An eBook on Marine Biology and Aquaculture

Chapter 1

A Comparative Study on Genetic Diversity of *Rachycentron Canadum* between Aquaculture and Wild Populations by Microsatellite Loci Analysis

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Abstract

Mei-Chen Tseng, Yan-Horn Lee, Tsair-Bor Yen (2020) The cobia, *Rachycentron canadum* is one of high economic fishes because of its rapid growth rate and a good adaptability in marine cage culture. Its artificial breeding technology has been matured; hence, most farms used their own inbreeded offspring as the seed fish for propagation program. A good genetic monitoring program can prevent the problems of inbreed due to recessive traits as well as reduce the impact of genetic decline. In the study, to understand the genetic deficiency in the aquaculture population of cobia, microsatellite loci were used to analyze the genetic diversities of the wild, distant relatives, and inbreeding samples. Each 30 specimens of distant relative and inbreed cobia were provided from Tungkang Biotechnology Research Center and 30 wild specimens were collected from Taiwan water by commercial fishing. The wild

samples revealed the highest mean values of number of alleles (*na* = 7.36 ± 3.11), effective number of alleles (*ne* = 3.88 ± 1.85), observed heterozygosity (H_O = 0.712 ± 0.161), and expected heterozygosity (H_E = 0.695 ± 0.147), while the inbreed sample had the lowest mean values of *na* = 5.46 ± 2.51, *ne* = 2.85 ± 1.24, H_O = 0.552 ± 0.234, H_E = 0.596 ± 0.172, and the highest inbreeding index (F_{IS} = 0.058) among three cobia samples. In conclusion, the results showed that genetic weakening occurred in aquaculture samples. In the future, it must be noted that following the principle of genetics to increase the genetic polymorphism of cobia and improving the selection and elimination of seeds is important for sustainable development of aquaculture farm.

Keywords: Cobia; Genetic Weaken; Inbreed; Observed Heterozygosity; Sustainable Development

1. Background

The cobia, *Rachycentron canadum* (Linnaeus, 1766) is a diurnal migratory fish which are mainly distributed in the Caribbean, the Atlantic, the Indian Ocean, and the Western Pacific [1]. The mechanism of natural spawning was established in the artificial breeding environment [2], and a yearling cobia can reach 6-10 kg in a proper condition was pointed out [3]. The complete aquaculture established and the fast-growth rate make cobia be one of Taiwan's major cage culture fish. In addition, many countries, including Australia, the United States, Dominican Mexico, Mexico, and Brazil, have been actively developing the cage culture of cobia [4].

Most seed fishes of the cobia at aquaculture farm in Taiwan had originated from the offspring of the wild population which was caught from Taiwan water in 1991. After more than ten generations, inferior qualities of fertilized eggs, *e.g.*, low fertilization ratio, low hatching ratio, and small egg diameter, were observed on propagation of cobia at farm; moreover, high malformation ratio, low starvation intolerance, slow growth rate, decrease disease resistance, and precociousness were also noticed [5]. Therefore, these inbreeding weaknesses had occurred in many aquaculture species as well. For example, the offspring of coho salmon *Oncorhynchus kisutch* (Walbaum, 1792) from inbreeding, their harvest weight declines by 10% in each generation [6]. Similarly, the inbreeding of white shrimp, *Litopenaeus vannamei* (Boone, 1931) leads to notable decrease of growth rate. Moreover, the inbreeding depression was more severe in more stressful environments [7,8]. In the present, inbreeding of cobia also has resulted in decrease of the total production of the cobia aquaculture industry in Taiwan.

In recent years, molecular genetic technology has been effectively applied to the breeding of many important economic aquatic organisms. Microsatellite DNA is a predominantly short (1-6 bp) and tandem repeated DNA sequences [9,10] which has many conveniences for studying

genetics, such as extensive distribution in the genome, easily been screened, high levels of genetic variability, codominant, parental inheritance, requiring only small amount of tissue, and uncomplicated analysis procedure. Therefore, many researchers today use microsatellite DNA as a genetic marker to investigate aquatic animal genetic diversity [11,12,13]. In the study, microsatellite loci were screened from the genome of the cobia and examined the genetic diversities among the inbreeding, distant relatives, and wild samples. A management strategy of aquaculture farm will be suggested based on these results.

2. Materials and Methods

2.1. Sampling

In total, 60 cobia specimens including distant relatives and inbreeding offspring were collected from Tungkang Biotechnology Research Center, Fisheries Research Institute of Taiwan in 2015. All 30 wild specimens of cobia were caught from southeastern Taiwan water.

2.2. Genomic DNA Purification

Muscle tissues from 90 specimens were preserved in 95% ethanol until DNA purification. A good quality and the quantity of genomic DNA was isolated and purified from muscle tissue of one wild individual for microsatellite library preparation. 0.5 grams of tissues with 1 mL lysis buffer was digested with 55 μL proteinase K solution [10 mM Tris-HCl (pH 8.0), 2 mM ethylenediaminetetra-acetic acid (EDTA), 10 mM NaCl, 1% sodium dodecylsulfate (SDS), 10 mg/mL dithiothreitol (DTT), and 0.5 mg/mL proteinase K]. DNA extraction was carried out using the method [14]. To conveniently isolate small amounts of genomic DNA from all specimens for genotyping, a Puregene core kit A (Qiagen, Valencia, CA, USA) was used in this study.

2.3. Screening of Microsatellite and Sequencing

High molecular weight genomic DNA of one individual was digested with the *Alu* I, *Hae* III, and *Rsa* I restriction enzymes (BioLabs, Ipswich, MA, USA) according to the manufacturer's instructions. Fragments of 200–800 nt were gel-purified using a Genemark DNA Clean/Extraction kit (Genemark Technology, Tainan, Taiwan) and then ligated to the *Sma* I blunt site of pUC 18 vector (New England BioLabs, Ipswich, MA, USA). Recombinants were transformed to a competent *Escherichia coli* DH5α strain. The library was plated onto 2YT medium plates, and colonies were lifted onto Whatman filters. These plates were placed in an incubator until the colonies were restored. All filters were dry in oven at 80°C for 2 hours and then preserved in -20 °C freezer until hybridization. Filters immersed in freshly prepared denaturing solution (0.5 M NaOH and 1.5 M NaCl) for 7 min, twice in neutralizing solution

(0.5 M Tris-HCl and 1.5 M NaCl, at pH 8.0) for 3 min, and in 2–4× SSC for 4 min. Probes used for hybridization were biotinylated oligo DNA $(GT)_{10}$ and $(GA)_{10}$. Hybridization was carried out overnight at 60 °C using a hybridization oven. After hybridization, filters were washed twice in primary washing buffer (1× SSC and 0.2% SDS) at room temperature, washed once in secondary washing buffer (0.5× SSC and 0.2% SDS) at room temperature, and washed twice in third washing buffer (0.1× SSC and 0.2% SDS) at 60 °C. These filters were immersed in blocking solution (Amersham Biosciences, Piscataway, NJ, USA) for 30 min and then washed three times in TBS buffer (0.05 M Tris-HCl and 0.15 M NaCl, at pH 7.5) for 10 min. The filters were incubated in 10 mL TBS buffer with 2 μL streptavidin-alkaline phosphatase (1 mg/mL) and 0.05% Tween 20 for 1 hour and then washed three times in TBS buffer for 10 min each. Deep-blue colonies were visualized by immersion in an NBT/BCIP solution for 1 hour. Positive clones were incubated, plated, and screened again. Final plasmid DNAs were isolated from positive colonies and were sequenced on an Applied Biosystems automated DNA sequencer 377 vers. 3.3 (ABI, Foster City, CA, USA) using a Bigdye sequencing kit (PE Applied Biosystems, Wellesley, MA, USA). T7 and SP6 primers were used in the sequencing reaction, and the PCR cycle parameters for sequencing were 35 cycles of 30 s at 95 °C, 30 s at 50 °C, and 1 min at 72 °C.

2.4. Primers Design and Genotyping

Paired primers of 18–22 nt long were designed using DNASTAR Primer Select software (vers. 4.0) (DNASTAR, Madison, WI, USA) for the 22 clones containing perfect and interrupted repeat sequences. A primary test of PCR was performed in a volume of 25 μL including ~10 ng genomic DNA, 10 pmol of the forward and reverse primers, 25 mM dNTP, 0.05–0.1 mM $MgCl_2$, 10× buffer, and 0.5 U *Taq* polymerase (Takara Shuzo, Tokyo, Japan) with Milli-Q water. The PCR products were subjected to a 1.5% agarose gel, and allelic sizes were checked by comparison with a DNA ladder and the length of the original sequence. After primary test, eight loci were specific amplification. Their forward primers were labeled with FAM, TAMRA, ROX, and HEX fluorescence tags. PCR amplifications were carried out in a Px2 Thermal Cycler (Thermo Fisher Scientific, Waltham, MA, USA) with the following temperature profile: 1 cycle of 95 °C for 4 min, followed by 38 cycles of 94 °C for 30 s and annealing at 56–64 °C for 30 s and 72 °C for 30 s. Each 5 μL of PCR product from three loci labeled with the different fluorescence tags were mixed and precipitated with 95% alcohol. Semiautomated genotyping was performed using a capillary ABI 3730XL DNA Analyzer (ABI). Genotypes were scored with GeneMapper 4.0 (ABI).

2.5. Data Analysis

Except the eight microsatellite loci cloned from this study, three previous published loci Rca 1-A11, B12, and E11 [15] were also genotyped for all samples. The total number of alleles

(*na*) and effective allelic numbers were estimated for each locus using the program, Popgene [16]. Observed (H_O) and expected (H_E) heterozygosities were independently calculated for each locus [17]. Deviations from Hardy-Weinberg expectations (HWEs) were examined by an exact test using GENEPOP [18]. Genetic differentiation index (F_{ST}) between samples and inbreeding index (F_{IS}) within each sample were estimated using Arlequin software [19]. Results of factorial correspondence analysis (*FCA*) performed in *Genetix* [20,21], showing multivariate relationships among samples using microsatellite variations.

3. Results and Discussion

3.1. Screening of New Microsatellite Loci

Among 1982 clones screened, 74 had positive signals. After sequencing, only 37 different microsatellite sequences were determined in this study. All 37 sequences include 21 GT, 1 GA, and incidentally 1 TA perfect microsatellites; the remainder contained both 13 interrupted and 1 compound microsatellite sequences. In fishes, GT repeats are more frequent than GA repeats [22], as was also found in this study. Microsatellites were distinguished into three categories based on the composition of major region: perfect, interrupted, and compound repeats [23]. In the study, most of cloned microsatellites consist mainly of perfect repeat sequences (23 of 37 microsatellite sequences, or approximately 62%). It is clear that perfect microsatellites were primarily selected for the study due to more easily count allelic sizes than interrupted and compound microsatellites. Classically, two allelic mutation models of these perfect microsatellites mainly have been considered [24], the infinite allele mutation (IAM) model [25] and the stepwise mutation model (SMM) [26]. The IAM predicts that mutation will lead only to new allelic states and may involve any number of repeat units. In contrast, the SMM predicts that mutation occurs through the gain or loss of a single repeat unit [27]. However, these mutant models of microsatellite loci were not determined in the present result. For many fish species, the number of alleles and heterozygosity values are considerably larger than those observed in mammals [27]. Microsatellite loci are useful molecular tools for studying population genetics and monitoring genetic variation for fish. Hence, microsatellites were cloned from cobia for application in examining genetic diversities among culture and wild populations.

Primers were designed for only 22 of the 37 microsatellite sequences. The remaining microsatellites sequenced were not used owing to the occurrence of a repeated sequence in one of the flanking region, the short sequences (< 90 bp, limited by the restriction site) and complicated repeat sequences. Primer sets of five loci did not produce amplifications as designated (null allele) which were probably caused by different molecular structures or unknown factors, even the extra efforts were made with different concentrations of $MgCl_2$, annealing temperatures, and other PCR conditions. After electrophoresis on 2% agarose gels,

the six loci still gave nonspecific and inaccurate size PCR products. Lastly, we succeeded in testing specific amplification on 11 loci that yielded a consistent PCR product corresponding to a single locus of the expected size. The primary test for genetic polymorphisms of these 11 loci in the randomly selected 30 individuals revealed that three loci have the single genotype; the remaining eight loci which contain six perfect and two interrupted microsatellites were variable. The individually designed primer sequences and annealing temperatures of PCR were able to effectively and accurately amplify the above eight new cloned loci shown in **(Table 1)**.

Table 1: Cloned microsatellite code, label fluorescence, designed forward (F) and reverse (R) primers, cloned sequence length, composition of major region, and annealing temperatures (T_a) of polymerase chain reaction in the study.

Locus	Fluorescence labeling	Primer sequence (5'→3')	Length of cloned allele (bp)	Major repeats	T_a (^{0}C)
COMS-1	HEX	**F:**TCCACCCTGTTTCTCTTGC	164	$(CA)_{23}$	56
		R:CCAACGACCCTATTATTGAC			
COMS-11	FAM	**F:**CTAGTGGCTGCCTTGAAGTC	175	$(GT)_{16}$	64
		R:GCCGCAGTGACAACAAATC			
COMS-14	ROX	**F:**GGTGTTCATAACTAGACCTCAC	162	$(CA)_{34}CT(CA)_3$	64
		R:AATCAATGTGCAAGAGCC			
COMS-15	TAMARA	**F:**TCCAACAGTTTACCGTCACC	225	$(GT)_8GC(GT)_{11}$	64
		R:CACTTCACAGTCTCAGACCTG			
COMS-17	ROX	**F:**ACCCAATGACTGCTAACACC	112	$(CA)_{20}$	58
		R:GAGCAACCGCTGTTTACATAC			
COMS-18	TAMARA	**F:**GATGTGAAACAGAACGCCTG	133	$(CA)_{13}$	64
		R:GAAGGCGTTGGTTGATTGAG			
COMS-20	HEX	**F:**GTGGTCAGCTGAATGAGATC	250	$(CA)_{19}$	58
		R:AGATAGGCAAGTAAGGGAGG			
COMS-22	HEX	**F**:TGTGACTGCTCATGTGAAGC	111	$(GT)_{13}$	60
		R:CTCATTTCTCCTGTTTGCCG			

3.2. Genetic Variations of Microsatellite Loci

Except for eight cloned microsatellite loci, three previously developed most polymorphic loci Rca 1-A11, Rca 1- B12, and Rca 1-E11 [15] also were used in this study. All allelic characters of 11 loci from a total of 90 cobia specimens were examined. The number of alleles of 11 loci ranged from 5 (COMS-20, COMS-22 & Rca 1-B12) to 15 (Rca 1-A11) **(Table 2)**. The sizes of the alleles in these 11 loci ranged from 96 bp (COMS-17) to 250 bp (COMS-20). The repeated number of dinucleotide in the motif region of all loci ranges from 5 to 41. The mutations at these eight microsatellite loci are due to the major change of copy number in the 2-bp repeat unit.

Table 2: Number (*na*) and size (bp) of alleles per locus, number of effective alleles (*ne*), observed heterozygosity (H_O) and expected heterozygosity (H_E) of cobia (N= 90). Accession numbers of microsatellite sequences also were list.

Locus	Range of allele size (bp)	number of repeats	*na*	*ne*	H_O	H_E	Accession no.
COMS-1	152-180	17-31	14	5.89	0.822	0.835	MF346860
COMS-11	171-181	14-19	6	3.57	0.589	0.724	MF346861
COMS-14	148-170	30-41	11	6.50	0.667	0.851	MF346862
COMS-15	211-231	12-22	8	4.02	0.867	0.755	MF346863
COMS-17	96-118	12-23	7	2.58	0.433	0.616	MF346864
COMS-18	129-145	11-19	7	1.76	0.511	0.435	MF346865
COMS-20	230-250	9-19	5	2.43	0.433	0.592	MF346866
COMS-22	103-113	9-14	5	1.88	0.533	0.471	MF346867
Rca 1-A11	161-193	11-27	15	4.37	0.967	0.776	AY721673.1
Rca 1-B12	174-182	5-9	5	2.04	0.600	0.512	AY721674.1
Rca 1-E11	165-191	8-21	10	4.28	0.678	0.771	AY721680.1
Mean			**8.45**	**3.56**	**0.646**	**0.667**	

A total of 93 alleles from 11 loci were observed in all 90 specimens. Twenty nine alleles were private **(Table 3),** i.e. found in only one sample. The frequency of the major alleles across the above 11 loci ranged within 90 specimen from 23.89% (COMS-14, allele F) to 73.87% (COMS-18, allele C). Observed heterozygosity (H_O) across 11 loci ranged from 0.433 (COMS-17 & 20) to 0.967 (Rca 1-A11), with a mean of 0.646, which is slightly smaller than the expected heterozygosities (H_E) of 0.435 (COMS-18) to 0.851 (COMS-14), with an average of 0.667. The average number of alleles per locus in the cobia was estimated to be 8.45 which is lower than those of anadromous fishes (10.8), marine fishes (19.9), and other freshwater fishes (9.1) [28]. The reason causing low allelic number may be population depression or fewer sampling size. In the previous report, genetic polymorphisms of 33 microsatellite loci were assessed for 24 individuals of cobia and showed an average number 7.1 of alleles per locus (range, 2-17), average H_O of 0.496 (range, 0-1), and average H_E of 0.563 (range, 0.043-0.943) [29]. However, mean number of alleles across loci in cobia population from the Gulf of Thailand and Andaman Sea ranged from 13.7 to 17.7 [30]. By contrast, the mean H_O (0.646) of cobia in this study is significantly smaller than the average of 0.77 for several species of marine fishes [28], but the mean H_O (0.779) of cobia reported [30] is similar to the result [29]. However, the mean numbers of alleles per locus and H_O in the study are lower than the results of previous studies [29] [30]. This might be due to the cobia in this study contained the inbreeding and distant relative samples from aquaculture farm where those cobia tend to have lower genetic diversity within their populations.

Table 3 Number and frequencies of all alleles in 11 polymorphic microsatellite loci from inbred (I), distant relatives (D), and wild (W) populations of cobia. Chi-square test for Hardy-Weinberg equilibrium; * indicated significance at α level of 0.05. Gray highlight are the private allele.

	COMS-1			COMS-11			COMS-14			COMS-15			COMS-17			COMS-18		
	I	D	W	I	D	W	I	D	W	I	D	W	I	D	W	I	D	W
Allele A			0.017	0.017	0.017	0.233			0.067			0.183			0.133	0.100	0.217	0.033
Allele B			0.033	0.067	0.217	0.033			0.067	0.033	0.017	0.017			0.150		0.033	
Allele C			0.017	0.250	0.183	0.417	0.017		0.067	0.133	0.150	0.183	0.033	0.050		0.833	0.650	0.733
Allele D		0.050		0.583	0.483	0.183			0.067	0.333	0.417	0.167	0.283	0.067	0.417	0.033	0.017	0.050
Allele E	0.133	0.217	0.183	0.033	0.017	0.033	0.033	0.033	0.283	0.017	0.017	0.117	0.683	0.750	0.250			0.017
Allele F	0.150	0.050	0.067	0.050	0.083	0.100	0.267	0.350	0.100	0.050	0.033	0.050		0.083	0.050	0.033	0.083	0.117
Allele G	0.367	0.317	0.250				0.050	0.167	0.167	0.433	0.367	0.250		0.050				0.050
Allele H	0.100	0.083	0.100				0.033	0.033	0.033			0.033						
Allele I			0.050				0.317	0.283	0.067									
Allele J			0.167				0.167	0.050	0.067									
Allele K	0.017		0.017				0.117	0.083	0.017									
Allele L	0.100	0.233	0.017															
Allele M	0.133	0.050	0.067															
Allele N			0.017															
Allele O																		
na	7	7	13	6	6	6	8	7	11	6	6	8	3	5	5	4	5	6
ne	4.70	4.63	6.77	2.43	3.11	3.65	4.59	4.13	6.84	3.12	3.01	5.71	1.82	1.73	3.59	1.42	2.09	1.79
H_O	0.600*	1.000*	0.867	0.633	0.500*	0.633	0.600*	0.733	0.667*	0.867*	0.800	0.933	0.167*	0.500	0.633*	0.333	0.700	0.500
H_E	0.801	0.797	0.867	0.599	0.690	0.738	0.796	0.771	0.868	0.691	0.679	0.839	0.459	0.428	0.733	0.298	0.531	0.450

	COMS-20			COMS-22			Rca 1-A11			Rca 1-B12			Rca 1-E11		
	I	D	W	I	D	W	I	D	W	I	D	W	I	D	W
Allele A			0.033			0.067	0.133	0.067		0.083	0.183	0.200	0.517	0.367	0.133
Allele B	0.267	0.200	0.617	0.017		0.017	0.283	0.100	0.050	0.717	0.683	0.617			0.017
Allele C			0.050	0.733	0.700	0.600	0.033	0.183	0.017			0.017	0.033	0.167	0.017
Allele D	0.533	0.750	0.283	0.250	0.233	0.317		0.033	0.050	0.017	0.017	0.167	0.283	0.333	0.300
Allele E	0.200	0.050	0.017		0.067		0.017	0.033	0.033	0.183	0.117				0.050
Allele F							0.333	0.467	0.483				0.017		0.317
Allele G							0.033	0.017					0.150	0.067	
Allele H							0.033	0.050	0.217						0.117
Allele I							0.067	0.033	0.017						0.050
Allele J									0.017					0.067	
Allele K							0.033								
Allele L									0.017						
Allele M							0.017		0.050						
Allele N									0.050						
Allele O							0.017	0.017							
na	3	3	5	3	3	4	11	10	11	4	4	4	5	5	8
ne	2.53	1.65	2.15	1.67	1.82	2.15	4.57	3.67	3.42	1.80	1.94	2.23	2.70	3.54	4.40
H_O	0.400*	0.333*	0.567	0.467	0.600	0.533	1.000	0.933	0.967	0.467	0.600	0.733*	0.533	0.700	0.800
H_E	0.615	0.402	0.545	0.406	0.459	0.544	0.794	0.740	0.719	0.453	0.494	0.561	0.640	0.730	0.786

3.3. Genetic Diversities in Culture and Wild Samples

Total allele number of 11 microsatellite loci ranged from 60 (inbred sample) to 81 (wild sample). All allelic frequencies of each microsatellite loci in each sample were listed in Table 3. Expect for COMS-11, Rca 1-A11, and Rca 1-B12 loci, eight loci have more allelic numbers in wild samples than in two aquaculture samples. The observed heterozygosity (H_O) ranged from 0.167 (COMS-17) to 1 (Rca 1-A11) with an average 0.552 and the expected heterozygosity (H_E) ranged from 0.298 (COMS-18) to 0.801 (COMS-1) with an average 0.596 in inbreed samples of cobia. In the distant relative sample of cobia, the H_O ranged from 0.333 (COMS-20) to 1 (COMS-1) with an average 0.673, while the H_E ranged from 0.402 (COMS-20) to 0.797 (COMS-1) with an average 0.611. On the other hand, the H_O in wild sample ranged from 0.5 (COMS-18) to 0.967 (Rca 1-A11) with an average 0.712 and the H_E ranged from 0.45 (COMS-18) to 0.868 (COMS-14) with an average 0.695 **(Table 3 & 4)**. The highest averages of H_O and H_E appeared in the wild sample, while the inbreed sample has the significantly lowest H_O and H_E (Table 4). However, it is certain that two cobia samples from the aquaculture farm present lower genetic diversities and allelic numbers than the wild cobia sample. The inbreeding coefficient (F_{IS} = 0.058) found in the inbreed sample indicated a significant inbreeding effect **(Table 4)**. The results showed that the genetic similarity among the samples varied from 0.784 (distant relatives vs. wild) to 0.958 (distant relatives vs. inbreed). The genetic distances among the samples was from 0.043 (distant relatives vs. inbreed) to 0.243 (distant relatives vs. wild) suggested genetic differences have occurred between wild and aquaculture samples. A FCA of the 11 loci clustered the genotypes into two distinct groups **(Figure 1)**. The clustered circles of inbreed and distant relative samples partially overlapped. The major axe 1 occupied 80.07%.

Table 4: Averages of allelic numbers (*na*), effective allelic numbers (*ne*), observed heterozygosity (H_O), expected heterozygosity (H_E) of wild, distant relative, and inbreed samples and inbreeding index (F_{IS}) from 11 microsatellite loci analyses of cobia.

	F_{IS}	*mean*			
		na	***ne***	H_O	H_E
Wild	-0.041	7.36 ± 3.11	3.88 ± 1.85	0.712 ± 0.161	0.695 ± 0.147
Distant	-0.120	5.55 ± 2.02	2.85 ± 1.06	0.673 ± 0.195	0.611 ± 0.149
Inbreed	0.058	5.46 ± 2.51	2.85 ± 1.24	0.552 ± 0.234	0.596 ± 0.172

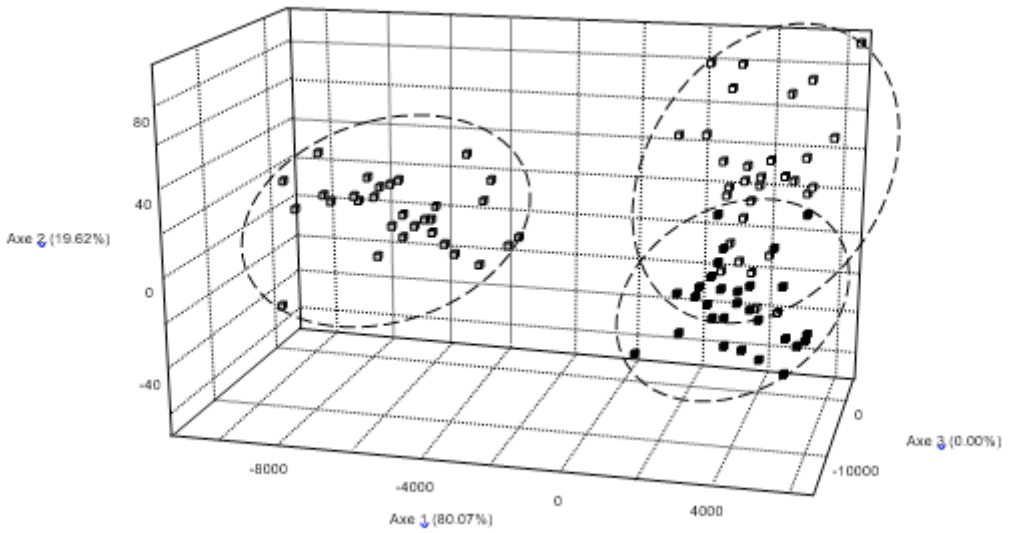

Figure 1: Factorial analysis of the correspondence of inbreed (black box), distant relative (white box), and wild (gray box) specimens using eleven microsatellite loci. Each point represents a multilocus genotype, and each circle represents the distribution boundary of specimens from the same population.

4. Conclusion

A total of 11 microsatellite loci were used in examining genetic diversities among wild, distant relative, and inbreed samples. In which, eight polymorphic GT/GA microsatellites were cloned from this experiment and three loci were previously published. The lowest averages of allelic numbers (*na*) and observed heterozygosity (H_O) occurred in inbreed samples from aquaculture farm as well as had the highest inbreeding index (F_{IS}) than other two samples. Nevertheless, the maximum amount of private alleles and the highest genetic diversity took place in the wild population. Results clearly indicated that markedly genetic weakening occurred in aquaculture samples. In the future, it is suggested that the breeding program should increase the genetic polymorphism of cobia by improving the selection and elimination of seed cobia to achieve the healthy and sustainable aquaculture farming.

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6. Authors' contributions

Yan-Horn Lee designed the study, and was responsible for collecting specimens for the study. Tsair-Bor Yen performed the statistical analyses and assisted writing the text. Mei-Chen Tseng performed genotypes analyses, wrote and submitted the manuscript. Yan-Horn Lee, Tsair-Bor Yen, and Mei-Chen Tseng participated in revising the manuscript. All authors read and approved the final manuscript.

7. Competing interests

Mei-Chen Tseng has received research grant from the MOST, Taiwan. All authors, Mei-Chen Tseng, Yan-Horn Lee, and Tsair-Bor Yen declare that they have no conflict of interest.

8. Availability of Data and Materials

All microsatellite loci cloned in the study had submitted to NCBI with accession nos. MF346860-67.

9. Ethics Approval Consent to Time Feed: All procedures in the research conducted in the manuscript have followed the ethic standards of the responsible committee on laboratory animal experiment by IACUC of National Pingtung University of Science and Technology (Approved no. NPUST-IACUC-102-013, Issued date: August 2013, Expired date: July 2017).

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