MORPHOLOGICAL POLYMORPHISM OF *Canarium urceus urceus* (Linnaues), 1758 (MOLLUSCA: GASTROPODA) IN MARINE AREAS OF CARAGA REGION, PHILIPPINES

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Abstract

A total of 877 individuals of Canarium urceus urceus were collected, measured and evaluated for their morphological attributes in the four (4) sites in Caraga Region: Lianga and Cortes Surigao del Sur; Nasipit, Agusan del Norte and Claver, Surigao del Norte. The shells were morphometrically measures in terms of its shell length (SL), body whorl length (BWL), shell width (SW), shell depth (SD), shell outer lip thickness (SOLT), aperture length (AL), operculum width (OW) and operculum length (OL). The morphometric comparison in different sites detected significant differences in all shell characters across sampling sites. The Principal Component Analysis (PCA) revealed that the most classified characters are the BWL, AL and SL across sampling sites. The most classified characters for male, female and imposex are the OW and SW according to canonical discriminate analysis (CDA). The operculum of imposex is much wider compared to female and male. However, the male gastropod operculum is wider compared to a normal female. In terms of SW of the gastropod, the females have much wider SW and globular than males that are slender in shape.

Keywords: canrium urceus urceus, morphometry, multivariate analysis

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1.0 Introduction

Gastropods are among the few animals that provide directly measurable connection of their life through their shells. The characteristic spiral structures produced by gastropods conveniently suit them as bioindicator for a variety of environmental conditions. Shells provide surfaces for muscle attachment and protection from predators, mechanical damage and desiccation (Yousif, 2012). The shell is composed of calcium carbonate structures that are accreted in a spiral growth pattern that records the ontogenetic development of the individual producing it and, therefore, serve as informative models for morphological research.

Morphometry is a complimentary method in studying morphology. Shell morphology involves growth from the beginning of postembryonic life, provides ontogenetic information, and is correlated with behaviors and habitats (Yousif, 2012). Because of these, morphometric analysis of several organisms can be helpful in the variation of biological shape and their covariation with other variables (Rohlf and Slice, 1990). According to Klingenberg (2011), the purpose of morphometry is to quantify the size and shape of organisms with the methods of multivariate statistics.

The *C. urceus urceus* is commercial seafood in the Philippines and Indo-Pacific Region (Erlambang and Siregar, 1995; Cob et al., 2007). This species is abundant wherever it occurs, and is generally associated with sandy mud bottoms and seagrass beds (Abbott, 1960; Erlambang and Siregar, 1995; Cob et al., 2007). It was the most abundant herbivorous mollusk within the study site. Also, these gastropod shells are unique, terminally elongating objects that result from accretionary growth and provide an ideal subject for shape analysis (Yousif, 2012). This study is very essential in evaluating its morphological structure, its taxonomic diversification of *C. urceus urceus* in Caraga Region. This would also formulate models of the relationships between environmental conditions and shell morphology.

2.0 Research Methodology

Study Sites

The study area was conducted at selected sites in Caraga Region. Four (4) sampling sites were established: Lianga and Cortes, Surigao del Sur; Nasipit, Agusan del Norte and Claver, Surigao del Norte.

Collection and Sampling

The sampling was done during the wet season of 2014, particularly in the months of November and December. The time of collection was characterized by strong winds and heavy rainfall. The collection sites were situated on the sea grass since this specimen is bound to rocks and in sea grasses.

The collection of samples was done through a random handpicking from identified stations. Also, the preference for such site was based on the majority of the shell collectors prefer to glean in the intertidal area.

Processing of Samples

The specimen were preliminarily narcotized with magnesium chloride solution 3.5% (Huet, et al., 1995) and preserved in a refrigerator. After 24 hours, the samples were placed in 10% formalin solution of seawater and kept frozen prior to analysis. The meat of the shell is obtained by pulling the soft body slowly without the need to break the shell. The use of this technique is proved very practical to less the secretion of the mucous (Cob et al., 2007).

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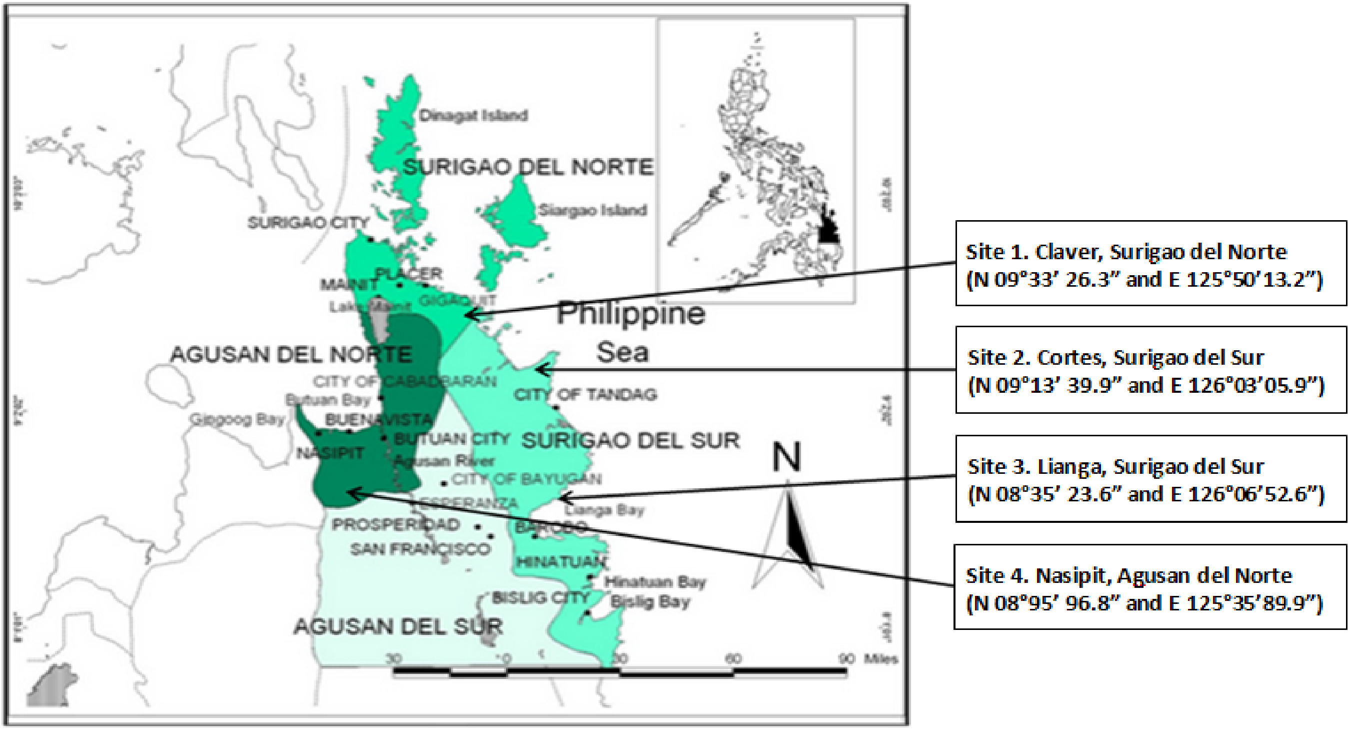

Figure 1. Map of Caraga Region showing the four (4) study sites

Morphometric Data Acquisition

Prior to the recognition of sexes of gastropod, the various linear morphological characteristics of the shell were measured to 0.01 mm using a vernier caliper. The measurements were the shell length (SL), body whorl length (BW), shell width (SW), shell depth (SD), shell outer lip thickness (SOLT), aperture length (AL), operculum width (OW) and operculum length (OL) as shown in Figure 2.

Recognition of Sexes

The *C. urceus urceus* individuals were classified as female, imposex female and male by measuring the penis length and examining the gonad color. Females have no penis and with yellow gonads while males are characterized by the presence of penis and red-orange gonads with whitish spot. Imposex females have penis and gonads that were either completely yellow or a mix of yellow and pale orange. Imposex females were examined morphologically by assessing morphological deformities such as symmetry in tentacles or abnormal eye growth on tentacles.

Data Analysis

Data were tested for normality and homogeneity of variances. Differences in morphometric variables between groups were analyzed via the non-parametric univariate methods such as the Kruskal-Wallis or H-test (with statistics distributed Chi-square) and Tamhane's test at $p<0.01$ probability levels. Variation between groups was further analyzed using multivariate approaches such as Canonical Discriminant Analysis (CDA) to identify the important characters that contribute to distinguishing

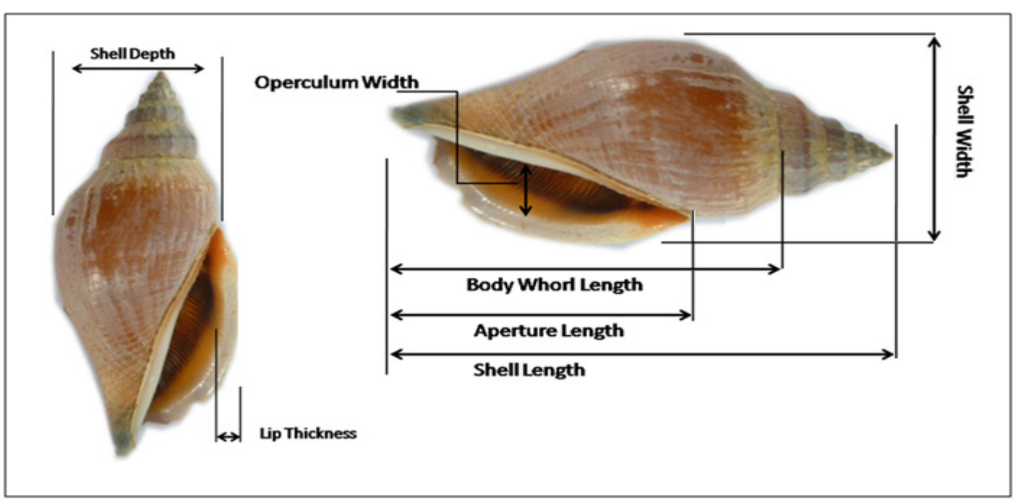

Figure 2. Measurements for comparing shell morphology in *C. urceus urceus*

shell characters and Principal Component Analysis (PCA) to generate components and vectors that account for variation in the data using SPSS software.

3.0 Results and Discussion

Shell Morphometry Across Sampling Sites

A total of 877 individuals of *C. urceus urceus* were collected and measured from the four (4) sites of Caraga Region: Lianga Surigao del Sur; Cortes Surigao del Sur; Nasipit, Agusan del Norte and Claver Surigao del Norte. Table 1 shows the morphometric variability of the shell characters of *C. urceus urceus* in the different sampling sites.

Shell morphometric characters have lowest means in site 1 but highest means in site 4 except for the lip thickness and aperture length. It is notable that lip thickness and aperture length were highest in site 1 when they were lowest in site 4. On the other hand, sites 2 and 3 have no significant difference in shell morphometry.

Table 2 shows the principal component analysis (PCA) of shell morphometric characters of *C. urceus urceus* in different sampling sites. Shell morphometry in site 1 is significantly determined by a uni-dimensional component of six (6) correlated shell variables listed in table 3 (excluding lip thickness and operculum length) explained by a total variance of 41%. Moreover, site 1 had highest loading for shell BWL (factor loading of 0.822); that is BWL primarily determines shell shape in site 1. Precisely, high SL, BWL, SW, SD and OW and low AL characterize shell in site 1. The BWL highly determines shell shape in site 1 but table 2 shows relatively shorter BWL in site 1 as compared with other sites.

This is noted that the sampling area has a strong wave that splashes in the intertidal area due to the southwest monsoon that brings strong winds and heavy rain. The BWL in site 1 is slightly shorter compared to other sites. The body whorl of the gastropod described as the most recently-formed of a spiral shell, terminating in the aperture. Most of the body of the soft parts of the animal fits into this whorl. The proportional size of the body whorl in gastropod shells differs significantly according to the actual shell morphology. For shells in which the rate of whorl expansion of each revolution around the axis is very high, the aperture and the body whorl are large, and the shell tends to be low spire. Since the revealed outcome, based on PCA BWL highly determines shell shape in site 1 but table 2 shows relatively shorter BWL in site 1 as compared with other sites. According to Trussell (1997) the shells that are shorter and wider, also been found to be more advantageous in high current environments to reduce drag.

PCA on the shell characters in site 2 reveals a two-dimensional shell morphometry that accounts to a substantial cumulative variance of 62.67%. The first component determined by SL, BWL, SW, AL and OL accounts for a variation of 48.22% of the shell morphometry in site 2 with high loading for BWL. This shell characterization in site 2 has few similarities with that in site 1 but the difference may be substantially explained with the presence of secondary characters – LT and OW in site 2. Thus, shell morphometry in site 2 is strongly characterized by moderate SL, BWL, SW and OL, and high AL.

For site 3, shell morphometry is strongly determined as uni-dimensionally by the correlated variables that account to 65.00% for the variance with highest loading for AL. Thus, shell shape in site 3 is substantially characterized by moderate SL, BWL, SW, SD and OL and relatively high OW and AL as best determinant.

The sites 2 and 3 have the same best determinant characters are the aperture length (best determinant). These sites have a contradicting description of the study site. The site 2 (Cortes) is the control area, it has no mining activity, no shipyard and far away from the households. However, for the Site 3, the collection of samples was done near shipyard activity and households. In terms for their oceanographic attributes, these sites, has a greater depth of almost 3 meters and have a sandy

Table 1. Summary on the morphometric variability of the shell characters of *C. urceus urceus* in different sampling sites

Variables	Lianga (Site 1) (mean±sd)	Cortes (Site 2) (mean±sd)	Nasipit (Site 3) (mean±sd)	Claver (Site 4) (mean±sd)	χ^2	*p*-value
Shell Length	^{bc}3.93±0.32	^{ab}4.01±0.40	^{b}4.04±0.33	^{a}4.14±0.48	24.798	<0.01
Body Whorl Length	^{b}3.05±0.23	^{b}3.08±0.24	^{b}3.09±0.27	^{a}3.31±0.45	83.800	<0.01
Shell Width	^{b}1.82±0.26	^{b}1.97±0.24	^{b}1.99±0.35	^{a}2.02±0.26	123.630	<0.01
Shell Depth	^{b}1.56±0.81	^{b}1.69±0.81	^{b}1.63±0.21	^{a}2.02±0.26	435.148	<0.01
Lip Thickness	^{a}0.36±0.06	^{b}0.29±0.24	^{b}0.30±0.08	^{b}0.26±0.29	189.855	<0.01
Aperture Length	^{a}2.81±0.26	^{a}2.86±0.26	^{a}2.86±0.23	^{b}2.62±0.38	106.731	<0.01
Operculum Width	^{b}0.32±0.05	^{a}0.59±0.15	^{a}0.58±0.17	^{a}0.57±0.07	418.852	<0.01
Operculum Length	^{c}0.65±0.37	^{b}2.66±0.22	^{b}2.66±0.22	^{a}2.96±0.39	574.219	<0.01

Note: Differing letters or letter combination means differing levels; a > b > c

Table 2. Principal component analysis (PCA) of shell morphometric characters of *C. urceus urceus* in different sampling sites

Variables	Lianga (Site 1) Component	Cortes (Site 2) Component		Nasipit (Site 3) Component	Claver(Site 4) Component
	1	1	2	1	1
Shell Length	0.790	0.723		0.895	0.935
Body Whorl Length	0.822	0.879		0.891	0.891
Shell Width	0.657	0.662		0.635	0.906
Shell Depth	0.249	-	0.830	0.785	0.906
Lip Thickness	-	-	-	-	0.302
Aperture Length	0.731	0.845	-	0.912	-
Operculum Width	0.472	-	- 0.567	0.593	-
Operculum Length	-	0.823	-	0.867	0.882
Total variance explained					
% of variance	41.62	48.22	14.45	65.00	68.11
Cumulative variance	41.62	48.22	14.45	65.00	68.11

Extraction Method: Principal Component Analysis.
Rotation Method: Varimax with Kaiser Normalization.
[a]Rotation converged in 3 iterations.

substrate. Also, these sites have a high occurrence of crabs that were observed.

In this case the aperture of the gastropods in these sites has a wider opening. It may be explained that since the aperture is the opening of the shell of the gastropods, there is a high occurrence of predation of crabs in these two sites. According to Vermeij et al., (1980) that opening of the gastropod may vary on the damage scars as the result from chipping due to crab predation (Vermeij et al.,1980). It may also this is an indicator that the crabs in the area is abundant (Hoagland, 1978). Also Currey and Hughes (1982) stated that the heavy surf on wave-exposed shores favor shells that are small, thin and have wide apertures, whereas the high risk of shell-breaking predation on wave-sheltered shores is thought to select for shells with small apertures and increased size and thickness.

Shell morphometry in site 4 is significantly determined by a uni-dimensional component of six (6) correlated shell variables listed in table 3 explained by a total variance of 68.11%. Moreover, site 1 had highest loading for shell SL (factor loading of 0.935); that is SL primarily determines shell shape in site 1. Precisely, high SW, SD, BWL and OL and low AL characterize shell in site 1. Site 4 the best determinant character is the shell length. The collection of samples was done near the mining area. The shell length and some of the shell characters has the highest mean. This could be explained that the gleaners in the area don't tend to collect the gastropod due to their perception that gastropods were already affected by the mining industry. In this case, since there are no gleaners, the gastropods in the area are abundant.

According to Carvajal-Rodriguez et al., (2005) the shell shape differs from the habitat they occupied. It depends on the different physical and ecological conditions. Environmental conditions differed in slope, wave energy and wave exposures may be the factors. The differences in shell shape across the sampling sites may be their mechanism for maintaining an extensive distribution and maximizing local success a variety of environments (Dunithan et al., 2012). Besides to water current, calcium levels affect snail shell morphology. The calcium levels in the environment act as limiting factors and selective pressures on snail shell morphology (Rundle et al., 2004). The wide variation of *C. urceus urceus* can also be attributed in taxonomic diversification. According to Poppe (2008) *C. urceus urceus* is an example of a possible complex of subspecies or a geographical cline. More research on this group is warranted. Attempting to understand the relationship between morphological and taxonomic diversification has become a central concern of both paleobiology and ecology (Latiolais, 2003). The Strombidae, a family of shelled marine snails, shows a wide variety of forms, making it an ideal group for such analysis. This study dealt a deeper analysis of *C. urceus urceus* across sampling sites and in terms of the sexes. The variation of *C. urceus urceus* in terms of geographic cline may have the influence on the habitat they occupied affect the shell morphology and colors of the gastropod.

The extreme diversity in shell shape of *C. urceus urceus*, have the influence in their diet and protection from predators. Their shell shape probably affect during their epifaunal locomotion, burrowing and passive protection from predators.

Shell morphometry and sex

A total of 515 females, 335 males and 27 imposex of *C. urceus urceus* were observed in the study. Table

3 shows the canonical discriminant functions on the morphometric variability of the shell characters among sex.

Table 3. Summary of the canonical discriminant functions on the morphometric variability of the shell characters among male, female and imposex of *C. urceus urceus*

	Axis 1	Axis 2
Eigenvalue	0.057*	0.016*
Percent (%) of variance	78.4	21.6
Cumulative % of variance	78.4	100.0
Eigenvectors:		
Shell length	.041	.488
Body whorl length	-.318	.058
Shell width	.183	.710
Shell depth	-.217	-.414
Lip thickness	.016	.213
Aperture length	.374	-.058
Operculum width	1.032	-.433
Operculum length	-.233	-.142

*The two discriminant functions were used in the analysis

Classification Results[(a)]

	Sex specific	Predicted Group Membership		
		Female	Male	Imposex
%	Female	38.7	32.7	28.6
	Male	31.0	42.0	27.1
	Imposex	3.4	10.3	86.2

[a]41.6% of original grouped cases correctly classified

The 1st canonical discriminant function coefficient accounted for 78.4% of the variance in morphology among the groups. Its eigenvector elements showed both positive and negative values, indicating that the CDA described differences in shape rather than size, where the operculum width (OW) was the most important discriminating parameters. The 2nd function accounted for 21.6% of the total variations, with shell width (SW) forming the most important selective character. The most classified characters for male, female and imposex are the operculum width (OW) and shell width (SW).

For the classification result in the predicted group membership among sex (Table 3) 86.2% was strongly classified as imposex in all of the population of *C. urceus urceus.* It implies that the shell characters of the imposex vary most in female and male. The operculum of imposex (figure 3) is much wider compared to female and male. However, the male gastropod operculum is wider compare to a typical female.

The most essential function of the operculum in gastropods is to allow signals to resist drying out, or desiccation. This is very important intertidal gastropods during low tide, and this also enables operculate gastropods to survive periods of drought and dry weather it can also serve as a protection against predators when the snail body is retracted. Also, operculum may serve as a sort of trapdoor to close the aperture of the shell when the soft parts of the animal during sexual mating.

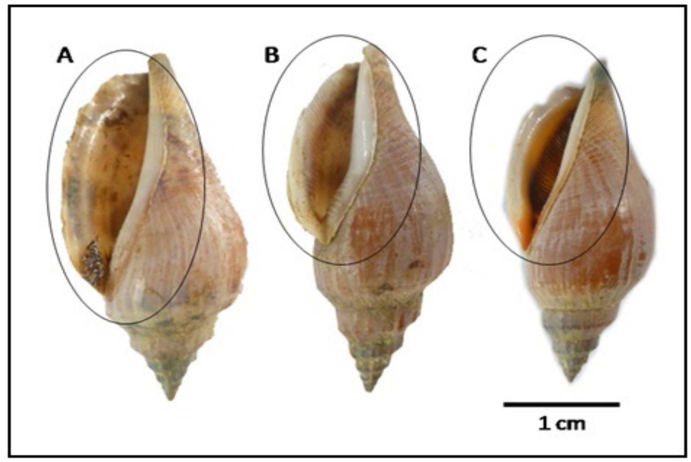

Figure 3. Morphological variabiability of aperture and operculum in *C. urceus urceus* in terms of sex (A. imposex; B. male; C. female)

In terms of shell width (SW) of the gastropod, the female is much wider than a male. However, the normal female is slightly wider compared to male. This result was supported in the different studies of Abbott (1949) in *Strombus gibberulus gibbosus* and Reed (1995) of *Strombus luhuanus*. This result suggests that *C. urceus urceus* male is slender compared to female. Female gastropod is globular and much wider in shape. This study also suggests that *C. urceus urceus* females allocate more energy to tissue and gonad production compared to males.

According to size comparison between males and females, the larger ones were females. In literature, studies that analyze this aspect report that females are bigger than males. According to Charnov and Bull (1989) that female fertility increases with size. Data on egg production for several species of calyptreaids make it clear that female fertility increases with size (e.g., Collin 2000; Chaparro and Flores, 2002). Most known cases of sexual dimorphism in molluscs are based on size differences, where female shells are usually larger than males (Buston et al., 2004) Therefore, it is likely that male decreases with size in these species. Such a decrease could be explained by the difference in mobility and reproduction.

4.0 Conclusion

The morphological attributes of *C. urceus urceus* differed from the habitat they occurred. The gastropods exhibit sexual polymorphism, the normal males and females, a 3rd morph composed of abnormal females with imposex characters were present.

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