - 31.7%), followed by T (28.4% -28.7%), A (23.9% - 24.5%), and most Slightly is G (15.3% -15.8%). The proportion of this amount is in accordance with the opinion of [30] that for the Birds and Fish group, the most common nucleotides are C followed by A, T and G. Beside that, an A-T base composition more than the C-G base composition. This is because A-T has two hydrogen bonds that are easily released and replicated compared to the three hydrogen bonds possessed by C-G, which

causes the A-T base composition more than C-G [15].

Table 2: Nucleotide substitution probability matrix Chromis fish in Indonesia dan Korea

	A	**T**	**C**	**G**
A	-	0.49*	0.53*	**18.76**^
T	0.4*	-	**25.53**^	0.27*
C	0.4*	**23.72**^	-	0.27*
G	**28.61**^	0.49*	0.53*	-

Note : ^ Tranversional, ***** Transisional

Diversity in life can arise due to genetic mutation factors that generate genetic diversity. Gene mutation is also called point mutation. This mutation occurs because of changes in the structure of genes or DNA [14]. Graur & Hsiung Li said that there are several types of mutations: (1) Substitution mutation is the replacement of a Nucleotide translation with another, (2) Recombination is a nucleotide with another, (3) Deletion is the movement of one or more nucleotides in DNA completion. Substitution mutations are important because basically evolutionary processes and sequences of DNAs (nucleotide sequences) are substitutes of a nucleotide with others during the time of evolution [5, 17]. Substitution mutations are divided into two types: transitions and transversions. The transition is a change between A and G (purine) or between C and T (pyrimidine). A transversion is a change between purine and pyrimidine. The result of matrix probability analysis of nucleotide substitution is bold number called transition substitution while the number in italics is called transversion substitution (Table 2). Transverse substitution mutations are smaller and less common than transition substitutions. The highest substitution mutation value is found in A base, followed by G, T and C base. This is closely related to the frequency of each nucleotide.

3.2. Close Relatedness

The genetic distance is used to see the close relationship of Chromis fish species in Indonesia and Korea. The genetic distance difference matrix consists of 15 species from Indonesia and 6 species from Korea that can be seen in Table 3.

Table 3: The genetic distance matrix of Chromis fish based on the location

No	**Spesies**	**1**	**2**	**3**	**4**	**5**	**6**	**7**
1	A. vaigiensis Buton							
2	A. vaigiensis Palabuhanratu	0.0983						
3	A. vaigiensis Sorong	0.0976	0.0018					
4	A. vaigiensis Korea	0.0973	0.0026	**0.0008**				
5	A. sexfaciatus Palabuhanratu	0.0985	0.0082	0.0070	0.0077			
6	A. sexfaciatus Pramuka	0.0976	0.0018	0.0000	0.0008	0.0070		
7	A. bengalensis Sorong	**0.1054**	0.0558	0.0541	0.0541	0.0590	0.0541	

Based on Table 3, the lowest genetic distance values are on species *A. vaigiensis* from Korean waters and *A. vaigiensis* from Sorong waters (Indonesia) ie 0.0008. While the highest genetic distance values are *A. bengalensis* Sorong waters (Indonesian waters) and *A. vaigiensis* from Buton waters (Indonesian waters). The value at the lowest genetic distance of 0.0008 means that in 10000 pairs of nucleotide bases there are 8 pairs of different nucleotide. The highest genetic distance value of 0.1054 means that in 10000 pairs of nucleotide there are 1054 pairs of different nucleotide. Based on these results, [18] explains that the higher the genetic distance is, the further the genetic relatedness and nucleotide differences. The lower the value of the genetic distance indicates a very close genetic relatedness. In addition, the higher the genetic distance of a population, the population has a high species diversity [7]. Phylogenetic reconstruction is a method to determine the rate of evolution and close relatedness of a species through a dendrogram based on the mitochondrial sequence data obtained. Phylogenetic reconstruction (Figure 1) shows the results of BLAST analysis according to the characteristics of the branch formed by the tree itself. Species with similar DNA chains form an adjacent branch and form a large clade.

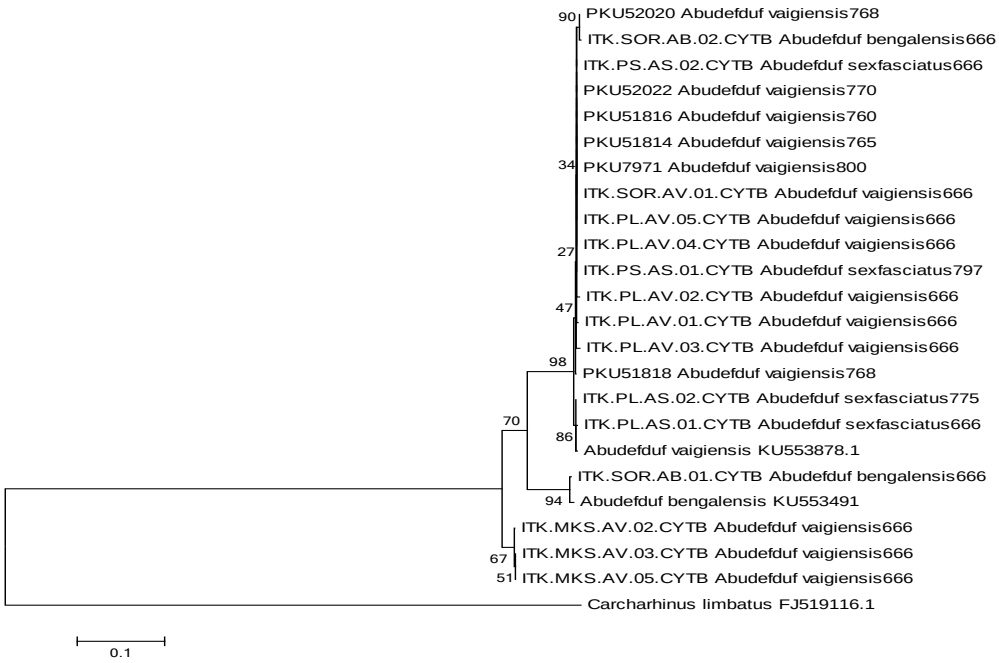

Figure 1: The Neighbour-joining tree on Chromis fish individual from Indonesiaand Korea, based on Cyt-B sequence data using Kimura-two-parameter substitution model with 1000 bootstrap

Results from the analysis of phylogenetic trees from Chromis fish (Figure 1) obtained 4 clades or a large group of descendants from one common ancestor, with 1 additional clade of outgroup taken from the species *Charcarhinus limbatus*. The addition of outgroup to the phylogenetic tree can make it easier to predict genetic distance between populations [32]. Genetic diversity can occur not only in intraspecies but also in interspecies. In each large clade, there is still a small clade indicating the difference in the composition of nucleotide bases between individuals. This can happen because each individual comes from a different population. When seen from Figure 1, two clades are formed from the *A. vaigiensis* species and one clade is formed from the *A. bengalensis* species. The first clade was formed from the *A. vaigiensis* in at Sorong waters (Indonesian waters),

Pelabuhanratu waters (Indonesian waters) and Korean waters, *A. sexfaciatus* in Pelabuhanratu and Pramuka waters (Indonesian waters) and *A. bengalensis* in Sorong waters (Indonesian waters). The second clade is *A. bengalensis* in Sorong waters (Indonesain waters) and the third clade of *A. vaigiensis* in Buton waters (Indonesian waters), by forming a clade. This condition makes it possible that the *A. vaigiensis* species in the Buton waters (Indonesian waters) is located due to regional isolation. The phylogenetic reconstruction based on Figure 1 is supported by the value of the genetic distance (Table 3) and the genetic relatedness of each of the three clades is likely to be affected by oceanographic conditions in each location. When viewed from pictures of phylogenic trees and genetic distance values, the *A. vaigiensis* species in Korea have a genetic close relatedness with *A. vaigiensis* in Sorong Waters (Indonesian waters) this is likely to be affected by the geographical conditions of the two locations, Jeju Island as the location of data collection in Korea is at Longitudes closer to Sorong Waters (Indonesian waters) than to other sampling locations in Indonesia. Genetic distances with geographical distances among populations may indicate that geographic isolation plays an important role in the determination of genetic differences between populations. While genetic differences occur between species due to mutation, transversion, deletion or inversion in the genetic coding of cells, causing the process to occur due to adaptation to world climate change occurring or even factors of population differences [22].

4. Conclusion

From the results of the study, it can be concluded that the genetic relatedness of chromis fish is divided into four clades with one clade outgroup. The close genetic relatedness rate between Chromis fish found in Korea and Indonesia occurs in the *A. vaigiensis* Korea waters and *A. vaigiensis* species in Sorong waters (Indonesian waters), ie, with a genetic distance of 0.0008. This genetic distance value means that in 10000 pairs of nucleotide there are 8 pairs of different nucleotide.

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