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Microsatellites reveal genetic structures of the slipper-shaped oyster, *Magallana bilineata* (Röding, 1798), populations in central Philippines

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Abstract

In the Philippines, the economically important slipper-shaped oyster *Magallana bilineata* (formerly *Crassostrea iredalei*) is valued for its tasty and creamy meat. Production of this shellfish in the country rely heavily on rearing natural spats through traditional techniques with focus on economically important traits. The need to advance culture technologies requires the use of genomic tools like microsatellites to preliminarily assess genetic structuring of oyster populations. Microsatellites are widely used low-cost molecular markers that can describe genetic variability among populations. Five identified microsatellite loci (CI.H09, CI.A08, CI.H10, CI.D01, and CI.B07) for *C. iredalei* were utilized to examine 8 oyster populations from Regions VI and VIII, central Philippines. Meat condition indices (CI) from morphometric and gravimetric data were used to correlate with the genetic information. Results from microsatellite analyses showed that all loci departed from Hardy-Weinberg Equilibrium (HWE) due to heterozygote excess with expected (*He*) and observed (*Ho*) heterozygosity ranging from 0.2096-0.7065 and 0.2333-0.9286, respectively. Negative inbreeding coefficients (F_{IS}) in several loci were observed in 7 populations with high *Ho* implying increased heterozygosity that is more likely to outbreed. There was little genetic divergence among all populations with a global F_{ST}=0.0086. These findings revealed a positive correlation between CI and *Ho* in each population, while a negative correlation was shown with its observed homozygosity.This study highlighted the potential application of microsatellite analysis for characterizing oyster populations from central Philippines, and identify markers that can be used to improve culture technologies, management practices and production.

Keywords: aquaculture, condition index, Hardy-Weinberg, population genetics

Introduction

Oysters (family Ostreidae) are among the extensively studied and cultivated shellfish due to their economic and ecological importance. Widely distributed in tropical and temperate regions worldwide, they inhabit various salinities from nearshore marine, estuarine, to brackish waters (Ruesink et al., 2005; Xia et al., 2009; Liu et al., 2011). As filter feeders, they are important ecosystem engineers reducing water turbidity, phytoplankton biomass, and suspended solids (Chang et al., 2016). Oyster assemblages also provide favorable habitats to various fish and invertebrates through nitrogen recycling and carbon sequestration (Grabowski et al., 2012). Moreover, oysters enable the stabilization and protection of these habitats as natural breakwaters (Scyphers et al., 2011).

Income from export and domestic sales of fresh oysters and oyster goods produces high market value providing more livelihood for fisherfolks as shown by the expansion of the oyster culture and farming industry over the last three decades (Laing & Bopp, 2019). In the Philippines, the slipper-shaped oyster or Philippine cupped oyster now known under a new name *Magallana bilineata* (formerly known as *Crassotrea iredalei*, Salvi and Mariottini, 2017), receives desirable farming attention as it grows at a faster rate with quality meat yield that is easily shucked from its straight shell margins (Samsin, 1988; Devakie & Ali, 2000). It ranked as the third most produced oyster species worldwide in 2004, with the Philippines identified as its major producer (Laing & Bopp, 2019). However, oyster production in the country has been erratic and reliant on wild harvest, and farmed through traditional culture practices according to the annual fishery report of the Philippine Bureau of Agricultural Statistics (2000-2017). A downtrend in production has been reported as an effect of red tide, water pollution and poor water quality, and frequent tropical cyclones destroying oyster farms that gradually deplete wild spats resulting in smaller oyster size harvest. With high demand and a booming oyster farming industry in many countries, oyster culture conditions are at risk from habitat degradation, diseases, poor water sanitation, detrimental species interactions, overharvesting, and environmental mismanagement (Ruesink et al., 2005; Gaffney, 2006).

Genetic marker-assisted selective breeding as a tool to better agriculture and improve livestock have gained popularity through the years (Williams, 2005; Collard & Mackill, 2008; Hollenbeck & Johnston, 2018). There are several selective breeding programs for mollusks using limited genomic tools (Astorga, 2014) and application still needs to be explored (Hollenbeck & Johnston, 2018). These genomic tools have found broad applications mainly in ecology, conservation efforts and taxonomy (Gaffney, 2006; Von Der Heyden et al., 2014; Yáñez et al., 2015). Genetic markers such as microsatellites are one of the widely used genomic tools. They are tandemly repeated motifs of variable lengths distributed throughout the genome in both coding and

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noncoding regions. Being highly polymorphic, codominant, and transferable between species, microsatellites potentially are the most informative genetic marker with the advantage of easy and low-cost detection by PCR (Chistiakov et al., 2006; Selkoe & Toonen, 2006). It has been used in association with phenotypic characteristics as a basis for marker-assisted selection of commercially important fish and shellfish species (Sun et al., 2015; Zhong et al., 2016; Xu et al., 2017). Other than *Crassostrea gigas* (now *Magallana gigas*), specific associations and/or correlations between genotypic and phenotypic characteristics of economically important oyster species have not yet been explored. In addition, studying genetic variation among populations has further biological implications. The deployment of selected enhancement stocks can improve degraded wild populations, and molecular markers such as microsatellites can be used to effectively monitor rehabilitation programs (Chauhan & Rajiv, 2010).

To date, no comprehensive study on the genetic structure of *M. bilineata* oyster populations using microsatellite markers have been done in the Philippines and little information is available using microsatellite markers to characterize populations of *Magallana* species globally. This study provides baseline genetic information associated with morphometric characteristics and meat condition index of *M. bilineata* populations in central Philippines using a low cost and easy method of microsatellite marker detection. Using identified microsatellite loci with sequences available in Genbank, sample oyster populations were assessed by PCR and population genetics described using conventional parameters: estimating genotypic heterozygosity, testing departures from HWE, and measuring genetic structure (F_{ST}) with inbreeding coefficient (F_{IS}). This assessment of genetic structure of oyster populations can preliminarily facilitate marker-assisted selection for selective breeding and stock restoration initiatives to foster advancements in oyster culture technologies and improve production.

Materials and Methods

Sample Collection

Oyster samples of 6-8 months old (as determined by the farmers) were randomly collected from eight different culture sites in central Philippines from 2014-2016 (see Table 1). These culture sites were only concentrated from the Philippine Regions VI and VIII. Region VI recorded the highest oyster production in the country. The culture sites are grouped according to provinces in each region with culture method and harvest practices noted. Culture methods include staking (S), hanging (H) (Samsin, 1988), and longline and raft (LR). Only Ivisan and Sapian (Capiz) farmers practice the staking method that uses individual bamboo stakes as spat collectors stabbed vertically in sediments of rivers and coasts. Other culture sites in Region VI follow the hanging method using empty oyster shells, old tires, and coconut husks as spat collectors tied to ropes that hang vertically in the water, each hanging from a long horizontal rope tied to bamboo poles at both ends. Samar oysters are farmed using longline and raft methods using bamboo poles tied together as rafts and with hanging spat collectors underneath. These rafts can freely float, tied to a specific location, and transferred by the farmer to the shore during bad weather conditions. Marketable harvest size of oysters according to farmers were from 6 to 8 months old counted from the time the spats attached to the collectors. Sample collection was assisted by the farmers in each culture site. Sampling permits were issued by the respective municipal/city agriculture offices.

Individual oysters were carefully brushed and separated from fouling organisms and any encrusting materials. The cleaned oysters were piled with moist towels in between stacks and stored on ice for transport. Oyster samples were kept alive during transport and were brought to the laboratory for DNA extraction and condition index determination.

Table 1. Surveyed culture sites in Visayas, Philippines from 2014-2016.

Culture Site	Province	GPS location	Culture method*	Marketable harvest (months)	Samples collected	
					Genetic analysis	Condition index
1. Sapian (Sap)		11^0 31’ 00.5“ N 122^0 35’ 12.5“ E	S	6-8	30	40
2. Ivisan (Ivi)	Capiz	11^0 32’ 10.5“ N 122^0 38’ 44.0“ E	S	6-8	30	20
3. Pan-ay (Pan)		11^0 35’ 20.2“ N 122^0 49’ 36.2“ E	H	6-8	30	40
4. Tarangan (Sam)	Samar	11^0 54’ 38.6“ N 124^0 48’ 08.2“ E	LR	6-8	30	40
5. New Washington (New)	Aklan	11^0 64’ 33.2“ N 122^0 42’ 29.3“ E	H	6-8	30	40
6. Batan (Bat)		11^0 35’ 41.30“ N 122^0 28’ 30.3“ E	H	6-8	30	40
7. Hinigaran (Hin)	Negros Occidental	10^0 16’ 36.5“ N 122^0 51’ 15.5“ E	H	4-12	30	40
8. Himamaylan (Him)		10^0 05’ 59.1“ N 122^0 52’ 02.9“ E	H	6-10	30	40
TOTAL					240	305

*abbreviations: S=Staking; H=Hanging; HS=Hanging and Staking; LR=Longline and Raft

Table 2. List of microsatellite markers.

Microsatellite	Primers	Tm (°C)	Repeat Unit	Accession Number
1. CI.H09	F: ACCAAGCGTAAAGCGGTAAA R: GTTTACAACTCCCAAAACGCTCAC	59.2	$(CT)_{22}$	JF833023
2. CI.A08	F: CAGTTGCTCGAGTGAAAACG R: GTTTCCATTGCAGGAATGTGGAAT	60.0	$(CT)_{15}$	JF833016
3. CI.H10	F: CCGTTGGAATAACTGCCAAA R: CAACGGCGCATAATTAGTGTT	56.1	$(GA)_{40}$	JF833024
4. CI.D01	F: CTTACGCGTGGACTAACTGAT R: TGAAAAATCCTCCAGCCATT	50.0	$(GT)_2(GA)_2(GT)_6$	JF833030
5. CI.B07	F: ATTGGTCCAGTGTTCTTGG R: GTTTGGGGTTTGTCCGACTCAAG	55.5	$(ACGG)_{16}$	JF833017

Sample preparation, morphometric data, and determining CI

Up to 40 samples from each culture site were dissected and the morphometric features measured. Before shucking the oysters, shell length and height of the left valve were measured to the nearest 0.1cm using a caliper. The samples were then allowed to dry in a drying chamber oven (Binder) at 40°C for 2-3 days. Total weight, shell weight, and body weight were measured before and after drying using a digital top loading balance to the nearest 0.01g. A gravimetric condition index protocol was used to compute CI using the following equations: CI1=Dry Tissue Weight/Wet Cavity Volume; CI2=Dry Tissue Weight/Dry Cavity Volume, and CI3=Dry Tissue Weight/Dry Shell Weight (Mercado-Silva, 2005).

DNA extraction

Thirty oyster samples from each culture site or population were dissected for genetic analysis. Genomic DNA was isolated from gonadal tissue using a modified phenol-chloroform-isoamyl alcohol (PCI) method and CTAB protocol (Caipang et al., 2011). Quality and quantity of extracted DNA was determined using Jenway Genova Nano UV-Vis spectrophotometer. DNA samples were stored at -20°C until further experimentation.

PCR amplification

Five identified microsatellite markers for *C. iredalei* (presently *M. bilineata*) sampled from Malaysia and obtained from registered sequences in GenBank database (https://www.ncbi.nlm.nih.gov/) were utilized as shown in Table 2. PCR primers and conditions were optimized for each microsatellite. Briefly, each reaction contained 50 ng DNA, 10 mM each forward (F) and reverse (R) primer, 10 mM dNTPs, 25 mM $MgCl_2$ (Vivantis), 500 um of Taq DNA polymerase (Promega) for 30 cycles using a MyCycler (Bio-Rad) thermal cycler. PCR products were visualized in 2.2% agarose gel electrophoresis as was done by Papetti et al. (2016) and Huvet et al. (2004) in detecting and confirming PCR fragments. These were viewed using Gel Doc XR+ (Bio-Rad) to discriminate the band fragments of the repeat regions. Analysis of fragment sizes was performed in the accompanying Image Lab Software.

Microsatellite analysis

Repeat regions of each microsatellite loci were determined from fragment size analysis and revealed as having 1 (homozygotes) or ≥2 (heterozygotes) bands with distinct sizes. Scoring was based on the number of fragments and its size for each sample in each population as previously described (Ashley & Dow, 1994). The highest number of alleles in each locus is equal to the total number of alleles found with allelic richness determined by comparing the number of alleles per locus in each population. The genetic parameters measured were estimation of genotypic heterozygosity based on allele frequencies, testing for departures from HWE, and determining genetic structure using F_{ST} with inbreeding coefficient F_{IS} (Brookfield, 1996; Wigginton et al., 2005; Holsinger & Weir, 2009; Meirmans & Hedrick, 2011). All genetic parameters were carried out using GENEPOP v3.4 (Raymond & Rousset, 1995; Rousset, 2008).

Statistical analyses

Differences in CI, allelic richness between populations, and between *He* and *Ho* was determined using Kruskal-Wallis test with Dunn's multiple comparison. Mean population CI was correlated with the observed heterozygosity and homozygosity inferred from each locus through a Pearson Correlation test (r-value). Data visualization and statistical analyses were performed at $p<0.05$ level of significance in GraphPad Prism version 7.0 for Windows, GraphPad Software, San Diego, California, USA.

Results

Population morphometric characteristics and CI

Morphometric measurements such as shell length, shell height, total weight and meat CI using three equations were determined (Fig 1). Aklan (Bat and New) and Samar oysters showed the highest morphometric characteristic shell length and total weight and were all significantly different from Negros Occidental (Hin and Him) samples. Capiz (Sap and Pan) oysters showed significantly highest shell height among other populations. Oysters from these sites also revealed comparable weight with Aklan and Samar samples. Negros Occidental oysters, however, showed very poor morphometric characteristics as exhibited by significantly smaller in dimensions and weight compared to other culture sites. Aklan (Bat and New) oysters had the highest CI across all CI equations, which significantly differed from other populations. Within Negros Occidental, Hin oysters were found to have the lowest CI, consistent with their small size and weight.

Microsatellite analysis

Five microsatellite loci previously developed for *C. iredalei* presently identified as *M. bilineata* were used to characterize the eight culture sites. The number of alleles per locus (N_A), expected (*He*) and observed (*Ho*) heterozygosity, as well as the inbreeding coefficient (F_{IS}) and genetic structure (F_{ST}) of the eight populations for the five microsatellite loci are summarized in Table 3. A total of 16 alleles were found in all 5 loci with CI.D01 having the most number of alleles (3 to 4) per locus. This was followed by CI.H09 and CI.H10 which showed 3 alleles per locus, and CI.A08 and CI.B07 with 2 to 3 alleles per locus. The locus CI.D01 showed 4 alleles in Capiz, Samar, and Negros Occidental populations. The lowest number of alleles were shown in CI.A08 having 2 alleles in Aklan and Negros Occidental populations, and in CI.B07 with 2 alleles in Pan-ay Capiz, Aklan and Negros Occidental populations In the mean alleles per locus, no significant difference among the populations and among the loci was observed. *He* ranged from 0.2096 (CI.A08, Sam) to 0.7065 (CI.DO1, Sap). New Washington samples had the highest mean *He* and was significantly different from Negros Occidental populations. *Ho* ranged from 0.2333 (CI.A08, Sam) to 0.9286 (CI.B07, New) with New Washington having the highest mean *Ho*, which significantly differed from oyster populations in Pan-ay

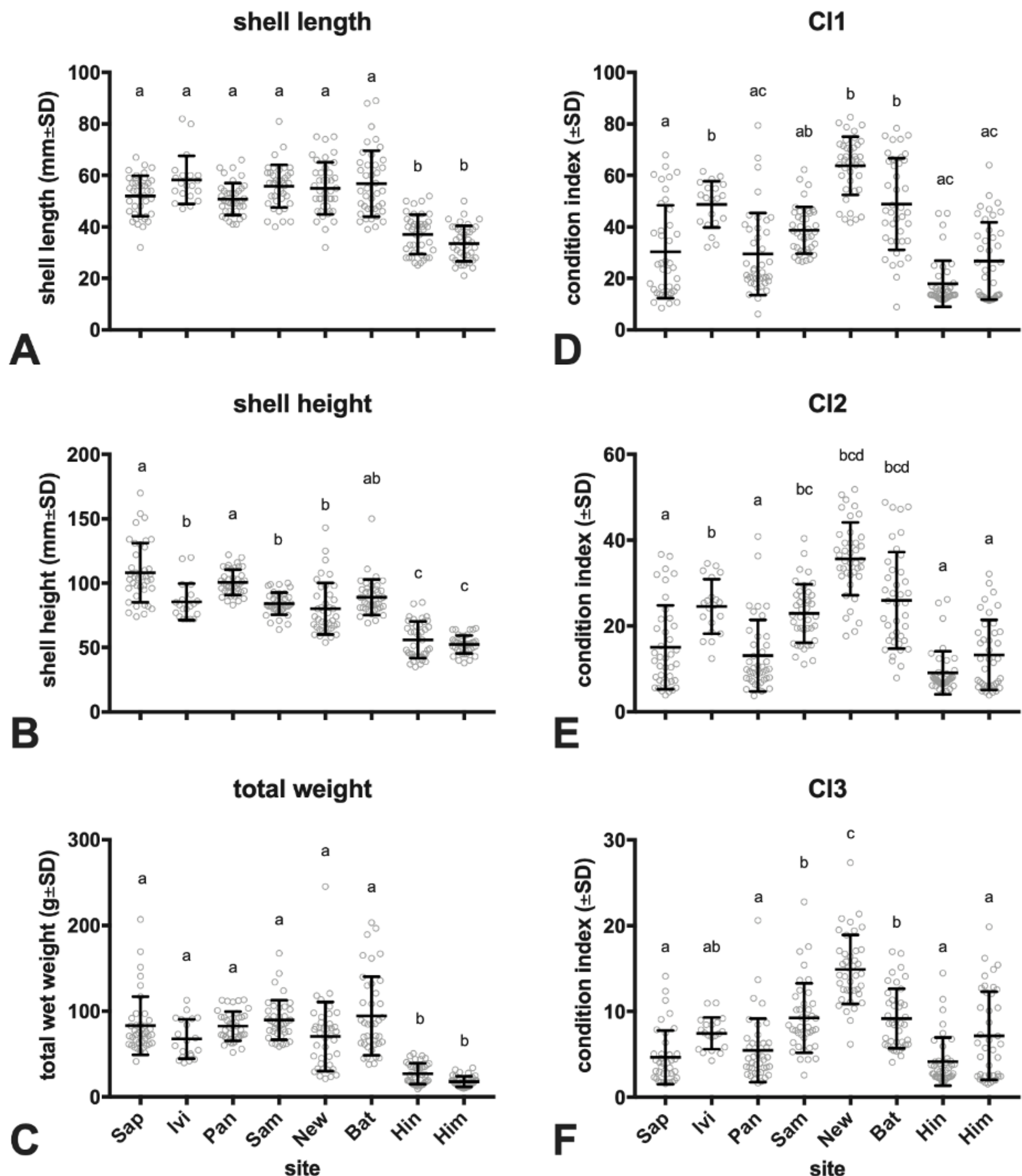

Figure 1. Morphometric data on (A) shell length, (B) shell width, and (C) total weight with corresponding (D-F) CI of *M. bilineata* samples from different populations in the Philippines. Lines indicate mean±SD with significant differences ($p<0.05$) between populations marked by different letters based on Kruskal-Wallis test with Dunn's multiple comparison.

and Negros Occidental. The global single-locus F_{ST} value was at 0.0086 with CI.B07 having the highest (F_{ST}=0.0247) and the lowest was with CI.D01 (F_{ST}=0.0037). Negative F_{IS} values were observed in almost all population-locus cases except in Ivi-CI.H09, Pan-CI.H09, New-CI.D01, Hin-CI.H09, and Him-CI.H10 with Hin-CI.H10 at F_{IS}=0. Departures from HWE was determined for the genotype frequencies using HWE exact test (Guo and Thompson, 1992) with estimation of exact p-values by the Markov chain method (1000 dememorization with 1000 batches at 10,000 iterations per batch). It was revealed that all five loci did not observe HWE. Among the 40 population-locus cases (8 populations and 5 loci), 9 significantly deviated from HWE – CI.H09 (New, Bat), CI.A08 (New), CI.H10 (New), and CI.B07 (New, Bat, Sap, Ivi, Him) due to heterozygote excess.

Table 3. Summary statistics of eight populations of *M. bilineata* screened for five microsatellite loci.

Population (N_o)		Microsatellite loci					
		CI.H09	CI.A08	CI.H10	CI.D01	CI.B07	Mean
Sapian (30)	F_{ST}	**0.0047**	**0.0057**	**0.0040**	**0.0037**	**0.0247**	**0.0086**
	N_A	3	2	3	4	3	3
	He	0.4084	0.3045	0.3669	0.7065	0.4721	0.4517
	Ho	0.4333	0.3667	0.4074	0.7777	0.6154	0.5201
	F_{IS}	-0.0620	-0.2083	-0.1128	-0.1030	**-0.3115***	0.1595
Ivisan (30)	N_A	3	3	3	4	3	3
	He	0.3226	0.3373	0.4604	0.6144	0.4891	0.4448
	Ho	0.3000	0.3667	0.4642	0.7083	0.6667	0.5012
	F_{IS}	0.0712	-0.0887	-0.0086	-0.1568	**-0.3724***	0.1395
Pan-ay (30)	N_A	3	3	3	4	2	3
	He	0.4367	0.3780	0.4085	0.4708	0.3620	0.4112
	Ho	0.4333	0.4333	0.4333	0.4737	0.4615	0.4470
	F_{IS}	0.0079	-0.1494	-0.0620	-0.0062	-0.2821	0.1015
Samar (30)	N_A	3	2	3	4	3	3
	He	0.4893	0.2096	0.4004	0.5706	0.5807	0.4501
	Ho	0.5333	0.2333	0.4074	0.6000	0.6250	0.4798
	F_{IS}	-0.0918	-0.1154	-0.0178	-0.0526	-0.0781	0.0711
New Washington (30)	N_A	3	2	3	3	2	3
	He	0.5153	0.4130	0.5153	0.6427	0.5065	0.5185
	Ho	0.6667	0.5667	0.6667	0.5455	0.9286	0.6748
	F_{IS}	**-0.3004***	**-0.3810***	**-0.3004***	0.1544	**-0.8621***	0.3997
Batan (30)	N_A	3	2	3	3	2	3
	He	0.4506	0.2750	0.4446	0.6106	0.4598	0.4481
	Ho	0.5806	0.3226	0.5000	0.6957	0.6818	0.5561
	F_{IS}	**-0.2950***	-0.1765	-0.1269	-0.1429	**-0.5000***	0.2483
Hinigaran (30)	N_A	3	2	3	4	2	3
	He	0.3678	0.2350	0.2759	0.5717	0.3214	0.3544
	Ho	0.3667	0.2667	0.2759	0.5862	0.3929	0.3777
	F_{IS}	0.0031	-0.1373	-0.0000	-0.0259	-0.2273	0.0787
Himamaylan (30)	N_A	3	2	3	4	2	3
	He	0.3466	0.2593	0.3612	0.5135	0.4442	0.3850
	Ho	0.3793	0.3000	0.3103	0.4737	0.6429	0.4212
	F_{IS}	-0.0961	-0.1600	0.1429	0.0795	**-0.4595***	0.1876
Average	N_A	3	2.4	3	3.6	2.4	3
	He	0.4171	0.3015	0.4041	0.5876	0.4545	0.4330
	Ho	0.4617	0.3570	0.4332	0.6076	0.6268	0.4973
	F_{IS}	0.1159	0.1771	0.0964	0.0902	0.3866	0.1732

Abbreviations: F_{ST}=single locus, N_o=number of samples, N_A=number of alleles per locus, *He*=expected heterozygosity, *Ho*=observed heterozygosity, and F_{IS}=inbreeding coefficient. Values in bold and with asterisk (*) have significant deviation (heterozygosity excess) from HWE based on exact HWE test (Guo & Thompson, 1992) and a Markov chain algorithm to estimate without bias the exact p-value (p<0.05) of the test. Expected numbers of heterozygotes and homozygotes were computed using Levene's correction. Values for F_{IS} and F_{ST} were estimated using Weir and Cockerham (1984).

Table 4. Condition index correlation with observed heterozygosity of the five loci.

Observed Heterozygosity	CI1		CI2		CI3	
	r	p-value	r	p-value	r	p-value
CI.H09	0.6395	0.0877	0.7028	0.0519	0.7882	**0.0201***
CI.A08	0.6144	0.1051	0.5443	0.1631	0.5556	0.1528
CI.H10	0.9393	**0.0005***	0.9133	**0.0015***	0.8375	**0.0095***
CI.D01	0.1895	0.6531	0.1753	0.6780	-0.1583	0.7081
CI.B07	0.8890	**0.0031***	0.8988	**0.0024***	0.8985	**0.0024***

abbreviations: CI1=Condition Index 1, CI2=Condition Index 2, CI3=Condition Index 3. Values with asterisk (*) are significantly different at $p<0.05$ in the two-sided test according to Pearson Correlation coefficient, r.

Table 5. Condition index correlation with observed homozygosity of the five loci.

Observed Homozygosity	CI 1		CI 2		CI 3	
	r	p-value	r	p-value	r	p-value
CI.H09	-0.6395	0.0877	-0.7028	0.0519	-0.7882	**0.0201***
CI.A08	-0.6144	0.1051	-0.5443	0.1631	-0.5556	0.1528
CI.H10	-0.9393	**0.0005***	-0.9133	**0.0015***	-0.8375	**0.0095***
CI.D01	-0.1895	0.6531	-0.1753	0.6780	0.1583	0.7081
CI.B07	-0.8890	**0.0031***	-0.8988	**0.0024***	-0.8985	**0.0024***

abbreviations: CI1=Condition Index 1, CI2=Condition Index 2, CI3=Condition Index 3. Values with asterisk (*) are significantly different at $p<0.05$ in the two-sided test according to Pearson Correlation coefficient, r.

The New Washington population among others exhibited the most number (4 of 5 loci) of significant departures from HWE. Among the loci, CI.B07 did not follow HWE in 5 of 8 populations.

Microsatellite-Condition Index Correlation

Both the genetic data on observed heterozygosity and homozygosity from the microsatellite loci were correlated to the CI of each of the 8 populations. Table 4 shows a summary of Pearson Coefficients (r) between the CI and loci. Positive correlations between microsatellite loci and CI were observed except for CI.D01 and CI 3. Among all the locus-condition index relationships, observed heterozygotes of the loci CI.H10 and CI.B07 showed significant positive correlation to all CI. Only CI 3, which measures dry tissue weight relative to dry shell weight showed significant positive correlations to loci CI.H09, CI.H10, and CI.B07.

Associations between the observed homozygotes of the 5 loci with CI are shown in Table 5. The same locus-condition index correlations shown in Table 4 showed significant correlations. However, based from the coefficients of each relationship, all CI revealed negative correlations with the microsatellite homozygotes except for CI.D01 and CI 3. Similar to the observed heterozygosity to condition index association, both CI.H09 and CI.B07 showed significant negative correlation in all CI, while only the CI 3 had a significant negative correlation with the CI.H09.

Discussion

Microsatellite markers have been previously used to describe oyster genetics and were applied as indicators of variability to infer levels of genetic diversity in natural populations (An et al., 2014; Xu et al., 2017). They are able to define population genetic structures as influenced by the number of subpopulations, frequency of alleles present in each subpopulation, degree of genetic isolation among subpopulations, and geographic distribution (Balloux & Lugon-Moulin, 2002; Väli et al., 2008). The present study collected slipper-shaped oysters from eight culture sites in the central islands of the Philippines to describe genetic structures of *M. bilineata* oyster populations based on previously identified microsatellite markers. The low-cost and simple PCR amplification method described in this study was able to discriminate microsatellite allelic diversity among the oyster populations with the estimation of allele frequencies based on band scoring during electrophoresis. These patterns of heterozygosity are measures of genetic variation with the expected and observed genotypes of homozygosity and heterozygosity estimated based on allele frequencies.

Departures from HWE among the studied microsatellite loci have been observed. The observed allele and genotype frequencies may have resulted from selective pressures caused by population fragmentation, migration, and selection (Wigginton et al., 2005). Departures from HWE are common in oyster populations and are mostly due to heterozygote deficits from inbreeding (Hedgecock et al., 2004; Li et al., 2006; An et al., 2014). Decreased heterozygosity reduces reproductive fitness leading to increased homozygote frequencies as mating is observed to be greater than expected by random mating. Such significant heterozygote deficits from HWE were also observed in *M. gigas* and *M. ariakensis,* (formerly *C. ariakensis*), as well as in *C. hongkongensis* (Hong Kong oyster), Pacific abalone (*Haliotis discus hannai*) and pearl mussel (*Margaritifera margaritifera*) (Li et al, 2006; Bouza et al., 2007; Xiao et al., 2010; An et al., 2014; Ma et al., 2021).

In contrast, this study describes heterozygote excess in eight *M. bilineata* populations relative to HWE, which are rare instances compared to the studies previously mentioned. Heterozygote excess in nine population-locus cases have been observed. Disagreement with HWE was found in four

loci in the New Washington, Aklan oyster population alone. Specifically, CI.B07 did not follow HWE in five of eight oyster populations studied, while CI.D01 had lesser *Ho* excess in the same five populations. Oyster populations from Samar, Pan-ay in Capiz, and Hinigaran in Negros Occidental did not significantly deviate from HWE. Only CI.H10 locus from Hinigaran oyster population followed HWE. The heterozygote excess in this study was exhibited by populations with high observed condition indices discriminated by CI.B07 loci. New Washington Aklan populations showed mostly excess heterozygosity which can be aligned with its significant morphometric measurements and condition indices. With the utility of microsatellite markers like CI.B07, it can be inferred that heterozygosity can be aligned with the morphological characteristics of oysters. Such significant HWE departures due to heterozygote excess was also observed in other oyster species including *C. virginica* in the coast of Mexico and in wild populations of *M. gigas* (Galindo-Sánchez et al., 2008; Meistertzheim et al., 2013). The excess heterozygosity found in those studies was however due to presence of null alleles, high microsatellite variability, genotyping errors, and repeated transfers of same seed stocks from aquaculture. In addition, excess heterozygosity in oysters is a possible evidence of polyploidy, although polyploidy is only induced in the Pacific oyster *M. gigas*. Moreover, polyploid individuals promote increased heterozygosity and are impossible to differentiate from diploid counterparts (Gong et al., 2004).

This study also utilized F_{ST} measures of Weir and Cockerham as an estimation of F-statistics to analyze the genetic structure of the different oyster populations. The F_{ST} depicts the correlation of subpopulations within the total population (Wright, 1965). Little genetic differentiation was found among populations with a global F_{ST}=0.0086 indicating that allele frequencies within each population are almost similar. CI.B07 loci had highest single-locus F_{ST} in 5 out of 8 populations that significantly deviated from HWE due to heterozygote excess. The lowest was observed in CI.D01 which revealed to be the only locus with no significant departures from HWE. This indicates that microsatellites with high F_{ST} value exhibit significant deviations from HWE in most population. Overall, no to little significant genetic differentiation is happening in the oyster populations investigated in this study. The inbreeding coefficient or F_{IS} measures the diversity of individuals within the subpopulation. The negative F_{IS} values in most loci were in populations with high observed heterozygotes (implying an increase in heterozygosity) is more likely to outbreed whereas positive values indicate inbreeding (Wright, 1965; Meirmans & Hedrick, 2011). The F_{IS}=0 in Hinigaran population supports its agreement with HWE in a specific locus, CI.H10.

Significant genetic differentiation between *C. hongkongensis* oyster populations in Southern China were due to high salinity sea area by summer upwelling which blocks the distribution of planktonic larvae (Ma et al., 2021). In contrast, minimal genetic differentiation was observed in populations of *M. gigas* and *C. virginica* because of anthropogenic interferences of transferring spats to other culture areas as well as restocking efforts (Galindo-Sánchez et al., 2008; Meistertzheim et al., 2013; An et al., 2014). Genetic homogenization in cultivated Pacific oysters in Japan is evident in aquaculture areas, while gene flow occurs due to larval dispersal by ocean currents especially in localities of near and same parent stocks (Kawamura et al., 2017). Moreover, marine species with long planktonic larval development stages like oysters have poor population genetic structures because of seasonal shifts in coastal currents and high levels of gene flow (Hellberg et al., 2002). It can be noted that *Crassostrea* oysters, wherein Asian species are presently identified under *Magallana* (Salvi and Mariottini, 2017), spend their larval life in the water column for about 2-3 weeks, dispersed from the parental stocks' site, colonize new locations, and lead to possible chances of genetic mixing (Avise, 1994). These can also explain the heterozygote excess of nine population-locus cases in this study. Same with the above-mentioned studies, it can be influenced by the negative inbreeding coefficient F_{IS}, probable human-mediated transfer of spats to neighboring and regionwide culture locations, and dispersal of planktotrophic larval phase. All of these can contribute to gene flow and mixing of the populations in the study. These assumptions, hence, result to little genetic differentiation as most culture areas were only concentrated in Region VI, except in one culture site in Region VIII (Samar) that showed no significant heterozygote excess.

Although significant genetic differentiation has been documented among oysters, it can be noted that these were found in distantly located populations and genetic mixing was blocked by high salinity and ocean upwelling. In this study, sampling locations were only limited to Regions VI and VIII with 7 and 1 culture sites, respectively. It has been mentioned that species geographical distribution with connecting water currents can affect genetic structure of populations, and populations in close proximity are genetically more similar than more distant populations. They are often genetically differentiated through isolation by distance as observed in previous studies (Balloux & Lugon-Moulin, 2002; Li et al., 2006; Xiao et al., 2010; Kawamura et al., 2017).

In this study, the association of five microsatellite loci with the CI was described. The observed heterozygosity and homozygosity of populations inferred from the analysis of microsatellite loci were correlated with CI. It was shown that there was an association between heterozygosity and oyster condition index among the populations surveyed. The index of condition used in the study expresses the fatness of an oyster as the percentage of shell-cavity volume occupied by its meat (Grave, 1912). Also, condition index is used to designate meat quality of particular marketed organisms in aquaculture (Lucas and Beninger, 1985). New Washington samples with the most abundant heterozygotes in all loci were found to have high condition indices. In contrast, Hinigaran samples had a relatively low CI and lesser number of heterozygotes. It can be inferred that increased population heterozygosity may lead to better CI, therefore improving meat quality (Lucas and Beninger, 1985). This study shows observations of heterozygote advantage in *M. bilineata* oysters from New Washington Aklan populations. Favorable gene combinations successfully inherited by New Washington Aklan oysters helped the organisms to be fit and adaptive to its environment through producing high meat condition index. Heterozygote advantage entails that heterozygote organisms are more fit than homozygotes (Bonner, 1988; Holsinger and Weir, 2009). Among the eight sites, New Washington is a potential source of brood stock culture producing good morphometric and meat condition index traits with high levels of heterozygosity.

And among the five loci examined, CI.B07 is a potential microsatellite marker useful for discriminating oyster populations for facilitating breeding program initiatives based from its microsatellite analysis and CI correlation.

Moreover, the alleles revealed that the surveyed oyster populations cannot fully achieve constant fitness wherein a possible population bottleneck is triggered by fluctuations of allele frequencies through time in response to changes in the environment (Launey et al., 2001). The differences in allele frequencies can be further explained by the high mutation rate of microsatellites. Being highly polymorphic and transferable between species, mutational dynamics varies among loci and can lead to its multi-allelic mechanism (Buschiazzo & Gemmell, 2006; Chistiakov et al., 2006).

Conclusions and Recommendations

The *M. bilineata* oyster populations in central Philippines revealed little genetic differentiation, implying marginal sub-structuring among populations. Deviations of loci from HWE were attributed to heterozygote excess. These findings revealed a positive correlation between the CI and the observed heterozygosity of each population, while a negative correlation was shown with its observed homozygosity. This study observed high heterozygosity among the oyster populations surveyed, a possible genetic response to attain fitness in order to cope with the changing habitat. Overall, this study highlighted the application of microsatellite analysis and its association with condition index to assess population genetic structure of oysters in central Philippines. Also, the utility of microsatellite markers reflects a potential marker-assisted selection tool to advance oyster farming technologies and better oyster production. Investigations involving larger and more distantly located populations across the country with the use of more polymorphic microsatellite markers with association studies on water current, sample demographic, and temporal sampling surveillance can be explored.

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