Relative Warp Analysis of Parasite–Induced Plasticity in the Shell Shape of Bithynia sp. (Bithyniidae)

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***Abstract*—Parasitism often influences the phenotype of gastropods. Many of the resulting changes are due to changes in resource allocation that come with infection. This investigation quantifies some aspects of the parasite-host relationship between cercariae and its intermediate host Bithynia sp. Noticeable differences in the shell shape of parasitized and uninfected Bithynia sp. snails were investigated using. Relative Warp Analysis, Discriminant analysis, and Kruskal-Wallis to determine shell shape divergence of the two populations. These shape divergence accounted for more than 35% of the variance in shell morphology relative to mean shape. Apparently, it may be that broader body whorl, wider aperture, conic to globose shape and bigger snails are more likely to become infected with *Bithynia sp.*, or that narrowly conic, reduced aperture, small-sized relative to the shell shape are more resistant to parasite infections. Hence, the methodology using landmark-based geometric morphometric methods proved to be more profound for the characterization of *Bithynia* sp. snails even at a subtle degree.**

***Index Terms*—Geometric morphometric analysis, relative warps, phenotypic variation, parasitism, Bithynia.**

I. Introduction

The *Bithynia* taxa are recognized as first intermediate hosts of trematode *Opisthorchis viverrini*, one of the most important human pathogens [1]. Interestingly, recent work on the genetic structure of geographically separated populations of *Bithynia* suggests species complexes [2]. Here, we provide a description of the morphology of the *Bithynia* species which are involved in the life cycle of medically important trematode to confidently predict specific outcomes of novel snail-trematode permutations.

Recent works have shown that parasitism can affect size in gastropods by altering the host's growth rate, inducing parasitic castration, alters behavior, fecundity and survivorship [3-13], but other morphological effects of parasitism have rarely been examined. In this study, the relationship between variation in host morphology and parasitism was examined in a population of the freshwater snail *Bithynia* species.

II. Materials and Methods

The study covered the representative barangays from Trento in the province of Agusan del Sur, Philippines (Fig. 1).

Figure 1. Map showing the study area, Trento, Agusan del Sur Province, Philippines.

With explorative-investigative study design, indefinite number of sample of *Bithynia* sp. snails was collected. Snail samples from the field were picked using forceps and kept in a sealed container from the field to the laboratory. Squash technique was used to observe for the presence of cercariae for all individual snail samples

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under a low and high power objectives compound microscope.

A total of thirty five (35) individuals of *Bithynia* sp. snails were collected and examined for cercariae. Of these, only ten (10) individuals (28.6%) were observed being infected with eye-spotted cercariae (Fig. 2). All samples of snails were obtained purposively for geometric morphometric analysis.

Figure 2. Eye-spotted cercaria observed under a compound microscope at 125x magnification.

Shells were photographed using a Nikon D5100 DSLR camera under a dissecting microscope with 20x magnification. Images of the shell were always in the same position with the columella at 90° of the x-axis in an aperture view or in the orientation in which the apex is visible. Obtained images were then subjected to geometric morphometric methods. In this study, landmark-based geometric morphometric method was used to obtain detailed shell shape pattern information between infected and uninfected snails of *Bithynia* species (Fig. 3). Digital images (ventral/apertural and dorsal views) were taken for each sample using a standardized procedure.

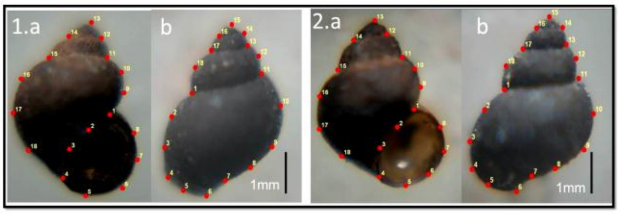

Figure 3. Landmarks used to describe the shape of the (a) ventral/aperture and (b) dorsal view of *Bithynia* species (1) uninfected, and (2) cercariae-infected shells.

Shell shapes of *Bithynia* species were deliberately studied using a landmark-based approach that eliminates the effect of variation in the location, orientation, and scale of the specimens, so that the remaining differences between individual or mean shapes are differences in shape only. Eighteen anatomical landmarks located along the outline of the ventral or apertural portion (Fig. 3, 1a & 2a) of the shell and also eighteen anatomical landmarks along the dorsal portion (Fig. 3, 1b & 2b) of shell were defined and used. This was achieved using an image analysis and processing software Tps Dig freeware 2.12 [14]. Tps Dig allows the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images. These coordinates were then transferred to Microsoft Excel application for organization of the data into two groups: uninfected and cercariae-infected shells. The two-dimensional coordinates of these landmarks were determined for each shell specimen. Then the generalized orthogonal least squares Procrustes average configuration of landmarks was computed using the generalized Procustes Analysis (GPA) superimposition method. GPA was performed using the software tpsRelw, ver. 1.46 [15].

After GPA, the relative warps (RWs, which are the principal components of the covariance matrix of the partial warp scores) were computed using the unit centroid size as the alignment-scaling method [16, 17] which may be visualized with a grid deformation [18]. The relative warps (RW) were computed to summarize the variation among the specimens. For visual representation of relative warp, rectangular grids are superimposed on a drawing representing a specimen, revealing the required "warping" from the mean shape to the specified configuration [19].

For statistical analysis and graphical presentations, the following applications were utilized through Paleontological Statistics (PAST) version 2.16 software [20]. Reduction of complex data as it reveals underlying morphological variables are the typical applications of Principal Component Analysis (PCA). Discriminant analysis and Hotelling's T^2 were also done for visually confirming or rejecting the hypothesis that two populations are morphologically distinct. To analyze whether or not the species differ significantly with regards to its shell shape, Kruskal-Wallis test was used [21]. Graphical representations showing a visual impression of the probability distribution of the two snail populations were generated with histogram and box plots. Histogram and box plots give a quick visual summary for comparing distributions between cercariae-infected *Bithynia* sp. shells and uninfected populations.

III. Results and Discussion

The analyses of geometric morphometrics of *Bithynia* sp. shell characters generated differences in shell shapes, by which landmark-based approach was used. In summary of the variation among the specimens, Fig. 4 and Fig. 5 present the geometric morphometric analysis (N=35) showing the consensus morphology (ventral/apertural and dorsal portions) and percentage variance produced by the relative warp (RW). This differentiation in shell shape was clarified by the dispersion of the landmark points related to the specimens from two different populations (uninfected shells and cercariae-infected shells) as compared to mean shape.

RW explains more than 5% of the overall variations both for ventral/apertural (Fig. 4) and more than 6% for dorsal (Fig. 5) parts of the shell. At the ventral/apertural view, the focal shell shape differentiation was greatly observed in the first relative warp (RW1) having percentage variance of 35.27%. Comparing deformation grids of Thin-plate spline (Tps) graphical models in RW1, shell samples with low negative (left) RW1 score have broader body whorl, the largest, last whorl of the spiral in a coiled shell, and bigger size relative to the shell shape. On the contrary, those with high positive (right) scores along the first relative warp axis have narrower body whorl with compressed spire and relatively small size based on the shell shapes. However, the apical sculpture of the shell both at negative and positive deviations is relatively tapered. Moreover, the aperture, opening of shell, situated at the last formed margin and providing an outlet for the head-foot mass tend to vary between infected (wide) and uninfected (narrow) shells as seen in RW 2, 3, and 4.

The group of (a) cercariae-infected shells spotted towards the morphological features of negative deviation, whereas the (b) uninfected shell samples characteristically more on the positive deviation as seen in the box-and-whisker plot diagram. However, the histogram did not reveal a discrete bi-modal frequency distribution of samples (Fig.4).

As for the dorsal view (Fig. 5), an extreme distant outlier established in box-and-whisker plot diagram resulted to a negatively skewed frequency distribution histogram. Morphologically, (a) cercariae-infected samples in negative deviation are broadly conic to globose, relatively larger size based on the shell shapes, and have wider shoulder portion. Whereas, (b) uninfected samples at positive deviation are broadly conic and small-sized relative to the shell shape. RW5 had the least percentage variation at 6.38% for the two extreme deviation axis; however, outlier of (a) infected-shells twisted its resemblance with the mean shape. Whether or not the two populations *of Bithynia sp.* (uninfected and cercariae-infected shells) differ significantly with regards to mean shape, Table 1 presents the Kruskal-Wallis test.

It revealed that all relative warps (RW) except for RW5 at ventral/apertural and dorsal views are insignificantly varied, manifested with the subtle distortion of apertural and body whorl shell sculptures as shown in Figs. 4 and Fig. 5.

Apparently, as all data points between two populations show their distribution pattern (Fig. 6b), an overlapping mean distribution can be observed which generally accounts to shell shape resemblances. Inversely, extreme distant points plotted along the axis of principal components can be implied with morphological dissimilarities of shell (Fig. 6a). In support, results in Principal Component Analysis (PCA) in Table 2 presents only three significant percentage variance (PC1, 46.07%; PC2, 21.45%; and PC3, 16.60%) for ventral/apertural and another three (PC1, 34.35%; PC2, 27.53% ; and PC3, 16.91%) for dorsal views. The Jolliffe cut-off value gives an informal indication of how many principal components should be considered significant, components with eigenvalues smaller than the Jolliffe cut-off may be considered insignificant.

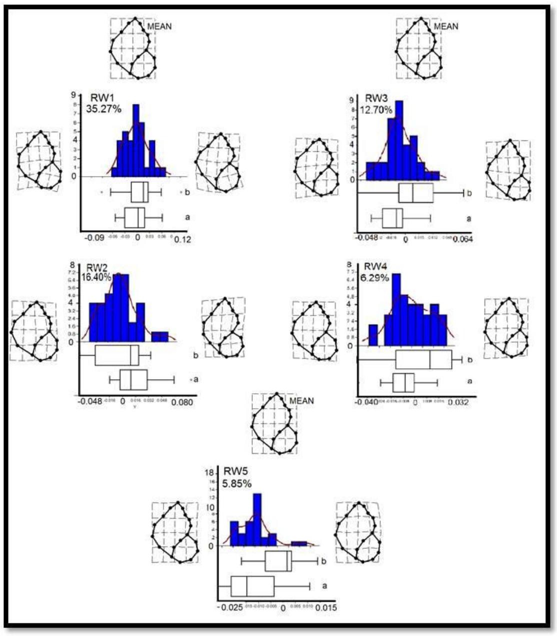

Figure 4. Relative warp box plot and histogram between (a) non-infected and (b) *cercariae*-infected shells of *Bithynia* sp. showing ventral/apertural shell variations in shape.

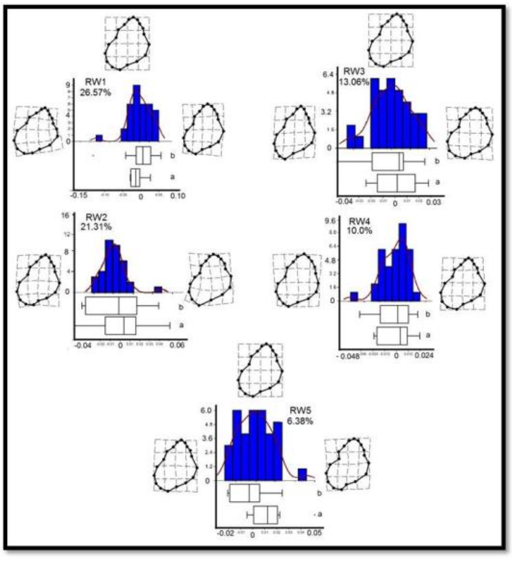

Figure 5. Relative warp box plot and histogram between (a) non-infected and (b) *cercariae*-infected shells of *Bithynia* sp. showing dorsal shell variations in shape.

TABLE I. RESULTS OF THE KRUSKAL-WALLIS TEST FOR SIGNIFICANT* DIFFERENCES AT 0.05 LEVEL OF SIGNIFICANCE IN MEAN SHAPES OF THE VENTRAL/APERTURAL AND DORSAL VIEW OF THE SHELLS BETWEEN THE UNINFECTED AND CERCARIAE-INFECTED SNAILS.

Relative Warp	Ventral/Apertural	Dorsal
1	0.6776	0.162
2	0.3847	0.7913
3	0.08897	0.7913
4	0.162	0.8501
5	0.04515*	0.0539*

TABLE II. THE PERCENTAGES OF VARIANCE ACCOUNTED FOR BY PRINCIPAL COMPONENTS IN THE VENTRAL/APERTURAL AND DORSAL PORTIONS OF *BITHYNIA* SP. SHELLS.

Principal Component	**Ventural/Apertural**		**Dorsal**	
	Jolliffe cut-off: 0.0005741		Jolliffecut-off: 0.0005195	
	Eigenvalue	**% Variance**	**Eigenvalue**	**% Variance**
1	0.00188918*	46.07	0.0012746*	34.35
2	0.00087956*	21.45	0.0010216*	27.53
3	0.00068109*	16.60	0.0006274*	16.91
4	0.00033730	8.225	0.0004805	12.95
5	0.00031347	7.644	0.0003061	8.251

Confirming or rejecting the hypothesis that two populations of *Bithynia sp.* are morphologically distinct, Discriminant analysis was performed. Fig. 7 shows the frequency histogram of the degree of dimorphism of *Bithynia sp.* based on two shell shape characters. Along the discriminant axis, the discrimination of the *cercariae*-infected (in red) from *cercariae*-free (in blue) is not absolutely separated; instead a superimposing population of *Bithynia sp.* is noticeable.

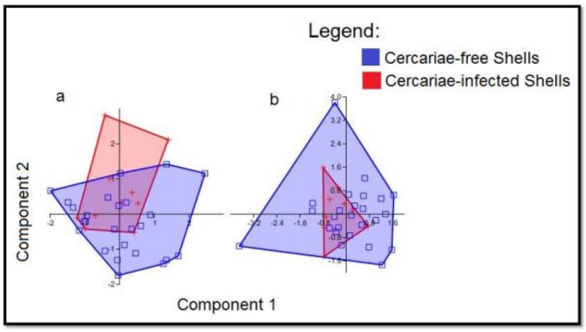

Figure 6. Scatterplot displays all data points between two populations, *cercariae*-infected (red) and *cercariae*-free (blue) shells of *Bithynia* sp. showing the distribution pattern at (a) ventral/apertural and (b) dorsal shell portions.

Statistically, there are instances wherein dimorphism could still be present even if the overlapping transpires in the histogram. Table 3 presents the percent correctly classified data based on the discriminant function scores of the ventral/apertural and dorsal portions of the shell. This percentage specifies how correctly trematode cercariae-infected shells are classified as cercariae-infected and how cercariae-free shells are classified as cercariae -free. Percentages greater than or equal to 75% of the correctly classified percentage is considered to be a cut-off for variation in structures, leading to dimorphism [16]. Remarkably, a high percentage of correctly classified shells population at ventral/aperture (85.71%) view detected in detail (Table 3) apparently explain for the morphological variations between the two populations of snails. In contrast, dorsal portion had a lower percentage of correctly classified shells population (74.29%), revealed with the overlapping populations in discriminant plot diagram (Fig. 7).

As a result, analysis of geometric morphometrics of the two populations (cercariae-infected and uninfected shells) of *Bithynia* sp. based on shell characters generally has demonstrated dimorphic features in shell shapes, yet limited at ventral/apertural view only.

TABLE III. PROPORTION OF VARIATION WITH THE DISCRIMINANT FUNCTION SCORES OF THE VENTRAL/APERTURAL AND DORSAL PORTIONS OF THE *BITHYNIA* SP. SHELLS.

Shell	**Ventral/Apertural**			**Dorsal**		
	C-i	C-f	CC %	C-i	C-f	CC %
cercariae -infected	8	2	80	7	3	70
cercariae -free	3	22	88	6	19	76
Total:			**85.71**			**74.29**
H t2:			35.284			16.382
p(same):			0.000503			0.03137

Legend: S-I, *Schistosoma*-infected; S-f, *Schistosoma*-free; CC, Correctly classified; H t2: Hotelling's

Recently, parasitism has been shown to alter shell growth, and thus shell morphology [22], [23]. The present study shows alterations of shell shape induced by parasite, trematode cercariae (Fig. 2) on *Bithynia* sp. (Fig. 3) populations. Snails infected with cercariae generally have bigger size relative to the shell shape compared to their non-infected shell population [24]. Accordingly, gigantism is a well-known phenomenon for host-parasite relationships, an adaptation by the parasites to increase the volume of available shell space [23-28].

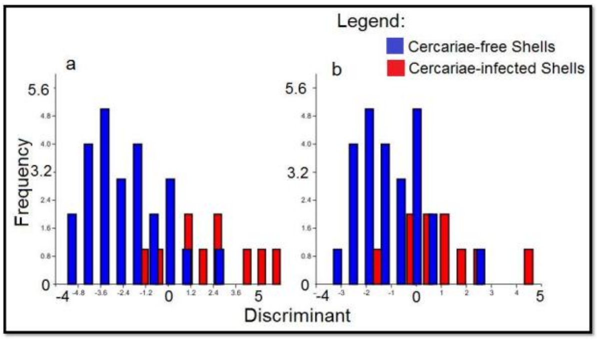

Figure 7. Frequency distribution histogram showing the variations in the (a) ventral/apertural and (b) dorsal portions in the shell patterns between *cercariae*-infected (red) and *cercariae*-free (blue) shells of *Bithynia* species

The magnitude of the effect associated with parasites was generally more pronounced in cercariae-infected shells having broader body whorl specifically at the shoulder region and wider aperture relative to body whorl (Fig. 4 and Fig. 5). Characteristically, mortality rate was higher among infected than uninfected snails [5], the smaller aperture observed (related to the shell profile) in the uninfected population protects the gastropods from desiccation or predation [29]-[31], suggesting that parasites are dominant drivers of phenotypic variation.

Contrariwise, in other gastropods, with infected snails were mostly having an elongated and narrower shell compared to non-infected group [8], [11]. Differential effects of parasites on shell morphologies of their gastropod hosts have been observed in other parasite–host systems [10] and host snails have been observed to become wider at the shell base in a few cases [9]. Though, here, no apical variations of shells observed between infected and uninfected snails. The absence of sutures or spires may be the consequence of the rerouting by the parasite of the snail metabolites allocated to growth [5], [6] or snails are often more likely to be blunted due to wear and tear.

IV. Conclusion

Parasitism often influences the phenotype of individuals-plastic response. Many of the resulting changes are due to changes in resource allocation that come with infection.

The application of a geometric-morphometrics (GM) method to the comparison of shell morphology allowed us to discretely identify shape variations even at subtle differences and low infection levels, 28.05% of the local populations being infected; the effects were even detectable at ventral/apertural portions. The parasite-induced morphologies were beneficial, as they precluded predation. Apparently, it may be that broader body whorl, wider aperture, conic to globose shape and bigger snails are more likely to become infected with *Bithynia sp.*, or that narrowly conic, reduced aperture, small-sized relative to the shell shape are more resistant to parasite infections.

Thus, using landmark-based geometric morphometric approach proved to be effective for the characterization between cercariae-infected and uninfected populations of *Bithynia* species. The changes in phenotype observed are likely to be adaptive for either the host or parasite and probably represent physiological by-products of the host-parasite relationship. This information should be taken into consideration for differentiating the species involved in the strategy for control of trematode infections in the country.

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