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Describing morphological and enzyme polymorphism in the Ribbed Venus Clam *Gafrarium tumidum* from five marine coastal locations in Mindanao, Philippines

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Abstract. This study was conducted to describe morphological variations and enzyme polymorphism in the Ribbed Venus Clam *Gafrarium tumidum* from five marine coastal locations in Mindanao, Philippines. Thirteen morphological characters and two enzymes were evaluated following standard methods of morphological measurements and enzyme pattern analysis. Results show variations in selected morphological characters and in the two enzyme loci among the populations examined. Enzyme polymorphism and the high heterozygosity in the populations examined can be attributed to the external fertilization and extended dispersal of planktonic larvae of this bivalve species. Factors such as heterozygote advantage during larval life, and the minimal effects if any of human exploitation can also be considered to explain the high heterozygote frequencies. Since only a few loci were investigated, it was postulated that the results may be different if a higher number of loci would be included in the evaluation of genetic variation in this species.
Key Words: enzyme polymorphism, *Gafrarium tumidum* Roding, morphological variations.

Introduction. *Gafrarium tumidum* Roding (Linnaeus, 1758) is a small sturdy clam with a ribbed pattern that sometimes are seen on some of coastal shores, on sandy areas in calm lagoons near seagrasses on intertidal shores with coarse sand. This facultatively mobile infaunal suspension feeder bivalve is one of the molluscan species that are delicious and protein rich food among the sea foods. This species of bivalve thriving in the coastline form an important source of cheaper food source in many countries of the Indian Ocean (Nayar & Rao 1985; King et al 1990; Jagadis & Rajagopal 2007) and South-East Asia (Nielsen 1976; Purchon & Purchon 1981; Davy & Graham 1983; Toral-Barza & Gomez 1985).

Knowledge of the parameters affecting the spatial distribution of edible bivalves is required for sound management of their exploitation (Davy & Graham 1983). The stocks of this species are being harvested in many parts of the South-West Pacific (Broom 1985) and in the Philippines, it is commonly and overly collected especially in areas where they are found to be abundant (Toral-Barza & Gomez 1985). It is unfortunate that there is a dearth of information regarding the distribution, abundance and ecological attributes of this species in the Philippines. No reported studies were undertaken to determine their biological and ecological characteristics and estimate their potential for commercial exploitation. With a view to the latter, assessments of existing natural stocks appear to be a priority objective (Baron & Clavier 1992). Since the factors affecting the spatial distribution of benthic species are complex and difficult to appraise, many authors show the importance of granulometric composition of the substrate on the distribution of benthic organisms (Sanders 1958; Rhoads & Young 1970; Bloom et al 1972; Thomassin

1978; Chardy & Clavier 1988). *G. tumidum* is sedentary, at least as adults thus their presence in an area is determined by larval recruitment and juvenile survival. *G. tumidum* is considered widespread and can be collected from muddy sediment pockets on the reef flat (Gibbs 1978), clean areas sheltered from waves and tidal currents (Purchon & Purchon 1981), no preference for muddy habitats, maximum biomass values generally associated with sediments comprising >50% medium and very fine sand, peebles and granules, and mostly mud (Baron & Clavier 1992). Since it is a short-siphoned suspension feeder, a relatively high fine sediment content would appear to limit its distribution. But considering that in many species, individuals tend to express different phenotypes (morphological, physiological, behavioral) when thriving in varied environments (Freeman & Herron 1998), studying variability in natural populations of this species will provide information on the status of the species in different ecological environments.

Today morphomeristic analysis combined with mathematics becomes a multipurpose approach (Solon et al 2012; Talu & Giovanzana 2011, 2012). Aside from morphomeristic analysis, one sensitive way to assess genetic variability is through enzyme electrophoretic analysis. Variation in the banding pattern can be directly equated to variation in a gene coding for the variant proteins being studied. Polymorphism can be studied by identifying the different allelic forms of a gene in a given population and measuring their relative frequencies (Rothe 1994). Protein profiles have been used to establish biochemical differences related to ontogenetic changes (Macaranas & Capuli 1994/1995; Lester & Cook 1987; Agerberg & Jansson 1995), geographic distance (Parkash & Yadav 1993), in elevated temperature (Schwantes & Schwantes 1982) and to other forms of environmental heterogeneity (Posthuma 1990; Benton & Guttman 1992; Tranvik et al 1994) thus was used in the current study. Enzyme heterogeneity was examined in different populations of this species to have clearer idea on the extent of their genetic variability and relate it to the possible factors affecting species distribution and management.

Fisheries management and conservation of commercially important marine species directed to ensure their sustainable exploitation must rely on a good knowledge of the biology of the species as well as population level genetic structure and gene flow among populations (Waples 1998). Many studies have been carried out on reproductive biology, ecology in *G. tumidum* (Davy & Graham 1983; Broom 1985; Toral-Barza & Gomez 1985), however, no studies have examined this species' population genetic structure. The economic potential of this mollusc can be further enhanced by relevant information on its genetic diversity and population structure as a basis for reasonable decisions by government and conservation policies as a whole.

Material and Methods

Sampling sites. *G. tumidum* were collected in four geographically distant sampling stations in Mindanao, Philippines based on the availability of the species. These include 1) Lugait, Misamis Oriental 2) Buruun, Iligan City and 3) Clarin, Misamis Occidental in Iligan Bay, and 4) Binuni (Maak and Lagoon) and Sagay in Camiguin Island (Figure 1).

Buruun and Lugait coastal areas in Iligan Bay are within the industrial zone and is considered to be the direct depository of some industrial wastes/effluents. Coastal areas of Lupagan, Clarin, Misamis Occidental, on the other hand, lie near the mouth of the Panguil Bay. Based on interview and actual observation, coastal area of Lupagan is the frequently visited fishing ground for dynamite fishing. Residents mentioned rampant dynamite fishing occurred during months of March to October. Sagay and Binuni coastal areas in Camiguin Islands are said to be pristine and/or relatively less disturbed in terms of pollution and illegal fishing such dynamite and cyanide fishing except for the Lagoon area where various aquatic organisms, invertebrates and fishes were cultured.

Iligan Bay is located at the southern part of Mindanao Sea, east of Panguil Bay and west of Macajalar Bay. It is bounded at the northeast by the coastal areas of Gitagum, Misamis Oriental and at the northwest by Plaridel, Misamis Occidental. The southernpart is bounded by Clarin, Misamis Occidental at the west and Maigo, Lanao del

norte at the east. Iligan Bay lies approximately between 8° 30' 31" north latitude, 123° 43' 15" east longitude. The mouth of Iligan Bay is approximately 350 miles. It has an estimated area of about 2000 sq. km (Camarao et al 1983).

Camiguin Island is a part of Mindanao and as a small island far north of Iligan City, it is less industrialized and serves as a tourist spot. It is located at the tip of Northern Mindanao and is separated from the main land by Macalajar Bay in the south and Butuan Bay in the east. The island is totally of volcanic origin, with an active Mt. Hibok-Hibok, a volcano which is 5,246 feet high.

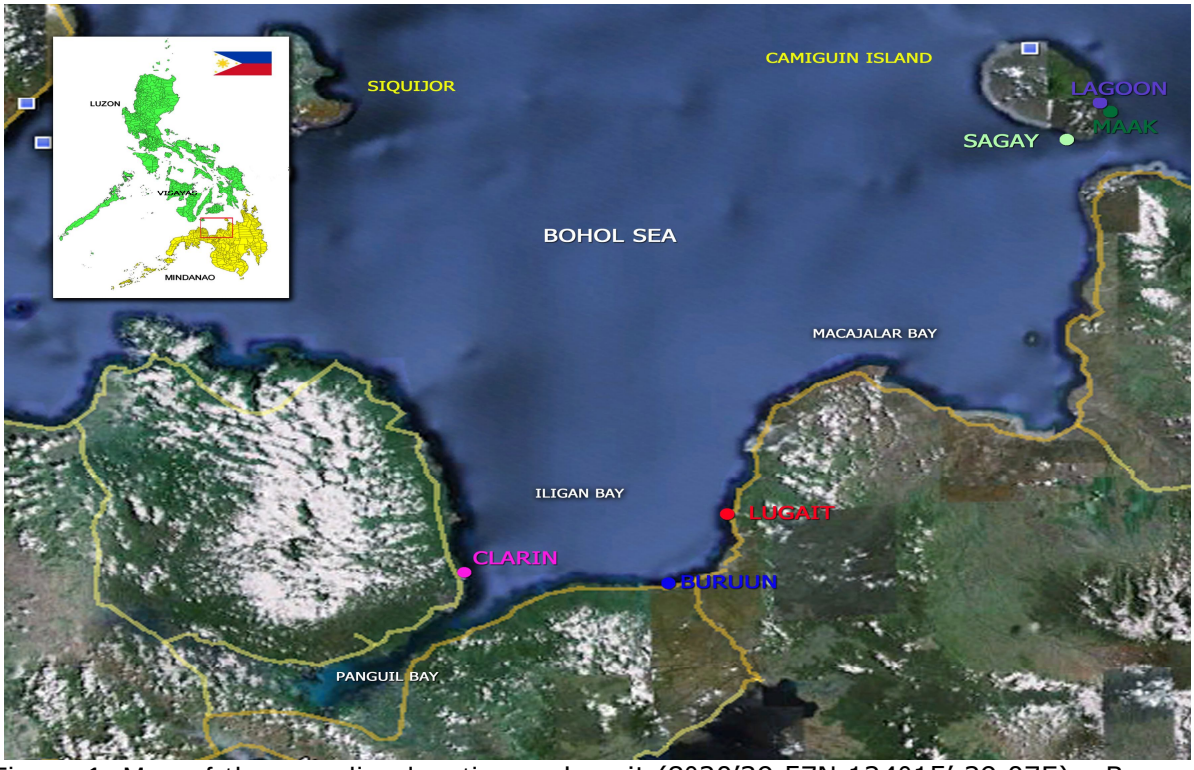

Figure 1. Map of the sampling locations: ●Lugait (8°20'39.57N,124°15' 29.07E), ●Buruun (8°11'28.3"N, 124°10'33.59"E), ●Clarin (8°11'46.89"N,123°5' 58.55"E), ●Sagay (9° 05' 40.01"N, 124° 45' 08.13"E, ●Maak (9° O7' 10.39"N , 124° 47' 08.52"E) and ●Lagoon(9° 07' 30"N, 124°47' 15.2"E).

The sediment profile of the five sampling areas where *G. tumidium* clams were collected is shown in Table 1. It can be seen from the data that the five sampling locations were different in percentage weight and grain types.

Table 1

Sediment profile in five sampling areas (Lugait, Buruun, Clarin, Maak and Sagay)

Mesh Size (mm)	*percentage(%) weight*					*Grain type*
	Lugait	Buruun	Clarin	Maak	Lagoon	
3.25 - 10.27	47.46*	62.30*	0.12	61.36^	25.34^	very coarse sand
1.19 - 2.0	22.22	21.09	9.75	21.42	22.98	coarse sand
0.6 - 0.85	14.19	3.00	15.40	2.19	5.37	medium sand
0.42 - 0.59	7.29	1.19	21.41	1.17	9.78	fine sand
0.30 - 0.40	6.80	12.25	42.58	8.86	28.5	very fine sand
< 0.15	2.04	0.07	10.74	4.99	7.98	muddy (silt/clay)

Wenworth grade scaling modified. (*-with coral rubbles, pebbles, granules ,^ - with fragments shells.

Choice and Collection of Samples. Approximately 50-80 live adult samples per population were collected. The live samples were brought to the Genetics Laboratory for processing. To avoid age effect, only sexually mature samples were used. Standard

sexually mature shell length range (Fig. 2) was used as the basis in collecting the samples.

Morphological Characterization. External and internal morphological features (meristic and morphometric traits) for each bivalve were characterized using the suggested data sheet (Table 1) for recording morphological characters. Two important meristic, ten morphometric characters and weight of the left shell were considered. All measurements of morphometric characters were done using a Vernier caliper and taken to the nearest 0.1 mm. Morphological characters are illustrated in Figure 2. A total of 67 to 82 sexually mature *G. tumidum* samples were conventionally measured.

Table 2

Measured morphomeristic characters of *Gafrarium tumidum*

Characters	
Number of frontal ridges	FR
Number of lateral ridges	LR
Shell width	SW
Shell length	SL
Shell depth	SD
Length of posterior adductor muscle scar	PAMS
Length of hinge plate	LHP
Distance between pallial line and ventral shell	PVM
Distance between inner portion of posterior of adductor muscle scar and inner portion anterior adductor muscle scar	PAMAM
Distance between lower tip of posterior adductor muscle scar and ventral shell margin	PAMV
Distance between umbo and posterior end ligament	UL
Length of lunule	LL
Weight (left side)	WL

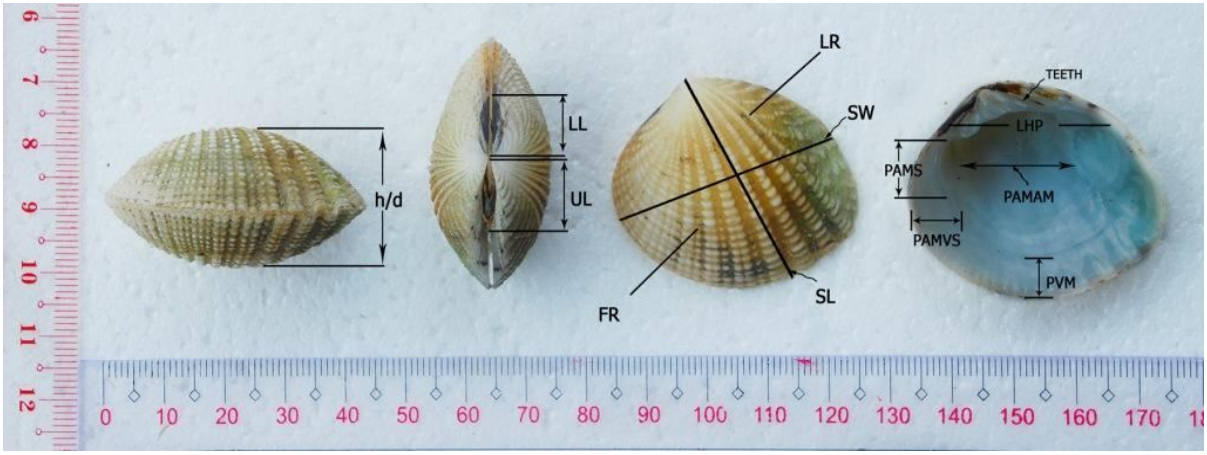

Fig. 2. Measured characters of *G. tumidum*.

Sample preparation. With the use of forceps and scalpel, the bivalve was forcefully opened to expose the internal organs. Gills and hepatopancreas was removed and washed with physiological salt solution. Then they were homogenized using tissue homogenizer with 1% glycerine. Homogenates were placed in properly labelled Eppendorf tubes and were centrifuged at 10,000 rpm for 5 minutes at 4^0C. Supernatant was then pipetted to new Eppendorf tubes and stored in the freezer until analysis. The pellet was discarded.

Enzyme electrophoresis. The different composition of the different buffer systems and staining solutions needed in the electrophoretic analysis of esterase and alkaline phosphatase gene loci were based from Shaw & Prasad (1970). All buffer solutions

prepared were kept refrigerated during storage. Stain solution was prepared fresh as much as possible.

Electrophoretic isozyme analyses of gills and muscle proteins was done in the Genetics Laboratory Room of the Department of Biological Sciences of MSU-IIT, using electrophoresis equipment (Model: OSP 135). Standard protocol and techniques in the loading of samples and electrophoretic run with some modifications was followed (Shaw & Prasad 1970; Doyungan 1995). The system of gene nomenclature for protein-coding loci developed by Shaklee et al (1990) was used in this study. This system is essentially founded on two recognized standards, the International Union of Biochemistry Nomenclature Committee (IUBNC 1984) for enzyme names and on several conventions used in human genetics.

To distinguish genes or loci having the alphanumeric symbols as the abbreviations of the proteins they code. In case of multilocus designation as in esterases, numbers was be assigned in sequential order in relation to electrophoretic mobilities beginning with 100 for the locus coding for the isozyme closest to the origin and proceeding towards the anode. The symbol Pl Est was used to refer for plasma esterase, for liver *(li)* and muscle *(mu)*: *Est* for esterase and *AklP* for alkaline phosphatase were used.

Alleles was designated with sequential Arabic number codes based on relative electrophoretic mobilities, beginning with 100 as the origin and add1 for the one closest to the origin. Symbols for alleles was always italicized and preceded by an asterisk, for example, 101, 102, 103, and so on. Genotypes were written in italics with an asterisk between the locus and the first allele in a genotype, for example, EST 101, 102, and so on.

The genotype and allelic frequency data was estimated based on the bands that were obtained from each locus. Each band represents an allele. The number of alleles was counted and out of the distribution (based on distance travelled) of these alleles, corresponding genotypes were determined. From the data gathered, genotype and allelic frequencies were computed for various presumptive gene loci, namely esterases (Est) and alkaline phosphatases (AP).

Identification and nomenclature of the protein-coding locus was patterned after Shaklee et al (1990). Genetic variability for the various gene loci was determined based on 1% criterion of polymorphism ($0.01 < q > 0.99$). Genotypic identity was determined in order to compare variation between natural populations of each species taken from different sampling sites. Due to limited number of loci that were electrophoretically studied, only the Nei's genetic identity was computed. This genetic identity was used as a basis in comparing genetic variability within and among population species. The normalized identity of genes or genetic identity (I) was computed (Nei 1978).

Measurements of genetic similarity and/or genetic distance was used to construct the genetic links of operational taxonomic units (OTUs) which may be population of one species in four/five sampling sites . The genetic identity values among populations, taken from selected stations, were used in the construction of dendrogram. Dendrograms were constructed based on the values of genetic similarity/identity for all possible OTUs which are presented in the form of matrix. The "unweighted pair-group arithmetic average clustering method" was used in the construction of dendrograms (Rothe 1994).

Results and Discussion. Figure 3 shows the box plot showing the measurements made on various morphological parts of *G. tumidum* from five sampling locations. The figure shows five higher mean values in Maak for LR, FR, SW, PAMAM and UL and Lugait for SD, LHP, PVM, PAMVS and LL. Having a greater number of variables with lowest mean values are those in Buruun like FR, PAMS, LHP, PVM, PAMVS and LL. In Lagoon, it is very evident that Wl (weight) (Figure 2l) has the highest mean as compared to other characters.

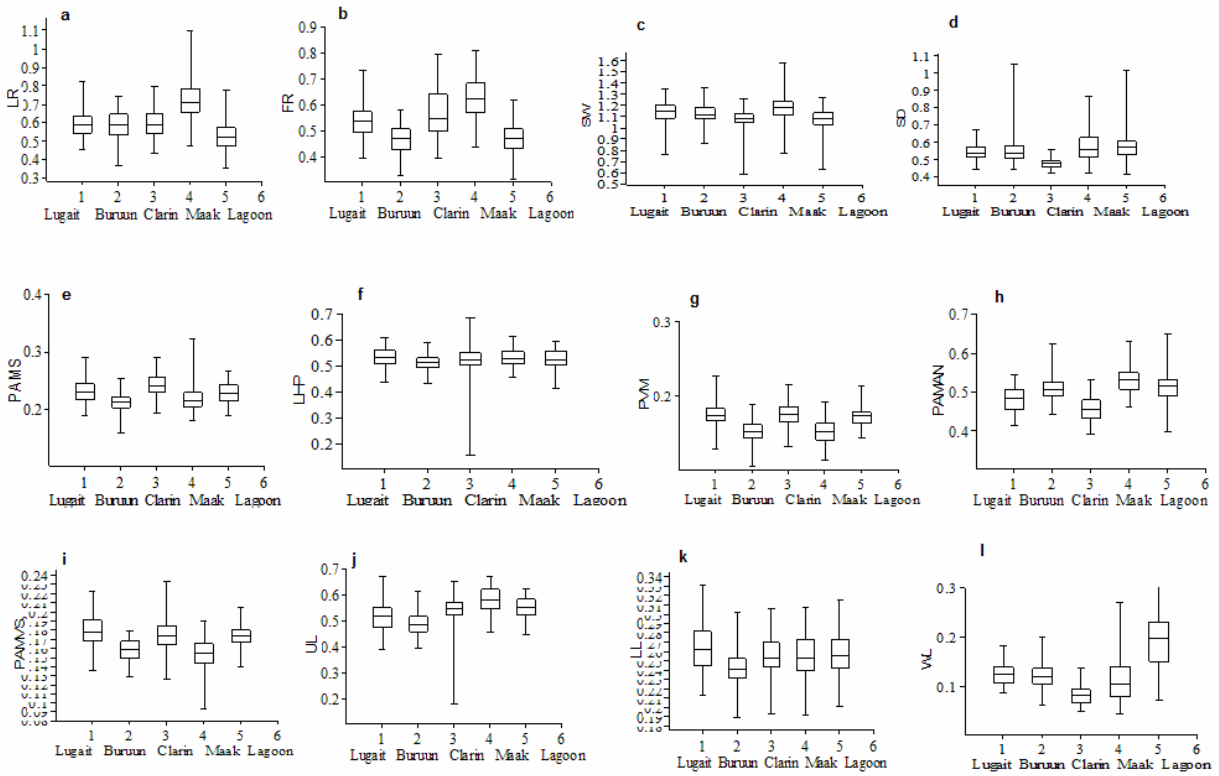

Figure 3a-l. Box plot for 12 variables from bivalve shells, *Gafrarium tumidum*, taken from five stations, namely, Lugait, Misamis Oriental, Buruun, Iligan City, Maak, Camiguin and Lagoon, Camiguin; LR – lateral ridges, FR – frontal ridges, SW –shell width, SD – shell depth , PAMS – length of posterior adductor muscle scar, LHP – length of hinge plate, PVM – distance between pallial line and ventral shell margin along shell, PAMAM – distance between inner portion of posterior adductor muscle scar and inner portion of anterior adductor muscle scar, PAMVS - distance between lower tip of posterior adductor muscle scar and ventral shell margin, UL – distance between umbo and posterior end ligament, LL – length of lunule, WL – weight.

Morphological differentiation in *G. tumidum* was assessed using multivariate analysis of variance (MANOVA) (Table 3). MANOVA reveals that there are significant differences on its overall morphological measurements. This is based on the results of the two statistics calculated from MANOVA which are the Wilks' lambda and Pillai trace. These results are further summarized by the canonical variate analysis (CVA). Canonical Variate Analysis is one of the more interesting applications of multivariate statistics. The technique is used to examine interrelationship between a number of populations simultaneously with a goal of objectively representing the interrelationships graphically in few dimensions (ideally two or three). In this study, two dimensions were utilized. The axes of variation are chosen to maximize the separation between the populations relative to the variation within each of the populations. In the CVA scatter plots presented, population points overlap around zero of the first and second axes. The CVA produces a scatter plot of specimens along the first two canonical axes, producing maximal separation and second to maximal separation between the five sampling locations evaluated (Fig. 4).

Table 3

MANOVA results showing significant differences in morphological measurements among the five populations of *G. tumidum*

	df1	*df2*	*F*	*P (same)*
Wilk' lambda (0.1157)	40	1371	26.26	5.142E-140
Pillai trace (1.551)	40	1456	23.05	3.247E-128

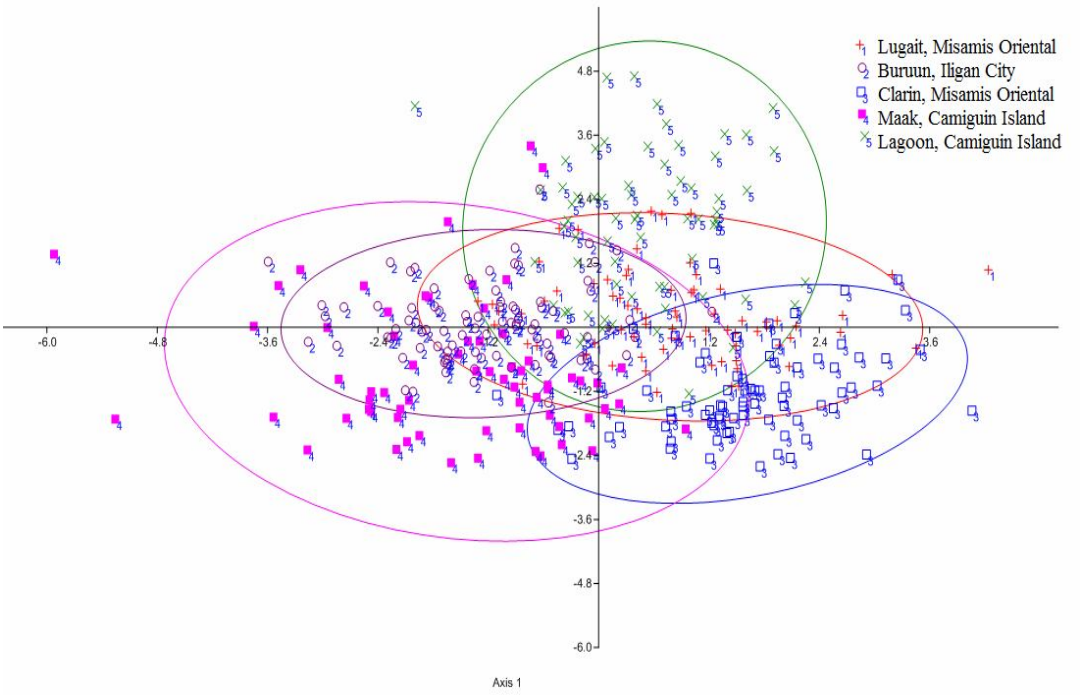

Figure 4. Distribution of the five populations of *G. tumidum* along the first two canonical variate axes.

Tukey's pairwise comparison shows highly significant differences among the five bivalve populations in selected characters (Table 4). A closer examination of the results shows that within a morphological character of the bivalve, three populations' (Lugait, Buruun and Clarin) were different from Maak and Lagoon in shell lateral ridges (LR). The Lagoon shell weight (WL) was found to vary ($p<0.01$) from the weights of those from other populations. No differences were observed between the length of hinge plate (LHP) of Lugait, Clarin, Maak and Lagoon but these three populations differ with Buruun, whose shell length of lunule (LL) also vary from the other four stations. For the five morphological variables LHP, PVM, PAMAM and PAMVS, differences were observed between Buruun and Maak but not between Lugait, Clarin and Lagoon.

Table 4

Pairwise comparison between populations of *G. tumidum*

	Lugait	*Buruun*	*Clarin*	*Maak*	*Lagoon*
Lugait	0	6.5846E-17	4.50114E-28	5.23598-28	4.85833E-23
Buruun		0	8.66924E-34	2.34584E-34	5.12437E-39
Clarin			0	8.11418E-33	6.32835E-19
Maak				0	5.57266E-31
Lagoon					0

Table 5 below shows discriminant values (beta coefficient) associated with each component. This table presents which variables contribute much to the discrimination between populations taken from two stations. The larger the beta coefficient, the greater the contribution it makes the separation between two populations. Buruun and Clarin (BC), being noted to be highly separated showed PAMS (-101.42), PAMAM (82.19) and PAMVS (-80.87). Other stations in between populations of *G. tumidum* also register similarly large beta coefficients like that of BC. Table 5 revealed that separation between two populations from different stations can be attributed largely to PAMS and WL. Only three populations however can be argued to be morphologically distinct based on the

results of classification matrix of all the individuals with greater than 70% correct classification (Table 6). It was shown from both discriminant and Tukey's pairwise comparisons in this study that the differences between populations of the ribbed mussel were due to differences in selected morphological characters.

Table 5

Discriminant values (beta coefficients) for each variable as compared *G. tumidum* between populations from five stations, namely, Lugait, Mis. Or., Buruun, Iligan City,Clarin, Mis. Occ., Maak, Camiguin and Lagoon (Binuni), Camiguin

CHARACTERS	*COMPARED POPULATIONS*									
	LB	LC	LM	LLa	BC	BM	BLa	CM	CLa	MLa
LR	- 17.06	6.43	-16.53	-5.50	18.18	-0.01	8.63	-29.72	-13.48	2.38
FR	**48.06**	3.25	-7.21	11.42	-29.08	-37.23	-23.20	5.9	**59.76**	21.26
SW	1.33	13.36	5.90	3.45	12.14	2.47	8.89	-7.30	-15.16	0.33
SD	-11.81	23.58	-6.22	-0.31	8.53	-6.30	13.47	-1.47	-12.36	14.14
PAMS	**63.46**	**-62.54**	**54.58**	0.64	**-101.42**	1.91	**-62.17**	**83.9**	**46.82**	**-50.58**
LHP	0.62	-4.47	-2.94	18.41	-6.48	2.08	14.6	**51.05**	10.61	1.04
PVM	36.18	-2.85	23.49	-15.16	**-59.59**	-10.64	**-77.37**	30.64	4.81	-70.36
PAMAM	**-53.14**	17.55	-31.68	-19.61	**82.19**	25.29	26.93	-43.49	-49.11	22.38
PAMVS	**104.46**	21.32	**66.50**	15.96	**-80.87**	25.94	**-76.89**	29.21	15.43	-38.06
UL	5.01	-4.64	-36.15	-12.20	-33.07	**-50.00**	-19.33	-1.77	15.37	32.78
LL	14.11	4.84	22.88	-1.69	**-50.0**	**51.96**	**-58.88**	27.42	2.31	**-43.55**
WL	**53.84**	**95.13**	14.48	-31.88	**68.52**	-2.27	**-58.28-**	**48.21**	**-82.29**	**-51.37**

Legend:

Stations: LB– Lugait/Buruun, LC- Lugait/Clarin, LM– Lugait/Maak, LLa– Lugait/Lagoon, BC– Buruun/Clarin, BM– Buruun/Maak, BLa – Buruun/Lagoon, CM –Clarin/Maak, CLa – Clarin/Lagoon, MLa – Maak/Lagoon.

Variables: (LR- lateral ridges, FR– frontal ridges, SW–shell width, SD– shell depth, PAMS– length of posterior adductor muscle scar, LHP – length of hinge plate, PVM – distance between pallial line and ventral shell margin along shell, PAMAM – distance between inner portion of posterior adductor muscle scar and inner portion of anterior adductor muscle scar, PAMVS - distance between lower tip of posterior adductor muscle scar and ventral shell margin, UL– distance between umbo and posterior end ligament, LL– length of lunule, WL– weight).

Table 6

Classification matrix of the *G. tumidum* individuals from five sampling locations

	PREDICTED GROUPS					
SAMPLING LOCATIONS	Lugait	Buruun	Clarin	Maak	Lagoon	N
Lugait	47 (62.67%)	7 (9.33%)	3 (4%)	4 (5.33%)	14 (18.67%)	75
Buruun	8 (10.53%)	65 (85.53%)	3 (3.95%)	0 (0%)	1 (1.32%)	76
Clarin	3 (4.48%)	4 (5.97%)	52 (77.12%)	3 (4.48%)	5 (7.46%)	67
Maak	16 (23.52%)	1 (1.47%)	2 (2.94%)	47 (69.11%)	2 (2.94%)	68
Lagoon	3 (3.37%)	1 (1.12%)	8 (8.99%)	5 (5.62%)	72 (80.85%)	89

Variability in the Esterase and Alkaline phosphatase loci Among Gafrarium tumidum populations. The electrophoretic analysis revealed that two gene loci for protein enzymes, esterase (Est) and alkaline phosphatase (AlkP), havepolymorphic expression except only for Buruun AlkP-2 locus (Table 7). Table 7 summarizes the allelic frequencies of alleles in each protein locus of gills and hepatopancreas of the *G. tumidum* collected from the five stations, Lugait, Buruun, Clarin, Maak and Lagoon. In gills and hepatopancreas Est and AlkP loci, polymorphism is governed by three to four codominant alleles. All bands were found to be anodally migrating.

Table 7

Allelic frequencies in each protein locus (*Est* and *AlkP*) of gills (*gl*) and hepatopancreas (*hp*) of *G. tumidum* from Lugait, Buruun, Clarin, Maak and Lagoon

Locus	*Tissue*	*Allele*					
			Lugait	Buruun	Clarin	Maak	Lagoon
Est-1	gl	110	0.290	0.141	0.250		
		112	0.50				
		114	0.468	0.50	0.546	0.391	
		118	0.242		0.313	0.359	
		117					0.469
		120					0.188
		125					0.344
Est-2	gl	103	0.375	0.594	0.266	0.422	
		108	0.625	0.406	0.734	0.574	
		112					0.359
		114					0.641
Est-1	hp	113	0.397			0.242	0.109
		118		0.442	0.234	0.370	0.391
		115	0.241				
		117	0.224		0.423		
		116				0.387	0.500
		120	0.138	0.5588	0.125	0.218	
Est-2	hp	103	0.348		0.258		0.131
		105	0.522		0.379		
		107	0.130	0.362	0.362		0.869
		113		0.281			
		115		0.720			
		104		0.167		0.286	
		108				0.410	
		110		0.833		0.304	
Est-3	hp	104		0.167			
		110		0.833			
Alkp-1	gl	105				0.250	
		112	0.533	0.433	0.167	0.250	
		117	0.67	0.567	0.367	0.313	
		107			0.267	0.312	0.375
		105			0.199		0.156
		115					0.469
Alkp-2	gl	105	0.107				
		107	0.893	1.000			
Alkp-1	hp	105	0.719	0.533	0.156		
		107	0.281	0.466	0.688	0.594	0.625
		109			0.156	0.406	0.375

Table 8 presents values on the total effective number of alleles (n_e), average heterozygosity (He) and total population differentiation (δ_T). These three values are measures of genetic variation within and among populations. The greater the number of effective alleles, the more the gene locus is more variable and shows higher heterogeneity. The table revealed that Clarin (2.55) and Maak (2.64) are more similar and in higher heterogeneity compared to other stations. Lugait (2.11) and Lagoon follow. Buruun (1.83) has the least number of effective alleles suggesting less gene loci variability. On the other hand, total population differentiation ($\delta_{T)}$ in a given population, individuals would be genetically different, more heterozygous and diverse from each other when δ_T would be equal to 1.0. Table 8 shows Clarin and Maak are highly genetically differentiated, followed by Lugait and Lagoon, and least differentiated, Buruun, having 42.01 $\delta_{T\%}$.

Table 8

Effective number of alleles (n_e), average heterozygosity (He) and total population differentiation ($\delta_{T\%}$) in each protein locus (Est and AlkP) of gills (gl) and hepatopancreas (hp) of *G. tumidum* from Lugait, Buruun, Clarin, Maak and Lagoon

Locus	*Tissue*	*Sampling sites*									
		Lugait		Buruun		Clarin		Maak		Lagoon	
		n_e	$\delta_{T\%}$	n_e	$\delta_{T\%}$	n_e	$\delta_{T\%}$	n_e	$\delta_{T\%}$	n_e	$\delta_{T\%}$
Est-1	gl	2.77	65.98	2.00	53.33	2.41	60.33	2.90	67.69	2.68	64.71
Est-2	gl	1.88	43.38	1.93	51.46	1.64	40.27	1.97	50.90	1.85	47.53
Est-1	hp	3.51	74.14	1.98	51.32	3.38	72.60	2.89	67.58	2.41	60.69
Est-2	hp	2.44	61.66	1.68	43.11	2.93	68.19	2.92	68.19	1.29	23.73
Est-3	hp			1.38	28.73						
Alkp-1	gl	1.99	53.41	1.97	55.66	3.66	77.87	3.66	77.49	2.60	65.62
Alkp-2	gl	1.24	20.61	1.0	0.0	-	-	-	-	-	-
Alkp-1	hp	1.68	43.12	1.99	52.50	1.92	51.23	1.93	51.46	1.88	49.90
Total n_e		2.11		1.83		2.55		2.64		2.06	
He		0.499		0.607		0.476		0.389		0.503	
Mean δ_T			51.76		42.01		61.75		63.89		52.03

Legend: He - average heterozygosity.

Within-population genetic diversity of *G. tumidum* in terms of n_e and $\delta_{T\%}$ showed moderate to high levels of genetic variation. The n_e was moderate in all populations, ranging from 1.83 to 2.64. However, the H_e was higher (0.3894 to 0.6069) than that reported for other marine bivalves (about 0.09 to 0.30) (Laudien et al 2003). The relatively high levels of genetic diversity of this exploited species can probably be explained by historically intact and large populations that have suffered a strong recent decrease because of anthropogenic deterministic effects of habitat destruction, water pollution or over-exploitation that are not linked to genetic selection. High genetic diversity is typically suggestive of a genetically healthy population, and therefore represents a positive asset for the recovery of the species.

Measurements of genetic similarity/identity was used to construct the genetic links of operational taxonomic units (OTUs) of the natural populations of *G. tumidum* in five sampling sites The "unweighted pair-group arithmetic average clustering method" (UPGMA) was used in the construction of dendrograms (Rothe 1994). Table 9 specifies probability of genetic identity of the gene loci, Est and Alkp, in gills and hepatopancreas of the populations from the five sampling sites. Based on this information, Figure 6 shows a dendrogram of the natural populations of *G. tumidum* from five sampling stations. Dendrogram gives similar results based on the presentation of the gene loci total genetic differentiation in Table 9. Cluster analysis revealed closeness of Lugait and Lagoon, Clarin and Maak, and Buruun being separated from the rest.

Table 9

Probability of genetic identity of gene loci, Est and AlkP, in gills and hepatopancreas of the natural populations of *G. tumidum* from five stations, Lugait, Buruun, Clarin, Maak and Lagoon

Station	*Est*			*Alkp*		
	gl	hp	gl/hp	gl	hp	gl/hp
Lugait	0.1084	0.1353	0.0147	0.2522	0.4366	0.1101
Buruun	0.1441	0.0925	0.0133	0.3790	0.3758	0.1424
Clarin	0.1107	0.0525	0.0058	0.1231	0.3193	0.0393
Maak	0.0483	0.0334	0.0016	0.3441	0.3842	0.1322
Lagoon	0.0853	0.1605	0.0137	0.2063	0.3921	0.0809

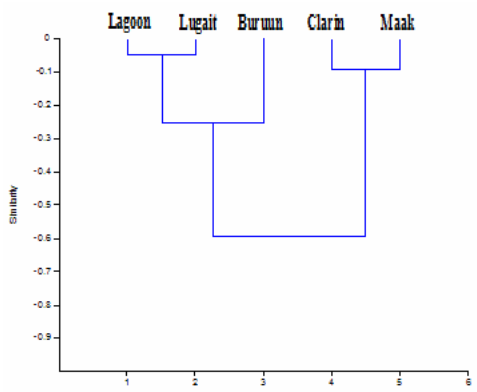

Figure 6. Dendrogram of the natural populations of *G. tumidum* from five sampling stations.

While heterozygote deficiencies relative to Hardy-Weinberg expectations in allozyme loci has been commonly reported in marine bivalves (Zouros & Foltz 1984; Gaffney et al 1990; Borsa et al 1991; Gosling 1992; Raymond et al 1997; Gallardo et al 1998; Passamonti et al 1999; Laudien et al 2003), our study show higher values. Reported low heterozygosity in many bivalve species can be attributed to selective mortality of genotypes, null alleles, inbreeding, and Wahlund effect (Borsa et al 1991; Gallardo et al 1998) and the observed high heterozygosity in *G. tumidum* can be due to external fertilization and extended dispersal of planktonic larvae of this bivalve species. Factors such as heterozygote advantage during larval life, and the minimal effects if any of human exploitation should also be considered to explain the high heterozygote frequencies (Mallet et al 1985; Gallardo et al 1998). It is important to note however that in this study, only a few loci were investigated. The results may be different if a higher number of loci would be included in the evaluation of genetic variation in this species.

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