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Relative Warp Analysis of Parasite–Induced Plasticity in the Shell Shape of the O. Quadrasi

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***Abstract*—The shell morphology of *O. quadrasi* is hypothesized to be driven through coevolution between *S. japonicum* infectivity capacities *with O. quadrasi*. This hypothesis predicts that measures of geometric-morphometric variations of snail populations parallel varied schistosome infections thus was evaluated in two provinces of Mindanao, Philippines where there were reported infections. Relative Warp Analysis was used in the investigation to determine shell shape divergence. For statistical analysis, Discriminant analysis and Kruskal-Wallis test were performed. The results showed that parasitism in *O. quadrasi* had significant effects on some morphometric dimensions of the snail- apertural and apical sculptures based on the shell shapes. These phenotypic variations accounted for more than 40% of the variance in shell morphology relative to mean shape. Even at low infection levels, 4.05% of the local populations being infected, the effects were even detectable. Analysis of geometric morphometrics of the two populations (cercariae-infected and uninfected shells) *of O. quadrasi* based on shell characters generally has demonstrated dimorphic features in shell shapes.**

***Index Terms*—Geometric morphometric analysis; relative warps; phenotypic variation; parasitism; *O. quadrasi*; *S. japonicum* .**

I. Introduction

Several experimental studies have been conducted in investigating the influence of infection with *S. japonicum* on growth of *O. quadrasi*, an intermediate host of the disease schistosomiasis. Accordingly, compared to uninfected control snails, mortality was increased among infected snails [1]-[5]. Growth was at first accelerated by the infection but eventually slowed down, often with the development of a distortion of the shell aperture. The reduced growth rate of the infected snail compared to uninfected ones was interpreted as the consequence of the rerouting by the parasite of the snail metabolites allocated to growth [3], [4]. Interestingly, it is not only the actual size of snails that is affected by trematode infection, but also the morphology of their shell. Recent studies have shown that trematode parasitism changes the morphometrics of the shell, i.e. the ratio of length to maximum width, as well as the degree of its ornamentation, such as the length of spines [6]-[11].

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II. Materials and Methods

The study covered the representative endemic barangays with prevalence of schistosomiasis from the provinces of Agusan del Sur (Bunawan and Trento Municipalities) and Bukidnon (Malaybalay city, Impasug-ong, and Quezon Municipalities), Philippines (Fig. 1). Comparatively, the non-infected barangays were also represented as pre-determined by Department of Health (DOH)-Schistosomiasis Control Office.

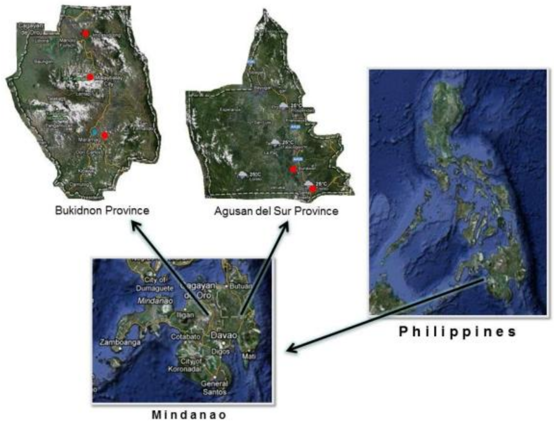

Figure 1. Map showing the study areas, Bukidnon Province (Impasug-ong, Malaybalay, and Quezon) and Agusan Province (Bunawan and Trento), Philippines.

With explorative-investigative study design, indefinite number of sample of *O. quadrasi* 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 *S. japonicum* cercariae for all individual snail samples under a low and high power objectives compound microscope.

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Figure 2. Fork-tailed cercariae of *S. japonicum* observed under a compound microscope at 125x magnification.

A total of one thousand six hundred seventy six (1,676) individuals of *O. quadrasi* snails were collected and examined for cercariae. Of these, only sixty eight (68) individuals (4.05%) were observed being infected with cercariae of *S. japonicum* (Fig. 2). One hundred thirty six (68 schistosome-infected & 68 schistosome-free snails) samples of snails were obtained purposively for geometric morphometric analysis.

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 *O. quadrasi* (Fig. 3). Digital images (ventral/apertural and dorsal views) were taken for each sample using a standardized procedure.

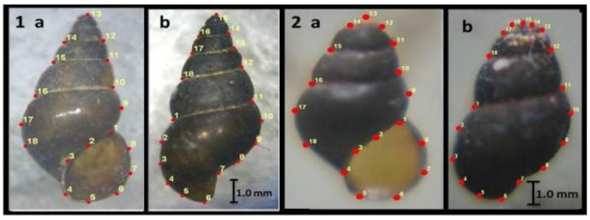

Figure 3. Landmarks used to describe the shape of the (a) ventral/aperture and (b) dorsal view of (1) uninfected *O. quadrasi*, and (2) *S. japonicum* cercariae-infected *O. quadrasi* shells.

Shell shapes of *O. quadrasi* 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. Tps Dig allows the statistical analysis of landmark data in morphometrics by making it easier to collect and maintain landmark data from digitized images [12]. 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 [13].

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 [14], [15] which may be visualized with a grid deformation [16]. 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 [17].

For statistical analysis and graphical presentations, the following applications were utilized through Paleontological Statistics (PAST) version 2.16 software [18]. 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 [19]. 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 *O. quadrasi* shells and uninfected populations.

III. Results and Discussion

The analyses of geometric morphometrics of *O. quadrasi* shell characters generated disparities in shell shapes, by which landmark-based approach was used. To summarize the variation among the specimens, Figures 3 and 4 present the geometric morphometric analysis (N=136) showing the consensus morphology (ventral/apertural and dorsal portions) and percentage variance produced by the relative warp (RW). This differentiation in shell shape was elucidated by the dispersion of the landmark points related to the specimens from two different populations (uninfected shells and *Schistosoma japonicum* cercariae-infected shells) as compared to mean shape.

RW explains more than 5% of the overall variations both for ventral/apertural (Fig. 4) and 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 30.27%. Comparing deformation grids of Thin-plate spline (Tps) graphical models in RW1, shell samples with low negative (left) RW1 score have broader aperture relative to body whorl 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 aperture and relatively small size based on the shell shapes. Moreover, the apical sculpture of the shell at negative deviation is relatively compressed while at the opposite deviation, tapered.

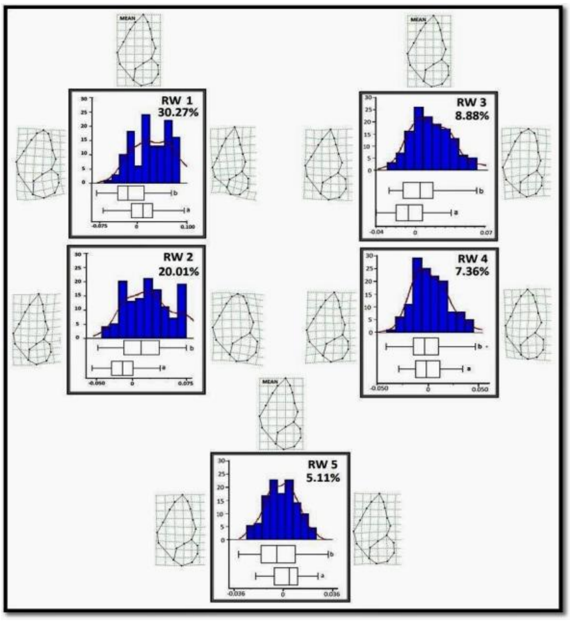

Figure 4. Relative warp box plot and histogram between (a) non-infected and (b) *Schistosoma*-infected shells of *O. quadrasi* showing ventral/apertural shell variations in shape.

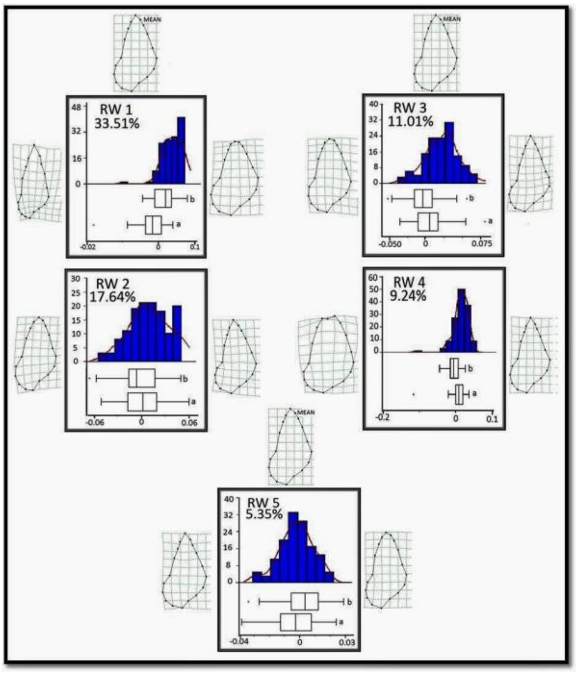

Figure 5. Relative warp box plot and histogram between (a) non-infected and (b) *Schistosoma*-infected shells of *O. quadrasi* showing dorsal shell variations in shape.

The group of (a) uninfected shells spotted towards the morphological features of positive deviation, whereas the (b) cercariae-infected shell samples characteristically more on the negative 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) uninfected samples at negative deviation are narrowly conic, small-sized relative to the shell shape, and tapered apical portion whereas, (b) cercariae-infected samples in positive deviation are broadly conic, relatively larger size based on the shell shapes, and have compressed apical portion. RW5 having the least percentage variation at 5.11% for the two extreme deviation axis, displayed the closest resemblance with the mean shape. Whether or not the two populations *of O. quadrasi* (uninfected and cercariae-infected shells) differ significantly with regards to mean shape, Table I presents the Kruskal-Wallis test.

It revealed that all relative warps (RW) except for RW4 and RW2 at ventral/apertural and dorsal views respectively are significantly varied, manifested with the distortion of apertural and apical shell sculptures as distinctively seen in Fig. 3 and Fig. 4.

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	4.54E-05*	1.425E-07*
2	1.165E-06*	0.06121
3	0.0002983*	0.03104*
4	0.5864	2.978E-08*
5	0.02389*	0.03441*

Seemingly, as all data points between two populations show their distribution pattern (Fig. 6), 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. In support, results in Principal Component Analysis (PCA) in Table 2 presents only two significant percentage variance (PC1,42% and PC2, 28%) for ventral/apertural and three (PC1,44%; PC2,23% ; and PC3,14%) 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.

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

Principal Component	Ventural/Apertural		Dorsal	
	Jolliffe cut-off: 0.0003979		Jolliffe cut-off: 0.0004257	
	Eigenvalue	% Variance	Eigenvalue	% Variance
1	0.00119996	42.253	0.00132848	43.694
2	0.000793263	27.932	0.000698795	22.984
3	0.000352005	12.395	0.000435945	14.338
4	0.000292109	10.286	0.000365418	12.019
5	0.00020262	7.1346	0.000211766	6.9651

Confirming or rejecting the hypothesis that two populations of *O. quadrasi* are morphologically distinct, Discriminant analysis was performed. Figure 7 shows the frequency histogram of the degree of dimorphism of *O.*

quadrasi based on two shell shape characters. Along the discriminant axis, the discrimination of the *Schistosoma*-infected (in red) from *Schistosoma*-free (in blue) is not absolutely parted; instead a superimposing population of *O. quadrasi* is noticeable.

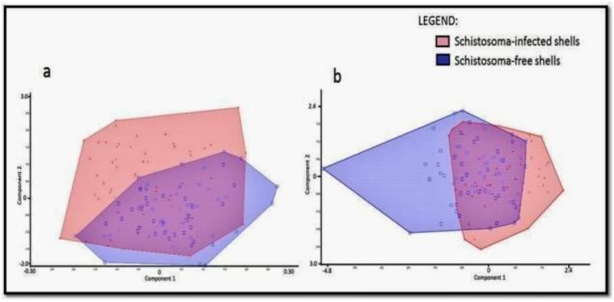

Figure 6. Scatterplot displays all data points between two populations, *Schistosoma*-infected (pink) and *Schistosoma*-free (blue) shells of *O. quadrasi* showing the distribution pattern at (a) ventral/apertural and (b) dorsal shell portions.

Statistically, there are instances wherein dimorphism could still be present even if the overlapping transpires in the histogram. Table III 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 *Schistosoma*-infected shells are classified as *Schistosoma*-infected and how *Schistosoma*-free shells are classified as *Schistosoma*-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 [14]. Yet, a reduced percentage of correctly classified *Schistosoma*-infected shells population both at ventral/aperture (70.6%) and dorsal (73.5%) views detected in detail (Table III) apparently explains for the overlapping populations presented in discriminant plot diagram (Fig. 6).

TABLE III. PROPORTION OF VARIATION WITH THE DISCRIMINANT FUNCTION SCORES OF THE VENTRAL/APERTURAL AND DORSAL PORTIONS OF THE O. QUADRASI SHELLS.

Shell	Ventral/Apertural			Dorsal		
	S-i	S-f	CC %	S-i	S-f	CC %
Schistosoma-infected	48	20	70.6	50	18	73.5
Schistosoma-free	6	62	91.2	7	61	89.7
Total:			**80.9**			**81.6**
H t2:			116.2			98.173
p(same):			3.11E-16			3.51E-14

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

More generally, however, there are 80.9% and 81.6% correctly classified discriminant function scores for ventral/aperture and dorsal views of *O. quadrasi* respectively, which are considered to be significant. As a result, analysis of geometric morphometrics of the two populations (cercariae-infected and uninfected shells) of *O. quadrasi* based on shell characters generally has demonstrated dimorphic features in shell shapes.

Recently, parasitism has been shown to alter shell growth, and thus shell morphology [20], [21]. The present study shows alterations of shell shape induced by parasite, *Schistosoma* japonicum (Fig. 2) on *Oncomelania quadrasi* (Fig. 3) populations. Snails infected with *Schistosoma japonicum* cercariae generally have bigger size relative to the shell shape compared to their non-infected shell population. Accordingly, gigantism is a well-known phenomenon for host-parasite relationships, an adaptation by the parasites to increase the volume of available shell space [21]-[25].

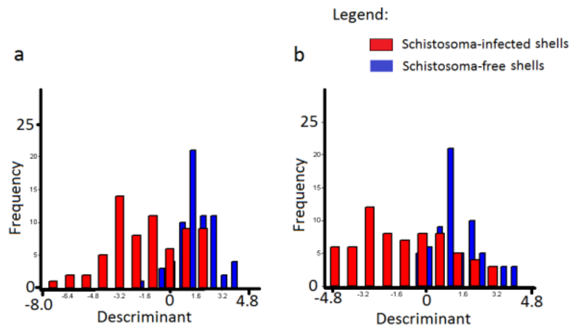

Figure 7. Frequency distribution histogram showing the variations in the (a) ventral/apertural and (b) dorsal portions in the shell patterns between *Schistosoma*-infected (red) and *Schistosoma*-free (blue) shells of *O. quadrasi*.

The magnitude of the effect associated with parasites was generally more pronounced in cercariae-infected shells having broader aperture relative to body whorl (Fig. 3). 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 [26], [27], suggesting that parasites are dominant drivers of phenotypic variation.

Inversely, in other gastropods, with infected snails were mostly having an elongated and narrower shell compared to non-infected group [6], [9] [10] [11]. Differential effects of parasites on shell morphologies of their gastropod hosts have been observed in other parasite–host systems [8] and host snails have been observed to become wider at the shell base in a few cases [7]. Though, here, compressed apical sculptured shells of infected snails may be the consequence of the rerouting by the parasite of the snail metabolites allocated to growth [3], [4] or snails are often more likely to be blunted due to wear and tear. Yet, other studies have found evidence for the actual involvement of parasites in shell variation [6], [28] and there is no reason why this mechanism should be different in *Schistosoma japonicum-Oncomelania quadrasi*, a parasite- host system.

IV. CONCLUSION

Parasites may be an important factor in explaining phenotypic variation in shell morphologies of *O. quadrasi*- a solitary intermediate host for *S. japonicum* cercariae in the Philippines. 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, 4.05% of the local populations being infected, the effects were even detectable. The parasite-induced morphologies were beneficial, as they precluded predation. Apparently, it may be that broader aperture, blunted apex, and bigger snails are more likely to become infected with *S. japonicum*, or that narrowly conic, small-sized relative to the shell shape, and tapered apex are more resistant to parasite infections. Nevertheless, other environmental factors are also known to affect gastropod shell morphology. Hence, the site factor possibly integrates a complex interplay of various environmental and genetic factors. However, the present study indicates that parasites can be one of the main drivers in this complex interplay.

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