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CERTIFICATE

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Presenter

Genetic Diversity Of Three Sunu Groupers Species (Plectropomus spp)

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Untuk melaksanakan tugas mengikuti seminar Internasional ***The 4th Annual Applied Science and Engineering Conference*** dengan tema ***"Integrating Inovation in Science and Engineering among Young Researchers*** sebagai presenter, yang akan dilaksanakan secara daring pada :

Hari / Tanggal : Rabu, 24 April 2019

Waktu : 09.00-13.00 WIB

Tempat : Daring (Zoom Meeting)

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Tembusan : Arsip

Genetic diversity of three sunu groupers species (*Plectropomus spp*)

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Genetic diversity of three sunu groupers species (*Plectropomus spp*)

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Abstract. The quality of fish seed may be decreased as a result of inbreeding that is a marriage between two individuals who have the same genetic trait. Fish seed quality improved with the genetic approach to parent selection and crossbreed. Quality of fish seeds is taking from natural populations are determined by the genetic diversity of a population. Inbreeding can decrease the quality of seed because it can increase the number of homozygosity and decrease the number of heterozygosity. This study aims to determine differences in genetic variation three species of sunu groupers (*Plectropomus maculatus*, *Plectropomus leopardus*, and *Plectropomus oligacanthus*) and know the kinship of the three species of grouper. While the benefits of this research can provide information on genetic diversity in natural coral trout grouper, expected inbreeding can be avoided. Muscle and liver tissue was used as a sample for electrophoresis analysis with twelve kinds of enzymes namely aspartate aminotransferase (AAT), Alcohol dehydrogenase (ADH), Estrase (EST), -Gliserofosfat dehydrogenase (-GPD), Glukofosfat isomerase (GPI), Isocitrate dehydrogenase (IDH), Lactate dehydrogenase (EST), Malic dehydrogenase (MDH), Malic Enzyme (ME), 6-phosphogluconate dehydrogenase (6- PGD), phosphoglucomutase (PGM), and sarcoplasmic enzymes (SP). Three polymorphic loci in *Plectropomus oligacanthus* species are 6-PGD, GPI-2, and PGM. In *Plectropomus maculatus*, and *Plectropomus leopardus* all monomorphic loci. The three polymorphic loci on *Plectropomus oligacanthus* meet the Hardy-Weinberg balance with X2 value for 6-PGD value is 0.123, GPI-2 is 0.028 and PGM is 0.028. *Plectropomus oligacanthus* has a polymorphism level of 0.188, the total number of the focusing allele is 1,154, and the average heterozygosity is 0.023. The genetic distance between the sepsis population based on twelve enzymes showed between *Plectropomus maculatus*, and *Plectropomus leopardus* had a spacing of 0.134, between *Plectropomus leopardus* and *Plectropomus oligacanthus* of genetic distance 0.196 and between *Plectropomus maculatus* and *Plectropomus oligacanthus* the genetic distance was 0.200. The larger the genetic distance between the species, the kinship relationship will be farther away. The distant kinship is owned by *Plectropomus oligacanthus*.

1. Introduction

Mutation, migration, population size, and selection influence levels of genetic diversity within and among populations of marine species [1]. Fish seed quality improved with the genetic approach to parent selection and crossbreed. Quality of fish seeds which taken from natural populations are determined by the genetic diversity of a population. Inbreeding can decrease the quality of seed because it can increase the number of homozygosity and decrease the number of heterozygosity [2].

Grouper is a reef waters fishery products that have a high economic value. Groupers are include in the family Serranidae, sub familia Epinephelinae consisting of fifteen genus and 159 species [3]. Fish seed quality may decline as a result of inbreeding in which marriages between the individuals who have the same genetic trait. Allel or genes possessed derived from identical DNA molecules. The quality and quantity of feed and environmental approaches for successful production, it is also important to improve seed quality. Seed quality improvement can be done genetic approach through the selection of mother and crossbreed. Quality seeds taken from natural populations are determined by the genetic diversity of a population is in the business information needed improved seed quality, management of biodiversity in the ecosystem and the management of genetic diversity within species gene polls [2].

The high chance of homozygosity allows no allel is lost. Allel is missing feared is allele important as allel controlling growth, resistance of fish to diseases and resistance to environmental changes. Need to research on genetic variations and phylogenetic relationship some species of grouper in nature as a baseline in order improved seed quality grouper coral trout. It is expected that cross-breeding can be avoided.

2. Material and methods

This study used 30 groupers (*Plectropomus spp*). Consists of three species taken from the fish auction Jimbaran, Bali. Chemicals used in this study is Starch potatoes in order, $MgCl_2$ 1M, KCN 0,1N, TC-8 buffer, the buffer CAPM-7, 7% citric acid, Fast Blue Marker, several kinds of enzymes such as aspartate aminotransferase (AAT), Alcohol dehydrogenase (ADH), Estrase (EST), -Gliserofosfat dehydrogenase (∞-GPD), Glukofosfat isomerase (GPI), Isocitrate dehydrogenase (IDH), Lactate dehydrogenase (EST), Malic dehydrogenase (MDH), Malic enzyme (ME), 6-phosphogluconate dehydrogenase (6-PGD), phosphoglucomutase (PGM) and sarcoplasmic enzymes (SP), and the dye solution. Sample preparation: The sample plate, scalpel, tweezers, deep freezer, bar, pipettes, knives, and digital balance.

The observed variables were the enzyme diversity of three species of grouper namely *Plectropomus maculatus*, *Plectropomus leopardus*, and *Plectropomus oligacanthus*. Analysed with electrophoresis from liver and meat tissue. Electrophoresis procedures follow procedures performed by Sugama [4], such as gelling, extraction tissue, applications tissue extract to gel, running, slicing, and staining.

2.1. Data interpretation

Naming locus and allele following the method of Allendorf and Utter [5]. Multiple loci that encode the enzymes are marked with numbers separated by a dash (-) behind the name of the locus, which shows the relative distance migration of these loci. Locus said monomorphic if any locus consists of only one band depending on the type of enzyme monomer, dimer, tetramer, and so on. Data obtained from such electrophoresis hereinafter zimogram used to calculate allele frequency, the degree of polymorphism, Heterozygosity (He), The Average Heterozygosity (H), Calculating the number of allel per locus, The Value of Chi-square Hardy-Weinberg Expectations Genetic Distance.

3. Results and discussion

Zimogram results of electrophoresis of 13 enzymes were attempted by CAPM buffer-7 clearer than the buffer TC-8. Of the 13 enzymes tested in the eye tissue, heart, liver, and muscle were detected 12 enzymes in the muscle tissue and the liver. For enzymes, the buffer system and the type of the detected tissue can see in Table 1. Type enzyme, locus, buffers, tissue species, and polymorphisms are presented in Table 2.

Table 1. Summary of the enzyme, codes for an enzyme, tissue-specific as well as the buffer used in *Plectropomus maculatus*, *Plectropomus leopardus* and *Plectropomus oligacanthus.*

No	Enzyme	E.C, No.	Buffer	Tissue
1	Alcohol dehydrogenase	1.1.1.1	CAPM-7	Muscle
2	α-Gliserofosfat dehydrogenase	1.1.1.8	CAPM-7	Muscle, Liver
3	Estrase	3.1.1.3	CAPM-7	Liver
4	Laktat Dehidrogenase	1.1.1.27	CAPM-7	Muscle, Liver
5	Malat Dehidrogenase	1.1.1.37	CAPM-7	Liver
6	Malat Enzim	1.1.1.40	CAPM-7	Muscle
7	Fosfoglukomutase	2.7.5.1	CAPM-7	Muscle
8	Glukosafosfo isomerase	5.3.1.9	CAPM-7	Liver, Muscle
9	6-Fosfoglukonat dehydrogenase	1.1.1.44	CAPM-7	Liver
10	Isositra dehydrogenase	1.1.1.42	CAPM-7	Liver
11	Aspartat aminotransferase	2.6.1.1	CAPM-7	Liver
12	Sarcoplasmic protein		CAPM-7	Muscle

Determination loci more than three species, namely *Plectropomus maculatus*, *Plectropomus leopardus and Plectropomus oligacanthus.* Enzymes are used 12 16 loci detected in muscle and liver tissue with buffer CAPM-7.

Table 2. The enzyme, locus, buffers, tissue, and polymorphism in *Plectropomus leopardus* and *Plectropomus oligacanthus.*

No	Enzyme	Locus	Buffer	Tissue	Polimofism		
					P.leo	*P.mac*	*P.olig*
1	Alcohol dehydrogenase	Adh	CAPM-7	Muscle	M	M	M
2	α-Gliserofosfat dehydrogenase	α-GPD	CAPM-7	Muscle	M	M	M
3	Estrase	Est	CAPM-7	Liver	M	M	M
4	Laktat Dehidrogenase	Ldh	CAPM-7	Muscle	M	M	M
5	Malat Dehidrogenase	Mdh	CAPM-7	Liver	M	M	M
6	Malat Enzim	ME	CAPM-7	Muscle	M	M	M
7	Fosfoglukomutase	PGM	CAPM-7	Muscle	M	M	P
8	Glukosafosfo isomerase	Gpi-1	CAPM-7	Muscle	M	M	M
		Gpi-2	CAPM-7	Muscle	M	M	P
9	6-Fosfoglukonat dehydrogenase	6-PGD	CAPM-7	Liver	M	M	P
10	Isositra dehydrogenase	Idh-1	CAPM-7	Liver	M	M	M
		Idh-2	CAPM-7	Liver	M	M	M
11	Aspartat aminotransferase	Aat	CAPM-7	Liver	M	M	M
12	Sarcoplasmic protein	Sp-1	CAPM-7	Muscle	M	M	M
		Sp-2	CAPM-7	Muscle	M	M	M
		Sp-3	CAPM-7	Muscle	M	M	M

Description: M = monomorphic, P = Polymorphic

3.1. Description of locus polymorphic and variations

3.1.1. Phosphoglucomutase (PGM: EC.2.7.5.1). Phosphoglucomutase active in muscle tissue and moves to the anode. Mobility or movement of the band shows a different picture. PGM scheme banding pattern of electrophoresis as shown in Figure 1.

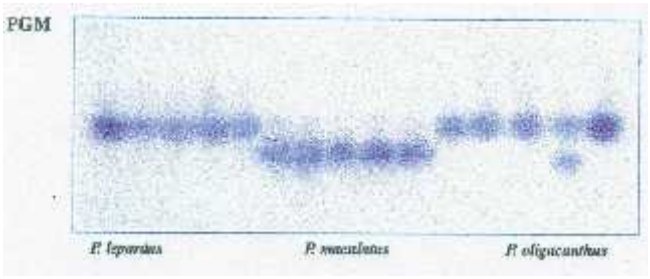

Figure 1. PGM band pattern scheme.

3.1.2. Glukosafosfo isomerase (GPI: EC.5.3.1.9). Glukosafosfo isomerase show 2 locus is Gpi-1 and Gpi-2. One locus appears in the anode region and one locus in the cathode region which is active in muscle tissue and controlled by 2 allel. GPI enzyme having phenotype with dimer structure. GPI locus scheme band pattern as in Figure 2.

Figure 2. GPI band pattern scheme.

3.1.3. 6-phosphogluconate dehydrogenase (6-PGD: EC.1.1.1.44). 6-PGD activity in liver tissue and detected at the anode region. Heterozygote individuals looked at *P. oligacanthus* demonstrated by the three bands and the homozygote by one band. It shows the 6-PGD enzyme phenotypes with a dimeric structure. Scheme pattern 6-PGD locus as shown in Figure 3.

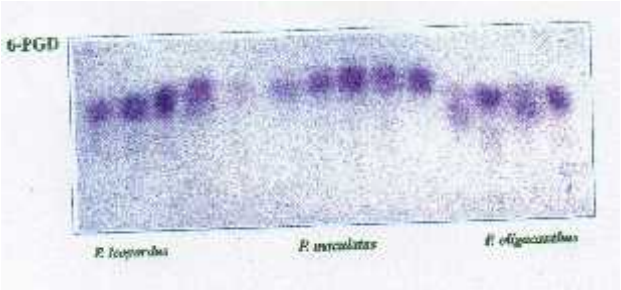

Figure 3. 6-PGD band pattern scheme.

3.1.4. Genetic variation. Twelve enzyme electrophoresis results of liver and muscle tissues of three species *Plectropomus spp* detected sixteen loci. Of the sixteen loci only three polymorphic loci such as a case in Table 2. Locus 6-phosphogluconate dehydrogenase (6-PGD), Locus Glukosafosfo isomerase (GPI-2), and Locus phosphoglucomutase (PGM) polymorphic only in *Plectropomus oligacanthus*. The

level of polymorphism of each population that is *Plectropomus leopardus* (P = 0), *Plectropomus maculatus* (P = 0), and *Plectropomus oligacanthus* (P = 0.188). The average number of allel per locus for *Plectropomus leopardus* and *Plectropomus maculatus* is 1 and *Plectropomus oligacanthus* 1.154. Three loci polymorphism allele frequencies of three *Plectropomus spp* population (Table 3). *Plectropomus oligacanthus* follow Weinberg Hardiy at the locus 6-PGD, Gpi-2, and PGM (<0.05). Heterozygosity each locus (He) and average heterozygosity show that in *Plectropomus oligacanthus*, three loci heterozygote obtained, namely 6-PGD (He = 0.180), GPI-2 (He = 0.095), and PGM (He = 0.095). In *Plectropomus leopardus* and *Plectropomus maculatus* no locus heterozygote but everything is homozygote. On average heterozygosity *Plectropomus oligacanthus* is H = 0.023.

Number of allel per locus for *Plectropomus oligacanthus* is 1.154. As for *Plectropomus maculatus* and *Plectropomus leopardus* allele number for each locus is 1.000. Genetic variation *Plectropomus maculatus*, *Plectropomus leopardus*, and *Plectropomus oligacanthus* can be seen in Table 4. The genetic distance (D) (Table 5), was calculated by the formula Nei showed genetic differences between species [6-8].

Table 3. Genetic variation P. leopardus, P.maculatus, and P.oligacanthus.

Parameter	*P.leo*	*P.mac*	*P.oliga*
Number of Samples	10	10	10
Total Locus observed	16	16	16
Total Locus Polymorphic	0	0	3
Polymorphic Degrees	0	0	0188
The number of allel per locus	1	1	1,154
Heterozygosity (H)	0	0	0023

The genetic distance (D) (Table 4), calculated by formula Nei showed genetic differences between species [8].

Table 4. Genetic distance (D) Inter-Species.

Inter Species	Genetic distance (D)
P. leopardus - P. maculatus	0134
P. leopardus - P.oligacanthus	0196
P. maculatus - P. oligacanthus	0200

Pletropomus leopardus and *Plectropomus maculatus* relatively close compared with *Plectropomus oligacanthus*,

From the results obtained UPGMA clustering with genetic distance matrix and grouping of species based on proximity to each other dendogram presented in Figure 4.

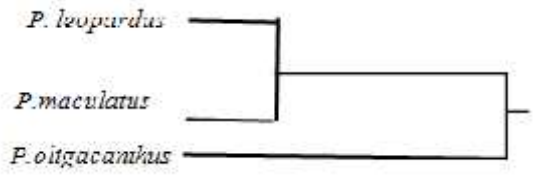

Figure 4. Genetic distance based on character dendogram enzyme.

Based on the genetic distance dendogram (Figure 4) shows that *Plectropomus leopardus* with *Plectropomus maculatus* has a genetic distance closer (D = 0.134) than Pletropomus leopardus with *Plectropomus oligacanthus* (D = 0.196). While the genetic distance *Plectropomus maculatus* with o *Plectropomus oligachantus* most distant family links (D 0.200).

Harris and Hopkinson, the primary function of the buffer is to support the change of pH during electrophoresis process, which is as sour on the positive pole (anode) and a base in the camp negative (cathode) [4]. The level of pH changes directly related to the duration of electrophoresis progresses, the voltage used and the electric current in the circuit. The buffer must have the required pH and enzyme must have stability and strength to neutralize the pH changes at the electrodes. Besides, the low voltage will produce a good resolution [9]. The electrophoresis result of liver and muscle tissue three species *Plectropomus spp,* used twelve enzyme detected 16 loci. Population inhabiting the same habitat or sustainable will reveal many similarities both phenotypic and genetic and among far-flung populations in different habitats will reveal many differences [10].

4. Conclusion

The highest genetic variation of the three species of grouper sunu is in *Plectropomus oligacanthus*. *Plectropomus leopardus* with *Plectropomus maculatus* have a close genetic relationship. Hybridization can breed *Plectropomus oligacanthus* with *Plectropomus leopardus* for increasing quality of seed fish.

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