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ORIGINAL ARTICLE

Shell geometric morphometrics in *Biomphalaria glabrata* (Mollusca: Planorbidae) uninfected and infected with *Schistosoma mansoni*

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Abstract Freshwater planorbid mollusks belonging to the genus *Biomphalaria* act as intermediate hosts for *Schistosoma mansoni*, the etiological agent of human intestinal schistosomiasis, in the Neotropical Region. Identification of *Biomphalaria* spp. are carried out based on morphological characters, and the *Schistosoma* infection are determined by the presence of cercariae (verified through microscope preparation and mounting). Recently, the geometric morphometrics has proven to be a useful tool for determining shape differences in disease vectors arthropods. Due to this, we used geometric morphometrics to determine *Biomphalaria glabrata* shell differences (shape and size) between uninfected and infected specimens. We digitalized 12 anatomical points over the shell left side (from umbilicus to the last whorl) by combining type I and II landmarks and sliding semilandmarks; the coordinates were aligned by generalized Procrustes analysis. Principal component analyses were implemented for examining main variation axes, and discriminant analysis for testing group membership significance. We found significant separation between infected and uninfected shell conformation. All specimens were 100% correctly classified. The main differences occur in the peristome. The Kruskal-Wallis test finds significant differences in shell isometric size among infected and uninfected specimens. These findings correspond to other studies of traditional morphometrics, that infected snails showed the reduction in shell size in contrast to those uninfected specimens.

Key words *Schistosomiasis*, Gastropoda, peristome, landmarks, Procrustes.

1 Introduction

Freshwater planorbid mollusks belonging to the genus *Biomphalaria* act as intermediate hosts for *Schistosoma mansoni* Sambon, 1907, the etiological agent of human intestinal schistosomiasis, in the Neotropical Region. The disease cycle begins when the parasite penetrates the snail tissue. Then, in the intermediate host, asexual multiplication occurs and another larval stage, which is infective to humans, is released into the water. The human infection occurs in populations that live near to water bodies (Faro *et al*., 2013; WHO, 2015). In particular, *Biomphalaria* infection occurs by means of active penetration of the *Schistosoma mansoni* larvae or miracidia, usually the base of the head (antennae) or foot (cephalopodal mass). In this process, the larvae undergo morphological and physiological changes, being transformed into primary sporocyst that remains in the muscular tissue near the penetration region. Then, the primary sporocysts generate

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the secondary ones, which migrate from the musculature to the digestive gland-gonad (hepatopancreas), where they undergo profound anatomic changes and their germinative cells can generate the cercariae. Finally, cercariae are released from daughter sporocysts and are released from the snail (Negrão-Corrêa *et al.*, 2007; Humphries, 2011). Traditionally, the detection of *Biomphalaria* snails infected with *S. mansoni* is performed by cercariae shedding induced by artificial light exposure or by squeezing snails between two glass slides. However, these methods are not able to detect the parasite neither in dead snails, or in molluscs with decomposed bodies or, yet, only empty shells, which precludes their identification and *S. mansoni* detection (Caldeira *et al.*, 2004). Geometric morphometrics is an approach that quantifies the phenotypic variation and its covariation with other biotic or abiotic variables (Bookstein, 1991; Zelditch *et al.*, 2004; Webster & Sheets, 2010). The geometric morphometrics is wide used in medical and forensic entomology, such as Triatominae bugs (Dujardin, 2008; Soto-Vivas *et al.*, 2011; Goncalves *et al.*, 2016), Culicidae (Jirakanjanakit & Dujardin, 2005), scorpions (Bechara & Liria, 2012), Calliphoridae adult and larvae (Vasquez & Liria, 2012; Nuñez & Liria, 2016) and so on. However, studies have not been performed using geometric morphometrics in schistosomiasis intermediate hosts, in particular with *Biomphalaria* snails. So in this article, we described the variation of shell configuration and size in uninfected and infected snails.

2 Material and methods

2.1 Specimens source and data acquisition

The *Biomphalaria glabrata* Say, 1818 specimens were collected from Brisas del Lago (10.22020°S, 67.62587°W; elev. 400 m), Maracay, Aragua State, Venezuela. In laboratory, 300 snails were maintained in controlled conditions following standard rearing protocols (Eveland & Haseeb, 2011). Then, the colony was divided into two groups: without infection of any trematode, and infected by *Schistosome mansoni* (Venezuelan JL strain), maintained in the Instituto de Investigaciones Científicas (Cesari *et al.*, 1989, 2014). Then, 30 days after the infection of *S. mansoni*, the individual snail infection was corroborated by means of cercariae shedding induced by artificial light exposure a direct light.

Thrity uninfected snails and 30 infected with *Schistosoma mansoni* were selected; each specimen was placed laterally on a board and photographed. Each image was created ten equidistant fans (Fig. 1) with MakeFan 7.0 software (Sheets, 2010a), centered on a circle of three landmarks (LM1, LM8 and LM11), in order to describe the shell curve (previous analyses showed that ten fans allow describing the outline of this structure). Twelve anatomical points (x, y coordinates) over the shell left side were digitalized with tpsDig 1.62 software (Rohlf, 2008). The combining types I and II landmarks follow Bookstein (1991) criteria: LM1 most extreme point of peristome, LM8 union between peristome and suture, LM10 union between intersection of lip (peristomal callus) and suture, and LM11 umbilicus. Other landmarks were considered sliding semilandmarks (LM2–LM7, LM9, LM12).

2.2 Morphometric analysis

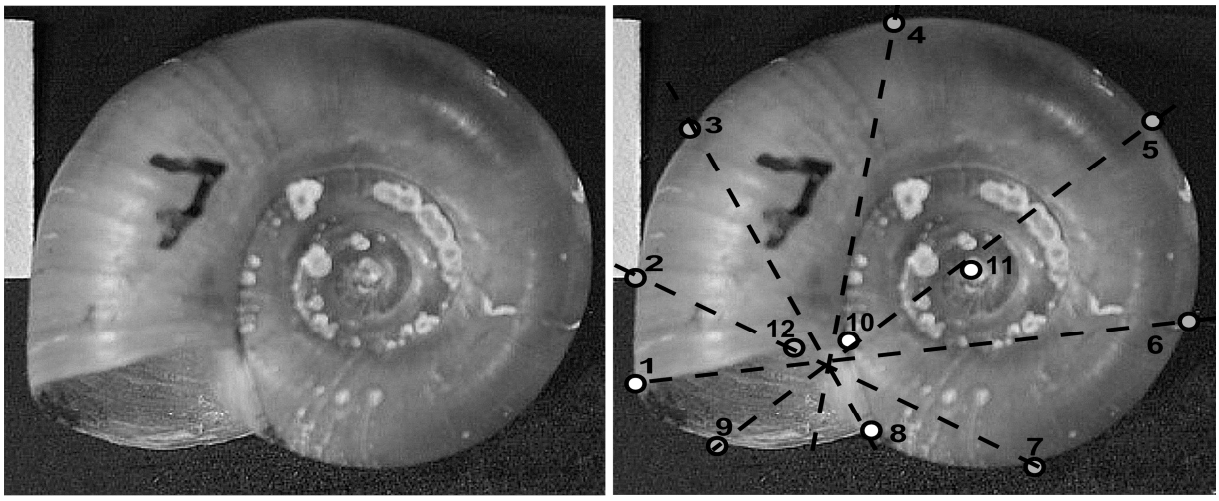

Figure 1. Shell of *Biomphalaria glabrata* showing the landmarks (LM1–LM12) disposition. White landmarks correspond to type I and II landmarks, while the gray to semilandmarks.

From 60 configurations matrix, the coordinates were aligned by generalized Procrustes analysis using tpsRelw software (Rohlf, 2016) to analyze sliding semilandmarks and extract the matrix configurations (Partial Warps = PW) and centroid size (CS). The semilandmarks are thus allowed to slide along their curve or surface in order to remove the effects of the arbitrary spacing by "optimizing" the position of the semilandmarks with respect to the average shape of the entire sample (Gunz & Mitteroecker, 2013). Later, MorphoJ 1.06d software (Klingenberg, 2011) was used to perform a principal component analysis (PCA) for examining main variation axes, and discriminant analysis (DA) for testing the group (uninfected or infected) membership significance with Hotelling's test. The group configuration differences were also estimated with TwoGroup 7.0 software (Sheets, 2010b), by means a Goodall test. The F test compares the difference in mean shape between two samples relative to the shape variation found within the samples (Zelditch *et al.*, 2004; Vasquez & Liria, 2012). For statistical significance, we perform an F test based on a Bootstrap analysis on 10000 random data permutations. Finally, CS differences were analyzed with PAST 2.10 software (Hammer & Harper, 2011) by means of non-parametric Kruskal-Wallis test at a significance level of 0.05 with Bonferroni correction.

3 Results

3.1 Centroid size

Significant differences ($\chi^2 = 42.125$, df 1, $P < 0.001$) are found among the shell centroid size; the uninfected specimens show larger size (142.09–343.96 mm, 216.55 mm in mean) in contrast to those infected (99.82–199.16 mm, 148.21 mm in mean).

3.2 Shell conformation differences

Figure 2 shows the PCA diagram with the first and second PCs, comprising together 60.2% of explained variance; the 60 specimens are separated into two groups: uninfected (black dots) and infected with *Schistosoma mansoni* (gray dots).

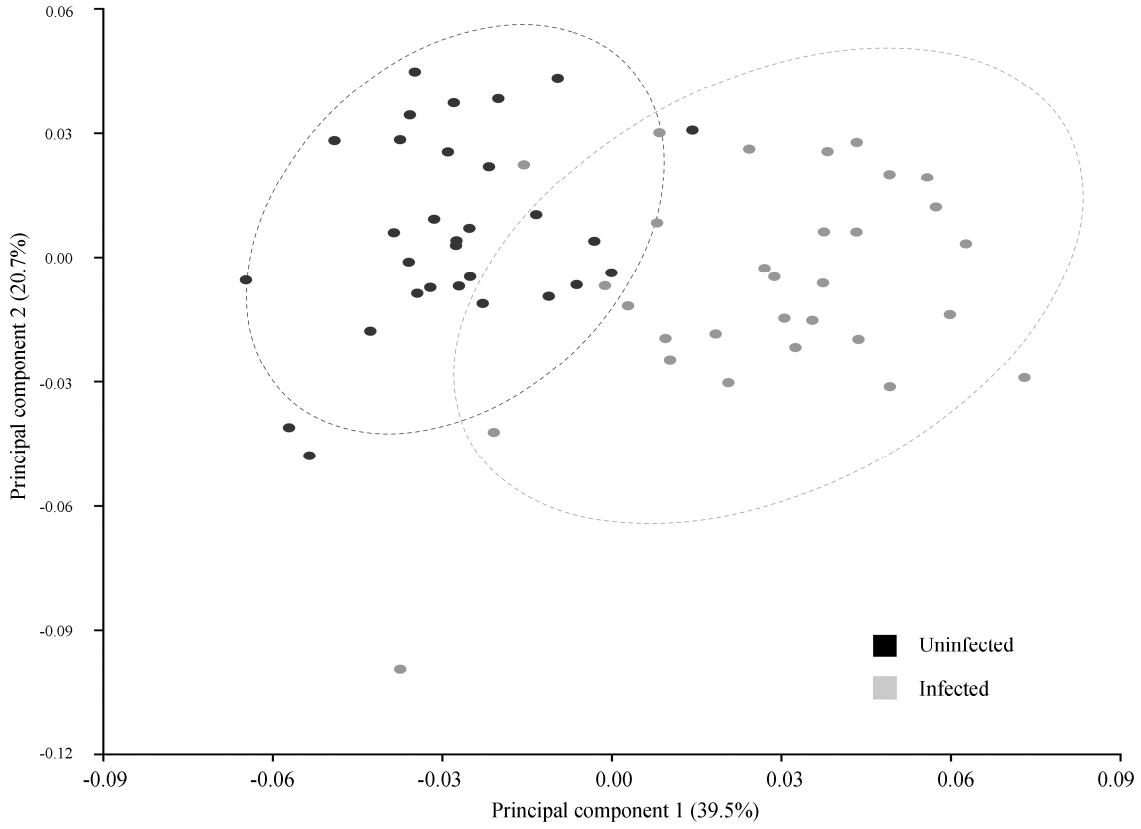

Figure 2. Principal component analysis diagram of the first two principal components (within percentage explained variance contribution) from 60 *Biomphalaria glabrata* specimens, uninfected (black dots) and infected with *Schistosoma mansoni* (gray dots). Ellipse encloses 90% of data for each group.

The main differences in shell conformation between uninfected and infected occur in the displacement of landmarks 8, 9, 10 and 12 (Fig. 3): displacement of union between peristome and suture, displacement of union between peristomal callus and suture, and displacement of shell curvature. The DA (T^2: 103561.32, $P < 0.0001$) and Goodall Test (F-Test: 23.80, df = 20.0, 1160.0, $P < 0.001$) show significant differences between uninfected and infected conformations. In the cross-validation DA, all specimens were 100% correctly classified.

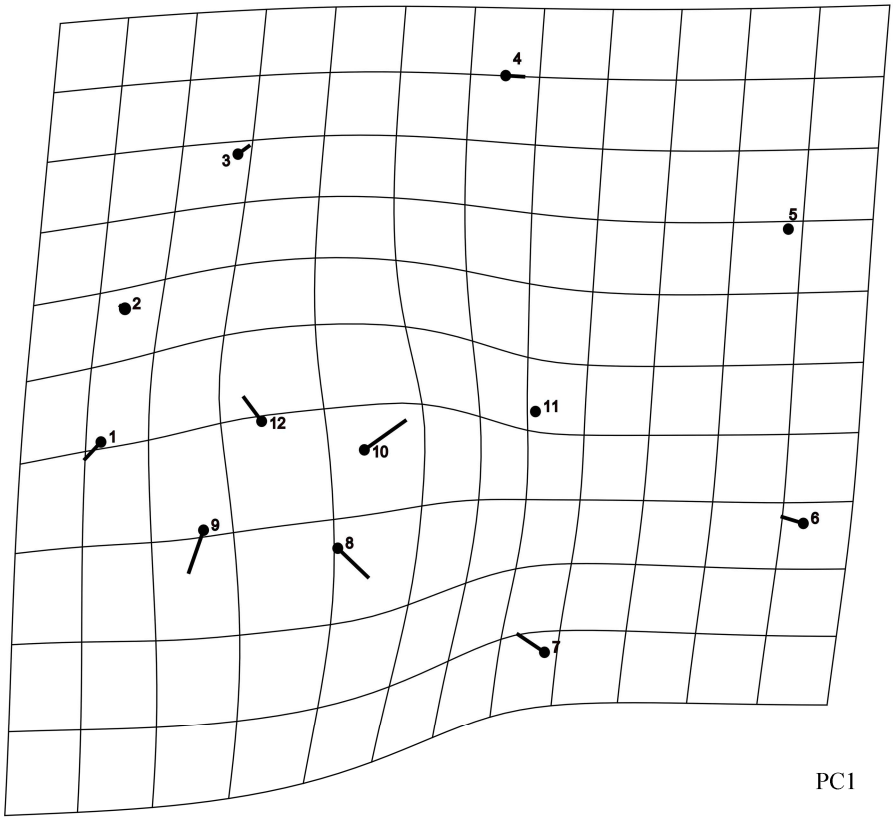

Figure 3. Grid deformation showing differences in the Principal Component 1 (PC1) between the mean configuration of *Biomphalaria glabrata* uninfected and infected with *Schistosome mansoni*.

4 Discussion

Several studies confirm the effect of *Schistosoma mansoni* infection in the *Biomphalaria glabrata* snails, which appeared in mortality, growth, metabolism, organic and inorganic elements, reproduction, and behavior (Blair & Webster, 2006; Negrão-Corrêa *et al.*, 2007; Humphries, 2011; Rowel *et al.*, 2015). In relation to growth, Sorensen and Minchella (2001) comments the effects between trematode infection and snails size. For example, *Lymnaea* exhibit gigantism when are infected, while in others genera *Biomphalaria*, *Bulinus* and *Helisoma* showed stunting on growth. The later findings correspond to our results, *Biomphalaria glabrata* infected specimens with *S. mansoni* showed a small centroid size.

In relation to shell shape in infected snails, our results demonstrated significant differences in the conformation between uninfected and infected populations. Zbikowska and Zbikowski (2005) in studies with traditional morphometrics (linear measures of height, width, and height spiral), demonstrated shell differences in Poland populations of *Lymnaea stagnalis* (Linnaeus, 1758) which infected with several trematodes. They reported a reduction in shell height on infected specimens, attributed to the age: young snails are more susceptible to parasite infection than older ones, which are manifested by their higher mortality. Vasallo *et al.* (2013) used geometric morphometrics for describing the shape in Philippines populations of *Oncomelania quadrasi* (Möllendorff, 1895) uninfected and *S. japonicum* (Katsurada, 1904) infected. They found significant differences in the snail-apertural and apical sculptures in infected snails. The magnitude of the effect associated with parasites was generally more pronounced in cercariae-infected shells by having broader aperture relative to body whorl. In contrast, Gustafson and Bolek (2016) examined the effects of trematode parasitism on shell characteristics (shape, size, and crush resistance) of *Physa acuta* Draparnaud, 1805 snails in different environments. In

their work, parasitism has no apparent effect on the shell conformation in stream or wetland snails, which demonstrated that habitat and/or flow treatment is the primary factor affecting the morphology and trematode parasitism plays a secondary role.

From a schistosomiasis program point of view, our study represents the first contribution that used geometric morphometrics for detection of *Biomphalaria* snails infected with *S. mansoni*, as a complementary tool when the traditional techniques or others such as DNA detection (Hamburger *et al.*, 1992; Caldeira *et al.*, 2004) are not efficient. However, more studies are necessary to evaluate how intraspecific variation overlaps with interspecific variation (Jarne *et al.*, 2011).

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