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Dendogram and Distribution of Bivalves Present in Mangroves and Seagrass Ecosystems of Clarin, Tubigon and Calape Bohol, Philippines

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Abstract: **A total of 35 species representing 4,658 bivalve individuals were collected from mangrove and seagrass ecosystems of Northwestern Bohol comprising three towns namely: Calape, Tubigon and Clarin. They were collected with the aid of belt transect which was 100 m long. There were six Orders of bivalves namely; Arcoida, Mytiloida, Pterioida, Ostreoida, Pectinoida and Veneroida and 14 Families explicitly: Arcidae, Mytilidae, Pinnidae, Placunidae, Pectinidae, Spondylidae, Cardiidae, Mactridae, Tellinidae, Lucinidae, Malleidae, Solenidae, Solecurtidae and Ostreoidae. These 35 bivalves' species fall under 23 Genera; Scapharca, Anadara, Modiolus, Atrina, Pinna, Isognomon, Malleus, Placenta, Decatopecten, Spondylus, Crassostrea, Allectryonella, Ensiculus, Trachycardium, Fragum, Mactra, Tellina, Placamen, Tapes, Austriella, Gafrarium, Animalocardia and Azorinus. The species of bivalves were identified based on the shell structures and were presented in a dichotomous key. Cluster analysis and distribution of bivalves were conducted using PAST software version 3.2. The cluster analysis showed a monophyletic relationship between taxa. This study will serve as guide for other researchers that may study them in the future.**

Keywords: **Identification, Distribution, Morphology and Nomenclature.**

I. INTRODUCTION

Bivalves are two-shelled mollusks which are bilaterally symmetrical and compressed. The shell is composed of calcium carbonate, the hard exoskeleton that is used for protection and support for muscle attachment. Their shell is joined by the flexible ligament known as a hinge enabling them to open and close their valves without being detached. They lack a head and a radula which differ them from other class of molluscan fauna (Campbell and Schmidt, 2001). They have a specialized organ ctenidia used for breathing and feeding (Morton, 2012).

The gill filaments of bivalves evolved progressively over time. Earlier the class was named Pelecypoda since their muscular foot are wedge-shaped and evolved into Lamellibranchia since most of their gills are plate-like and thin. In the study of Yonge (1976) it was suggested that the notch at the bottom of each side of the wedge, which lined up with similar notches on adjacent filaments to form a food groove that extends the length of the underside of the gill was probably preceded folding of the gill filaments. Both of these modifications increased the surface area of the gills (Barnes, 1980). Changes in both ciliation and water circulation followed. The exploitation of filter feeding made it possible for bivalves to colonize a wide variety of habitats that had hitherto been inaccessible to their protobranch ancestors (Gosling, 2008).

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Moreover, bivalve species are usually identified based on the shell morphology. In this study, the color, texture, radial ribs, umbones, shape of valves, anterior and posterior edges were the bases for the nomenclature of these species. Identification of bivalve species is very important because they are all sedentary animals (Barnes *et al*., 1988). They have burrowing behavior that enables them to hide on sediments. The can also be tracers of the past because of their fossilized shell.

When the earthquake happened in Bohol last October 15, 2013 caused by reverse fault (Kobayashi, 2014), the bivalves were affected. The upliftment of intertidal areas particularly in northwestern part where the epicenter was located caused these bivalve species to decline in number as per interview of fishermen in the area. For this reason, this study was made to identify them before these species go extinct. Unfortunately, there have been no documented studies on the species of bivalves present in the intertidal areas of Bohol. Henceforth, the result of this study would also be useful in the identification of these species as well as a guide for students who like to conduct further studies about the different bivalve species.

Objectives of the Study

This study mainly focuses on composition of bivalves present particularly in Clarin, Tubigon and Calape Bohol, Philippines. Specifically, this study aims the following:

1. Analyze the relationship of each species of bivalves found in the area.
2. Determine the distribution of bivalves in three towns Clarin, Tubigon and Calape.

II. LITERATURE BACKGROUND

Molluscs are diverse group of organisms comprising about 100,000 species (12,000 freshwater, 35,000 terrestrial and 53,000 marine) (Haszprunar, 2001). Their body is soft and unsegmented enclosed in a glandular mantle usually covered by a shell. The shell is secreted by a mantle covering the upper surface. The underside consists of a single muscular "foot" (Ruppert, 2004). The muscular foot is used by snail for creeping over surfaces; the clam uses for burrowing soft sediments; and the squid uses it for seizing prey (Engenmann and Hegner, 1981). The visceral mass, or visceropallium, is the soft, nonmuscular metabolic region of the mollusc. It contains the body organs (Hayward, 1996). They are divided according to their symmetry and the characteristics of their foot, shell, mantle, gills and nervous system are the features used for their identification (Storer *et al*., 1979).

Bivalves are two-shelled molluscs which are bilaterally symmetrical and compressed. The shell is composed of calcium carbonate, the hard exoskeleton that is used for protection and support for muscle attachment. Their shell is joined by the flexible ligament known as a hinge enabling them to open and close their valves without being detached. They lack a head and a radula which differ them from other class of molluscan fauna (Campbell and Schmidt, 2001). They have a specialized organ ctenidia used for breathing and feeding (Morton, 2012).The gill filaments of bivalves evolved progressively over time. Earlier the class was named Pelecypoda since their muscular foot are wedge-shaped and evolved into Lamellibranchia since most of their gills are plate-like and thin. In the study of Yonge (1976) it was suggested that the notch at the bottom of each side of the wedge, which lined up with similar notches on adjacent filaments to form a food groove that extends the length of the underside of the gill was probably preceded folding of the gill filaments. Both of these modifications increased the surface area of the gills (Barnes, 1980). Changes in both ciliation and water circulation followed. The exploitation of filter feeding made it possible for bivalves to colonise a wide variety of habitats that had hitherto been inaccessible to their protobranch ancestors (Gosling, 2008).

Majority of bivalves are filter feeders and sedentary or sessile animals. Most bivalves bury themselves in sediment, where they are relatively safe from predation. Some of the bottom-dweller bivalves are cockles, soft-shell clam and bent-nose clam. Others lie on the sea floor or attach themselves to rocks or other hard surfaces such as mussels and oysters. A few bore into wood, clay, or stone and live inside these habitats. Some bivalves, such as the scallops, can swim (Wells, 1998).In addition, some bivalves are herbivores, planktivores while others feed on particulate nutrients in sea water or by sucking up detritus (Noren *et al*., 1999). This is one of the reasons why burrowing habit is mostly exploited by bivalves. Their siphons enable them to maintain contact in water columns while burrowing. For bivalves with short siphon they tend to burrow deep enough just to cover the shell while others with long siphon burrow very deeply.Cockles (e.g. *Cardium* spp.) are shallow burrowers, while many clam species, e.g. razor clams (*Siliqua* sp., *Ensis* sp.), burrow as deep as 60cm (Gosling, 2008). Most have separate sexes and their reproductive cells are release in the water for external fertilization to take place. Larvae have long free-swimming planktonic life followed by transformation leading to the definitive benthonic mode of life. However, some bivalve species exhibit different forms of hermaphroditism and fertilization may occur in the pallial cavity, sometimes with protection of eggs or larvae in a “brooding chamber”. The planktonic larval stage may be reduced or totally absent, and then young hatch directly as benthic organisms (Carpenter and Niem, 1998). They also exhibit high fecundity, 16 - 60 million eggs at a time. For clams and cockles it takes about a year for a juvenile bivalve to grow and two to three years to attain marketable size of about 2cm to 3.5 cm (Nie, 1982). In bivalves, growth is indeterminate and the asymptotic maximum size is habitat dependent (Sebens 1987). Food availability and other environmental factors affect the physiological costs and influence maximum size (Sebens, 1987).

Bivalves are ecologically important because they are devoured primarily by fish, mammals, crustaceans, birds and are also utilized for human consumption (Salanki *et al*., 2003). Besides, being a food source, they are collected for ornamental purposes and commercial uses (Shanmugam and Vairamin, 2005). Bivalves are also consumed by humans since they are good source of protein. A study has found 60% protein content in *Perna viridis* soft tissue (Choo & Ng, 1990). Bivalves are also good for bone health (Zalloua *et al*., 2007). It contains high vitamins (vitamin B6, niacin, thiamin and riboflavin) and essential minerals (such as Ca, Fe) (Yoshino *et al*., 2005). Futhermore, they are used as bioindicators due to their sedentary life which is important in monitoring contaminants in different ecosystem (Feldstein *et al.*, 2003). They also play a role in food chain and recycling of organic matters, detoxifying pollutants, dispersion and burial and secondary production (Borja *et al*., 2008). Through suspension and deposit-feeding activities, bivalves can cycle large amounts of particulate matter within the environment, converting some of it into flesh and gametes, depositing varying amounts to the benthos and cycling complex molecules into inorganic forms (Ward and Shumway, 2004). Suspension feeders play such a key role in water filtration that regulates light availability and growth thus, contributing substantially to maintaining water clarity and density. They are critically important for the ecosystem goods and services they provide to nearshore tropical and subtropical ecosystems (Souter and Linden, 2000).

III. MATERIALS AND METHODS

Design

Observational method was used in the study. This is a type of descriptive method wherein researchers view the natural environment setting (Jackson, 2009). The number of individuals were observed using a 100-m belt transect method.

Environment

The study was conducted in mangroves and seagrass areas of Northwestern, Bohol Philippines. Bohol is an island province of the Philippines (Figure 1) in Central Visayas region which is famous of its biodiversity and was the epicentre of the earthquake. As a small island, it was affected greatly when a 7.2 magnitude earthquake calamity happened last October 15, 2013. The three municipalities of Calape, Tubigon and Clarin were the most affected areas. Clarin was the nearest with 8 kilometers away from earthquake epicenter (Sagbayan), followed by Tubigon (15kms) and Calape (33kms) respectively. Station 1, Calape is located at 9° 53' 20" North, 123° 53' 0" East. This is the third income class municipality in the province. Municipal water of the area is about 12,486 hectares and 21.68 kilometers shoreline length excluding offshore island (Green *et al*., 2002).

Station 2, Tubigon (9° 57' 6" North, 123° 57' 43" East) is the second class municipality and the nearest seaport in Bohol to Cebu. It is a coastal town with 12 coastal barangays and 6 islands in which 40% of the population rely on fishing as a livelihood. Mangroves were found along the coast with seagrass beds on its intertidal areas (Green *et al*., 2002). Sediment observed in the area were soft substrata comprised of sand and mud preferable for burrowing organisms.

Finally, Clarin (Station 3) is located at 9° 53' 20" North, 123° 53' 0" East. This is the third income class municipality in the province. Municipal water of the area is about 12,486 hectares and 21.68 kilometers shoreline length excluding offshore island (Green *et al*., 2002). Large mangroves and seagrass beds abound the coastal area.

Procedures

Sampling was done by setting the transect line seaward perpendicular to the coastal line (English *et al*., 1997). This was adopted in the study of Katsanevakis and Thessalou-Legaki (2009) but with certain modifications. In the latter study, they used 50 meters line transect length with .5 m transect quadrats. However, in this study a 100 meters line transect used in each sites . Each of 100 meters transect line was overlaid with 1m x 1m quadrat with 10 meters interval from each quadrat. All bivalves present within the quadrat were collected and preserved in 95% alcohol for identification.

There were six transects deployed in each site, three replicate transects in both ecosystem each with 100 meters interval (Figure 2). A total of 90 transects were installed in all sites from September 2016-January 2017. The starting point of the line transect were randomly selected along the area.

Sampling and collection of bivalves was conducted from September 2016 to January 2017. Samples were collected in mangrove and seagrass ecosystems of Calape, Tubigon and Clarin. Collections and observation was carried out during low tides through gleaning (Dolorosa & Dangan-Galon, 2014). For sandy and muddy substrates, the surface was slightly swept or dug up for burrowing bivalves. For every area, the number of individuals of each species encountered was recorded. A voucher specimen from each encountered species was brought to the laboratory for identification. The soft tissues were removed and the shells were cleaned and photographed.

Species Identification of Collected Samples

Identification of bivalves was based on shell morphology (Bouchet *et al*., 2010). Specifically, outer structures such as color, shape, texture, number of radial rays and arrangement of ridges and inner shell structures like cardinal teeth, form of palial sinus, nacreous layer and scar of adductor muscle. Specimens collected were identified to the species level using different references, web search, and previous studies. Reliable identification guides were used in the study such as Mangrove Environments and Molluscs (Lozouet, 2008), Guidelines for Identifications of Bivalves and Gastropods (Carpenter & Niem 1998), Guidelines for Bivalves in Asia (Lam *et al*., 2009), FAO Species Identification Guide Baldwin (2003) and Guide to Marine Gastropods of the Philippines (Ilano & Sotto, 2008). Species identification was verified by the experts at the Marine Invertebrate Collections, Biological Museum, University of San Carlos, Cebu City and institutions such as South East Asia Fisheries Development Center (SEAFDEC), Iloilo, Philippines. The collected samples were preserved using 90% alcohol and deposited at the marine research station of the University of San Carlos, Mactan Cebu.

Data Analysis

The relationship among bivalve species was analyzed using cluster analysis in PAST SOFTWARE VERSION 3.2.

IV. RESULTS AND DISCUSSIONS

This dichotomous key of bivalve species was confirmed in the study of Carpenter & Niem (1998). However, there were three species of bivalves described in this study that were not described in the studies of Carpenter and Niem (1998), Baldwin (2003), Ilano & Sotto (2008) namely: *Katelysia cf hiantina*, *Katelysia recens* and *Meretrix cf lyrata*. These species are common in the Philippines with very similar features on *Katelysia hiantina* and *Meretrix lyrata* however they have different color forms (cf) which could be the reason why they were considered separate species. *Katelysia recens* is like a combination of *K.hiantina* and *K. cf hiantina* while *Meretrix cf lyrata* is close variety of *Meretrix lyrata.* Similarly, *K. cf hiantina* is a close species variety of *K.hiantina (*Huang *et al*., 1990).

The shown dendogram (Figure 1) was created using Jaccard's index of similarity and bootstrap method which appears to be monophyletic. However, the method used created almost overlapping clades (indicated with box) due to minute differences in the similarity index which means that the encompassed organisms were morphologically similar but not 100% with reference to the chosen characters.

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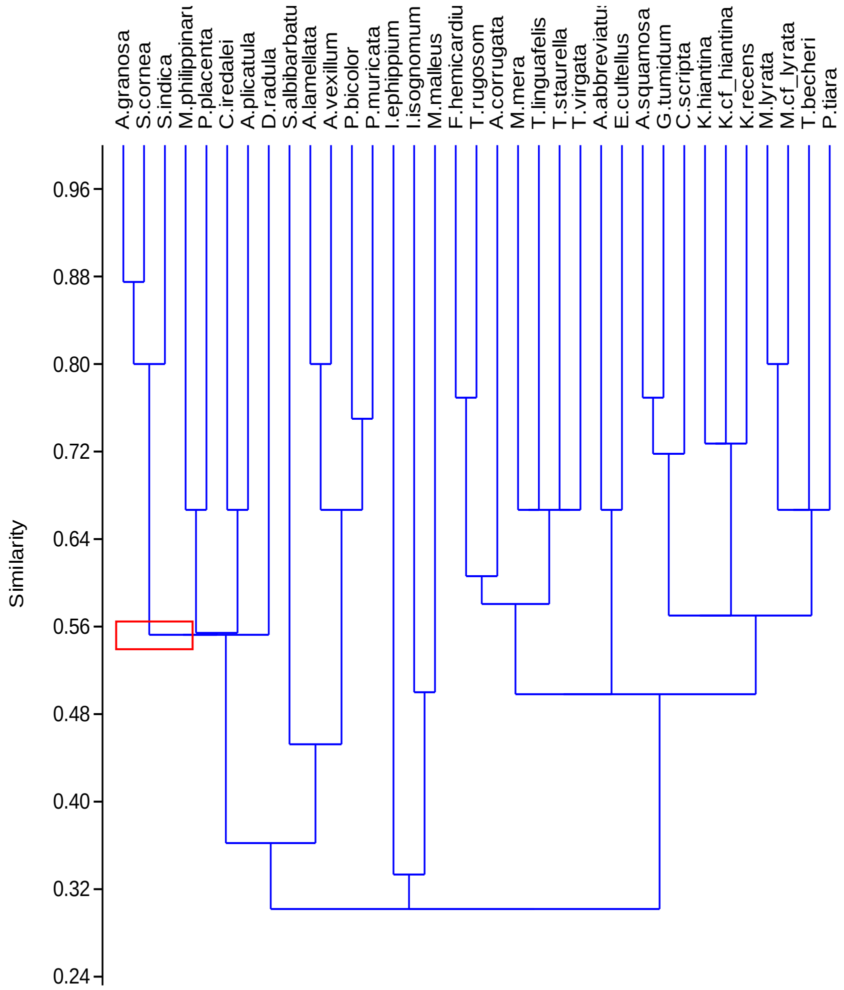

Figure 1: Cluster Analysis of Bivalves in Bohol, Philippines

Out of the six orders, Arcoida had a very strong bootstrap support with 80%. Arcoida was also noted as the basal group among all extant bivalves. This result was similar to that comparative anatomy study of Allen and Downing (1986). This group includes *A. granosa*, *S.cornea* and *S.indica* which have synapomorphies of having prominent radiating plates and arc-shaped shell. The sister taxa closely related with Arcoida was Mytiloida and Ostreioda with 65% bootstrap support. Both shared a dense shell anterior and inner white layer character like that of Arcoida. Study of Campbell (2000) on 18S DNA of bivalves strongly support the same close relationship noted between Mytiloida represented by *M. philippinarum* and Ostreioda represented by *P. placuna*, *C. iredalei* and *A.plicatula* respectively. The orders Pectinoida and Pteroida also showed close relationship. Wherein, the genus *Spondylus* was sister taxa to *Decatopecten* while genus *Atrina* and *Pinna* were sister clades. They both have a posterior adductor muscle and nacreous layer on inner shell.

However, the longer branches of *Spondylus* compared to that of *Atrina* and *Pinna* signified that there were many underlying differences between two clades. The difference could be on its shell shape since *Spondylus* was fan-shaped while the two were triangular. Although this was based on morphological description, result of this study coincided with the study of De Gaulejac *et al*., (1995) on acrosomal vesicle where Pectinoida and Pteroida were sister taxa. They also both established homology as they have apical wedge-shape zone. The Family Malleidae represented by *Malleus* and *Isognomon* belonging to Order Pectinoida were also sister group and showed the lowest bootstrap support (45%). They both have nacreous layer in inner shell.

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In addition, the same long branches were described by Orders Pteroida and Veneroida with less than 50% bootstrap support. This was comprehensible due to its high diversity. The more species in a clade the less robust it would be. Although, they have differences but still they have seen plesiomorphic character which was having dense anterior layer.

Meanwhile**,** distribution of bivalve species (Figure 2) had contributed to diversity of three sampling sites namely: Clarin, Tubigon and Calape. In this figure it also showed that there were similar species distributed widely in Clarin and Tubigon than Calape. This model exhibited a clear grouping and separation of the ordinance plot distribution of bivalves in Clarin, Tubigon and Calape.

The close points of the plot reflected sample similarity in species composition and distant plot showed differences (Clarke and Gorley, 2006). The difference level of percentage between Clarin and Tubigon was significantly different with p value 0.05 and global R of 0.3.

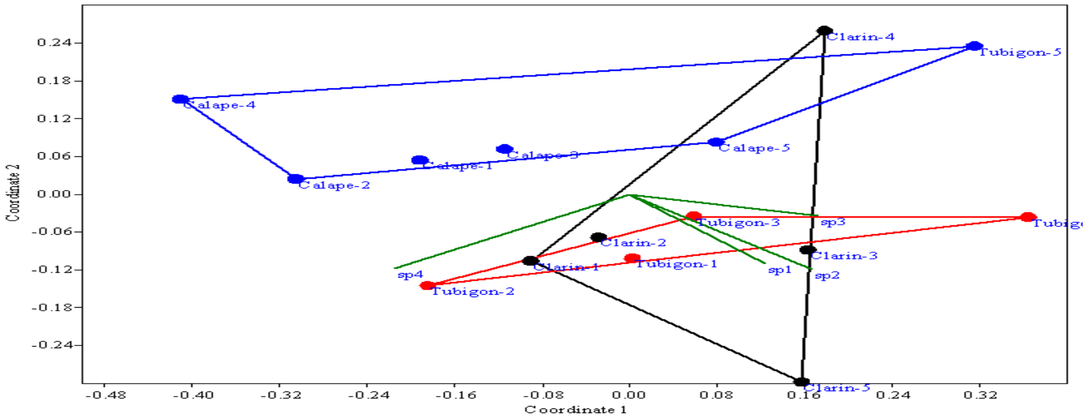

Figure 39. Bivalves nMDS plot in Mangroves and Seagrass Ecosystem of Northwestern Bohol, Philippines (black dot indicating Clarin, red for Tubigon and blue for Calape; Sp1-*S. cornea*, Sp.2 -*A. granosa*, Sp3-*S.indica*, Sp4-*M.philippinarum*)

The percent similarities within sites were 45.68 and between sites 56.07. This Similarity of Percentage (SIMPER) of bivalves was shown in Table 2.

Table 1: Mean Similarity (%) and Species contributing to similarity of Clarin, Tubigon and Calape

Mean Rank	Mean Similarity (%)	Species Contribution (%)
Within Sites	45.68	Sp.1- *Scapharca cornea* (51.16%), Sp.2- *Anadara granosa* (7.05%), Sp. 3- *Scapharca indica* (3.99%) and Sp.4- *Modiolus philippinarum* (3.5%)
Between Sites	56.07	

Out of the 35 species, there were 4 species that showed highest contribution on the similarity of these sites namely; *Scarpharca cornea*, *Anadara granosa*, *Scapharca indica*, *Modiolus philippinarum*. These means that these were the species commonly distributed in the area. High distribution of these species in Clarin and Tubigon could be due to their muddy substrate preference. This could also be attributed to their mode of feeding which is suspension feeding that is why they were usually found in locations were sediments particles are very fine (Rhoads and Young, 1970). Another reason on the distribution of the latter bivalve species in the seagrass bed was a high area of stability thus they have high survival rate (Narasimham, 1985). Besides, the dominance of these species over other bivalve species could also be due to their high tolerance to certain environmental stress like atmospheric exposure, fluctuation in pH and temperature (Yennawar *et al.*, 2014).

Bivalve community structure between sites Clarin, Tubigon and Calape found average dissimilarity of 35.21. The species contributing to this difference were Sp2-*A.granosa* (7%), Sp15- *Fragum hemicardium* (5%), Sp.16-*Trachycardium rugosum* (4.8%), and Sp28- *Tapes belcheri* (4.4%). The other species not mentioned were of very low distribution such as *Atrina vexillum*, *Atrina lamellata*, *Pinna bicolor*, *Pinna muricata*, *Isognomon ephippium*, *Isognomon isognomum*, *Placuna placenta*, *Decatopecten radula*, *Spondylus arbibarbatus*, *Mactra mera*, *Tellina linguafelis*, *Tellina* staurella,

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Austriella corrugata, *Gafrarium tumidum*, *Anomalocardia squamosa*, *Circe scripta*, *Katelysia recens*, *Katelysia hiantina*, *Katelysia cf hiantina*, *Meretrix lyrata*, *Meretrix cf lyrata*, *Malleus malleus*, *Placamen tiara*, *Azorinus abbreviatus*, *Tellina virgata*, *Crassostrea iredalei*, *Alectryonella plicatula*, and *Ensiculus cultellus*.

Their low distribution in the three towns could be due to their patchily distributed behavior, high predation of other organisms like sea stars which were observed dominant especially in Calape. According to Mcdonald *et al*., (2015), a need of extensive sampling is a must for patchily distributed organisms. However, sampling collection in this study was made only by handpicking, bolo and "mata-mata" technique with the common gleaners.

V. CONCLUSION

There were 35 species of bivalves identified only in Northwestern Bohol areas over 100 species reported in the entire province of Bohol which means 65 species could be present in other areas. Cluster analysis showed a monophyletic relationship which means that most of the species could evolved on the same basal group. Distribution of bivalves in three towns is patchy which could be due to its high exploitation in these areas.

VI. RECOMMENDATIONS

1. Further studies may be taken to get the overall record of species in Bohol province.

2. Molecular analysis may be conducted to strengthen the monophyletic relationship of bivalves and to evaluate if the color morphs variation means completely another type of species.

3. There may be a law regulating the collection of these resource as they are wild in nature and could be depleted if no mitigation has been conducted.

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