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Describing intermediate host snails in Kapatagan, Lanao del Norte, Philippines: a phenetic analysis

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Abstract

Many species of freshwater snails served as intermediate hosts of highly infective fluke (trematode) larvae of the genus *Schistosoma* which cause Schistosomiasis however, the nature of populations remained ambiguous. This study shed light on the nature of populations of intermediate hosts snails from two barangays: Curvada and Tiacungan, Kapatagan, Lanao del Norte, Philippines. This study determined and described species composition of populations and correlates positive and negative infected snails with size and shape morphology. Phenetic analysis was used to describe and delineate morphology of snails based on important shell characters. A total of 42 characters were used to construct the character matrix. The important characters in species delineation were mainly on the suture, last or body whorl width, color, basal lip, shell size, body whorl banding patterns, spire coloration and the presence of spines. The generated phenogram revealed the existence of two possible clusters based on overall phenotypic similarity. Results revealed the presence of five (5) morphotypes belonging to three genera: *Oncomelania, Tricula* and *Thiara,* which was contrary to local knowledge that a single genus was the sole intermediate host snail. Noteworthy, an important character that helped in species delineation was body whorl banding patterns. Shell size was evidently reduced in infected populations. Parasite infected snails has narrow body whorl, arch-shaped apex and pronounced narrow apertural opening.

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Introduction

A number of species of freshwater snails served as intermediate hosts of highly infective fluke (trematode) larvae of the genus *Schistosoma* which cause Schistosomiasis (or bilharziasis). The World Health Organization (WHO), estimated that Schistosomiasis and geohelminths represent more than 40% of global diseases that burden tropical countries and the third most devastating tropical disease in the world (Olveda *et al.*, 2013). In the world, freshwater snails of the genus *Biomphalaria* are intermediate hosts for flatworm parasites of the genus *Schistosoma*, causative pathogens of human schistosomiasis, in South America, the Greater and Lesser Antilles, Africa, Madagascar and the Arabian Peninsula. *Biomphalaria glabrata*, a neotropical snail, is the major intermediate host of *Schistosoma mansoni*. Apparently, snails from the genus *Bulinus* served as an intermediate host of *S. haematobium,* and a vector also of parasite species *S. intercalatum, S. bovis and S. mattheei* which are prevalent *in Africa*. In Asia, *Oncomelania* sp. is the unique intermediate host of human blood parasite *S. japonicum*, which causes schistosomiasis widespread especially in the Philippines. Meanwhile, Schistosomiasis cases have been prevalent in Kapatagan, Lanao del Norte and control measures of the disease are currently ongoing. However, no information is available regarding the nature of populations of snails in the area (Caldeira *et al.*, 2004; Duncombe *et al.*, 2012). A preconceived notion is that there is only one genus of snail behind recorded incidence of Schistosomiasis, which is *Oncomelania* in the area. The hypothesis remains that there are other species of snails that served as intermediate hosts. The true nature of snail populations in the area remained ambiguous thus, the importance of this study.

In addition, determination and description of species composition of populations and correlating positive and negative infected snails with size and shape morphology is pertinent. According to Mayr and Ashlock (1991), organisms can be highly similar in nature such that no single character is good enough or may have absolute diagnostic value herewith a combination of characters will allow correct assignment. Delineating species based on overall similarity is appealing. Hence, a combination of two or more characters is often adequate for diagnosis. In this respect, phenetic analysis can be useful to describe and delineate morphology of snails. A character matrix and character state table will be utilized along the process (Kim *et al.*, 2009).

Moreover, several studies on vector snails have focused on its internal composition and shell destruction in determining the presence of parasites. However, information of identifying the vector snails with the presence of its corresponding parasite using its morphologic composition such as the size and shape is still lacking. Studies showed that parasitism can influence shell morphology and affect size in gastropods by altering the host's growth rate. Many of the resulting changes are due to resource allocation that comes with infection. It has been documented, that parasites can bring about various phenotypic alterations in their hosts that appear to increase their own fitness at the expense of that of their hosts (Webster *et al.*, 2007; Thieltges *et al.*, 2008; Zhao *et al.*, 2010). Thus, this study also characterized shell shape of parasite-infected and non-infected intermediate hosts snails.

This study provides knowledge and information on the variation and nature of species of intermediate hosts snails of *Schistosoma* that caused Schistosomiasis. Information obtained may also aide in the development of tailor-fit preventive and health programs.

Materials and methods

Study area

The study area was in the municipality of Kapatagan. It is a second class municipality in the province_of Lanao del Norte, Philippines, total land area of 25,048.41 hectares which include the area that is now being contested by the Municipality of Lala with an approximate area of 759 hectares. Snail samples were collected from two (2) different barangays: Curvada and Tiacungan, with geographical coordinates of N ˚7 ˚53 ˚0.61 ˚ E 123”47134.8” and N 7 ˚52’ ˚41.1” E 123 ˚47’36.6” respectively (Fig. 1). These two barangays were chosen because of recorded high occurrence of Schistosomiasis incidence in the area as monitored by the Municipal Health Office (MHO) of Kapatagan. Assigned health officer accompanied the sampling procedure to identify specific areas where snail colonies were present.

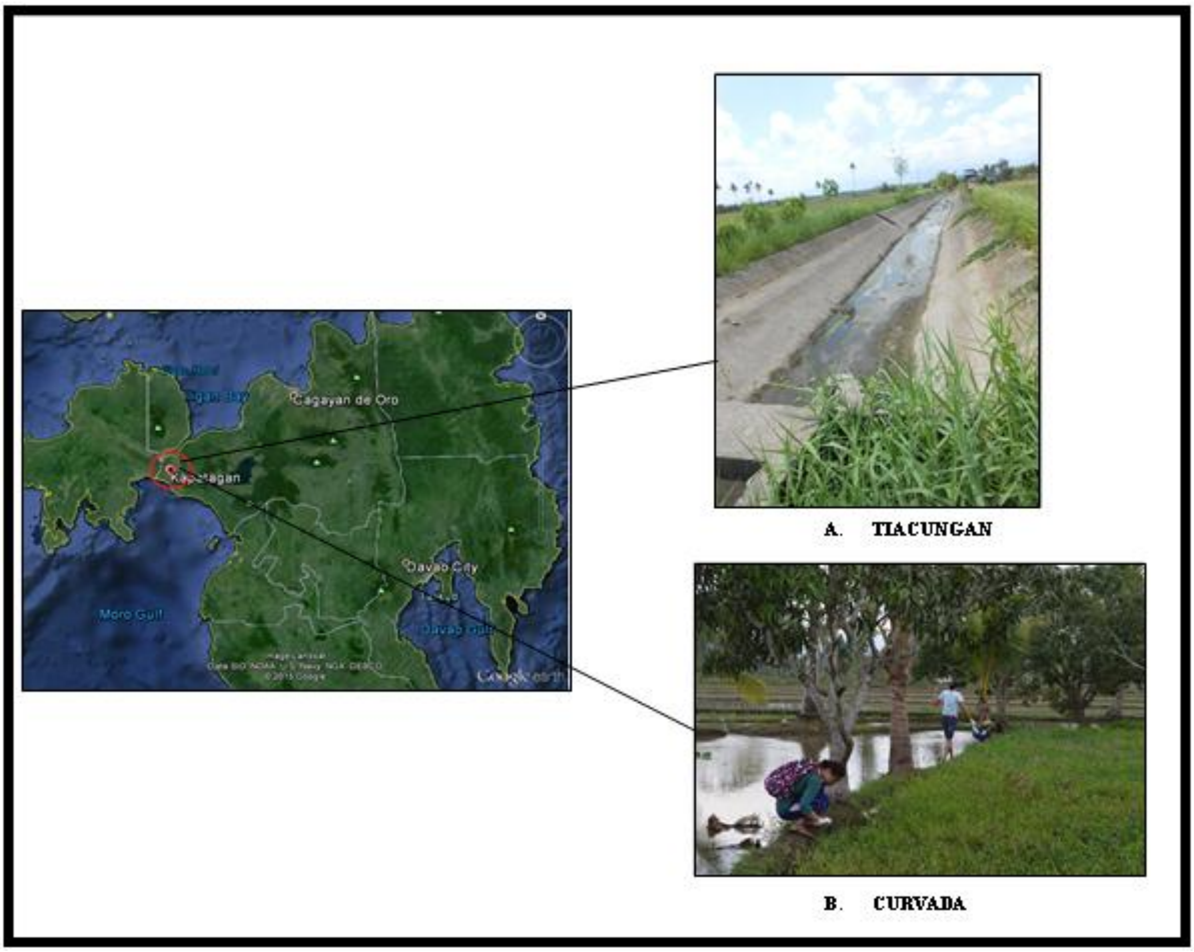

Fig. 1. Study site: Map of Kapatagan, Lanao del Norte, (A) Tiacungan and (B) Curvada.

Collection, preservation and processing of specimens
Freshwater snails had been considered as bioindicator species. In this study, shell colonies were chosen and identified with the help of an inspector officer from the Municipal Health Office (MHO).

Shell colonies were widely distributed and adapted to different environment.

Thirty (30) samples of snails per population were collected per barangay. Snail samples from the field were hand-picked using forceps and kept in plastic containers with distilled water then transported from the field to the laboratory**.** The water samples in these containers were checked under a stereomicroscope for any presence of parasite which had emerged from the host. Identification of samples was done using taxonomic keys, field guides, monographs and consultation with experts.

The randomly collected *Oncomelania* sp., *Tricula* sp. and *Thiara* sp. snails were examined for parasites. All samples of snails obtained were processed for phenetic analysis (Vasallo Jr. and Gorospe, 2015). Shells were photographed using a Nikon D3200 DSLR camera under a stereomicroscope with 20x magnification for documentation purposes and further analysis.

Phenetic analysis

External shell morphological data were utilized in order to prepare the character state table and the character matrix. All of the observed characters were assigned values of 0, 1, 2 or 3 and these values were used to conduct the phenetic analysis using Statistical Package Social Science (SPSS) for IBM, version 23.

In order to construct the phenogram, the characteristics of the species/groups were then equally weighted and treated unordered for quantification, objectification and efficient classification (Tabugo *et al.*, 2013). For the analysis, the names of the species were substituted with the Operational Taxonomic Unit (OTU) code numbers in order to be suitable for computer processing.

The raw data inputted in the spreadsheet was subjected to Hierarchical Cluster Analysis via average linkage between groups method and Squared Euclidean Distance (Distance (X × Y) = Σ(Xi – Yi)2) algorithm was employed to calculate the dissimilarity coefficient and then categorized the ones with the lowest coefficient and connected them to the one with the higher coefficient in order to prepare the phenogram. A total of 42 characters that include qualitative and quantitative characters were utilized for the five (5) identified morphotypes (Kim *et al.,* 2009).

Results and discussion

Most of the snails that served as intermediate hosts were found in wet and shallow areas. Snails from Tiacungan were collected from the rice fields ready for planting nearby an irrigation system while snails from Curvada were collected from a flowing creek nearby a non-cultivated rice field and Mango farm. There were five morphotypes classified during the sample collection belonging to the three genus: *Oncomelania*, *Tricula* and *Thiara*.

Morphological Description

In the description of gastropods, the shell is traditionally used in classifications and taxon descriptions. Shell morphometry and sculpture are regarded as essential for species discrimination (Kohler, 2003).

The external form of the shell was the principal character used in gastropod species-level taxonomy (Gutierrez *et al.*, 2011). Descriptions were made for collected samples.

Results revealed the presence of five (5) morphotypes belonging to three genera: *Oncomelania, Tricula* and *Thiara,* which was contrary to local knowledge that a single genus was the sole intermediate host snail. Generally, *Oncomelania* sp. has dark and light color, medium size shell, narrowly conic/ subglobose shell shape, dextral in the direction of coiling of gastropod shells, transverse/ growth lines or striae shell surface sculpture, shell with well-rounded whorls and indented sutures, thinner lip. *Tricula* sp. has light color, medium sized, cylindrical shell shape, dextral direction of coiling of gastropod shells, spiral raised lines or stria in shell surface sculpture, shell with rounded periphery, thin outer lip and pointed basal lip. *Thiara* sp. has light and dark color, small sized, narrowly conic, fusiform/spindle-shaped shell shape, dextral direction of coiling of gastropod shells, presence of spines in shell surface sculpture, shell with shouldered whorls, pointed basal lip and thin outer lip (Table 1).

For the infected and non-infected snails based on the dorsal portion of the shell, the difference in the posterior margin of the outer dorsal lip is more or less the same for both.

The parasite uninfected has a conical dorsal shell shape and highly elevated basal lip than that of the parasite infected. Dorsal parasite infected snails had narrower body whorl and arch-shaped apex. Parasite uninfected has longer shell length of the dorsal side than the parasite infected. Also, parasite infected snails have smaller shells compared to shells of the uninfected snails.

Herewith, parasitism often influences the phenotype of gastropods.

Table 1. Description of different morphotypes in the population A-B.) *Oncomelania* sp.; C-D.) *Tricula* sp.; E.) *Thiara* sp.

	MORPHOTYPES				
	PARASITE INFECTED		PARASITE NON – INFECTED		DESCRIPTION
	VENTRAL	DORSAL	VENTRAL	DORSAL	
A					**Genus:** *Oncomelania sp.* **Color:** Dark and light **Shell size:** Medium sized (15mm) **Shell shape:** Narrowly conic/subglobose **Direction of coiling of gastropod shells:** Dextral (shell coiled to the right) **Shell surface sculpture:** Transverse/growth lines or striae **Others:** Shell with well – rounded whorls and indented sutures, thinner lip
B					**Genus:** *Oncomelania sp.* **Color:** Dark and light **Shell size:** Medium sized (15mm) **Shell shape:** Narrowly conic/subglobose **Direction of coiling of gastropod shells:** Dextral (shell coiled to the right) **Shell surface sculpture:** Transverse/growth lines or striae **Others:** Shell with well – rounded whorls and indented sutures, thicker lip
C					**Genus:** *Tricula sp.* **Color:** Light colored **Shell size:** Medium sized (15mm) **Shell shape:** Cylindrical **Direction of coiling of gastropod shells:** Dextral (shell coiled to the right) **Shell surface sculpture:** Spiral raised lines or stria **Others:** Shell with rounded periphery, thin outer lip, pointed basal lip
D					**Genus:** *Tricula sp.* **Color:** Light colored **Shell size:** Small sized (10mm) **Shell shape:** Fusiform/spindle-shaped **Direction of coiling of gastropod shells:** Dextral (shell coiled to the right) **Shell surface sculpture:** Nodules **Others:** Shell with shouldered wh pointed basal lip, thin outer lip
E					**Genus:** *Thiara sp.* **Color:** Light and dark **Shell size:** Small sized (9mm) **Shell shape:** Narrowly conic, fusiform/spindle-shaped **Direction of coiling of gastropod shells:** Dextral (shell coiled to the right) **Shell surface sculpture:** Spines **Others:** Shell with shouldered whorls, pointed basal lip, thin outer lip

Many of the resulting changes are due to resource allocation that comes with infection. Parasites can bring about various phenotypic alterations in their hosts that appear to increase their own fitness at the expense of that of their hosts (Littlewood *et al.*, 2010).

In particular, parasites with complex life cycles often modify the behavior and/or the appearance of their intermediate hosts (Davis *et al.*, 2006). Thus, according to the 'parasite manipulation' hypothesis, the ability of a parasite species to modify its host's phenotype is the product of natural selection acting on the genes of the parasite (Lefevre and Thomas, 2008). Moreover, early studies suggests impact of parasites on the phenotype of their host typically focused on a single trait such as modified reaction to light (Bethel and Holmes, 1973), reduced fecundity (Skorping, 1985), respiration (Rumpus and Kennedy, 1974) or altered pigmentation (Oetinger and Nickol, 1981**)**. There is evidence to date that has also shown that the ribbed-shelled collections of individuals are highly susceptible to infection with the parasite, whereas smooth-shelled populations have lesser potential to be infected, categorizing to total resistance (Davis *et al.*, 2006).

Table 2. Morphological character selection and coding for intermediate host snails.

	CHARACTERS
1	Worm-like body shape: (0)Yes;(1)No
2	Shell: (0)present;(1)absent;(2)reduced
3	Tentacles around mouth: (0)present;(1)absent
4	Shell number: (0)less than 1 piece;(1)at least 1 piece
5	Body asymmetry: (0)bilateral;(1)unilateral
6	Shell coiling: (0)coiled;(1)uncoiled
7	Shell shape: (0) rounded or oval; (1) projected
8	Holes in the shell: (0) more than 2;(1) at least 2
9	Ribbed lines on shell: (0) distinctive;(1)regular
10	Hinge: (0)without;(1)with
11	Shell shape with an inner tube: (0)straight; (1)spiral
12	Aperture of shell:(0)closed by an operculum;(1)not closed by an operculum
13	Shell form:(0) neritiform; (1) not neritiform
14	Spire:(0) indistinct; : (1) pronounced
15	Inner surface of operculum: (0) smooth;(1) rough
16	Elongate attachment processes on its inner surface: (0) without;(1)with
17	Adult shell: (0) small;(1) medium;(2)large
18	Length: (0) less than 1 cm;(1)more than 1 cm
19	Operculum: (0) paucispiral;(1) multispiral
20	Body whorl margin: (0)crenate; (1)entire
21	Aperture direction: (0) Aperture follow the shell's initial spiral symmetry;(1) Aperture did not follow the shell's initial spiral symmetry
22	Increase in size: (0)discontinuous; (1) gradual
23	Whorl direction: (0)circular;(1) straight
24	Keel/ carina: (0)with;(1)without
25	Direction of coiling of gastropod shells:(0)Dextral(shell coiled to the right);(1)Sinistral(shell coiled to the left
26	Periphery:(0)angular ;(1)rounded
27	Suture :(0)indented;(1)shallow/not indented
28	Last/body whorl width:(0)thin;(1)wide
29	Aperture opening:(0)wide ;(1)narrow
30	Color :(0)dark;(1)light
31	Columellar lip:(0)thin;(1)thick
32	Basal lip:(0)pointed;(1)semi-pointed;(2)arched-shaped
33	Whorl structure :(0)well-rounded;(1)flattened;(2) shouldered
34	Shell surface sculpture:(0)transverse/growth lines/striae;(1)spinal raised lines or stria;(2)nodules;(3)spines
35	Shell size:(0)medium(15 mm);(1)small (10 mm)
36	Shell shape with respect to length and width:(0)Narrowly conic/subglobose;(1)cylindrical;(2) Fusiform/spindle-shaped;(3) Narrowly conic, fusiform/spindle-shaped
37	Body whorl banding patterns: (0)Horizontal dark bands distant from each other and fading light-colored bands in between; (1) Flame-like structure from the first suture to the headpart before the basal lip;(2) Uniform dark axial costae in semi-horizontal pattern in the body whorl;(3) Punctuate, unmodified and thick vertical bands on the lower left side of the body whorl fading to the right;(4)Punctuate spreading of dark brown bands in semi-horizontal position from left to right in the middle of the body whorl to the top near in the basal lip with thick distant dark brown bands above the suture
38	Axial costae: (0)absent;(1) Dark brown axial costae;(2)Black axial costae
39	Pigmentation: (0)none; (1) On the right side of penultimate whorl;(2) Unmodified pigmentation in the suture
40	Dark coloration at anterior portion: (0)present;(1)absent
41	Spire coloration: (0) Brownish;(1) Dark Brown to black;(2)Black;(3)Light brown to black
42	Spines: (0) Present;(1) Absent

Phenetic analysis

In this study, five (5) morphotypes belonging to the three (3) genera were considered for phenetic analysis using 42 characters. External morphological data were utilized in order to prepare the character state table and the character matrix (Table 2). Phenetic Analysis revealed the relationships of the 5 morphotypes based on the most useful characters selected on the species level and were then incorporated into a data matrix to allow them to be coded (Table 3).

Table 3. The Character data matrix for phenetic analysis.

	CHARACTERS																																									
OTU	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42
A	1	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	1	0	0	1	0	0	1	0	0	0	0	0	0	1	0	0	1
B	1	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	1	0	1	0	0	1	1	0	0	0	0	1	0	0	0	3	1
C	1	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	1	1	1	0	0	0	2	1	1	0	1	2	1	0	1	1	1
D	1	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	1	0	1	1	1	0	1	0	1	1	2	0	2	3	1	0	1	1	1
E	1	0	1	1	0	0	0	1	0	0	1	0	1	1	0	0	1	1	0	1	0	1	0	1	0	1	1	0	1	1	0	0	2	3	1	3	4	0	2	0	3	0

Within the 5 morphotypes of *Oncomelania* sp, *Tricula* sp and *Thiara* sp, the lowest dissimilarity indices were found between morphotype A and B, morphotype B and C, morphotype C and D. Moreover, the highest dissimilarity values were found between morphotype A and E, morphotype B and E, morphotype C and E and morphotype D and E which indicated that they less likely resemble each other based on their overall phenotypic similarity (Table 4).

Table 4. Dissimilarity matrix in the combined dataset of intermediate host snails. The numbers below in the diagonal are the divergence values corrected for multiple substitutions using SPSS for IBM, version 23.

	SQUARED EUCLIDEAN DISTANCE				
OTU	A	B	C	D	E
A	-				
B	14.00	-			
C	15.00	13.00	-		
D	26.00	22.00	5.00	-	
E	55.00	45.00	34.00	21.00	-

The generated phenogram revealed the existence of 2 possible clusters based on the overall phenotypic similarity (Fig. 2), dividing the morphotypes into 2 clusters and one unclustered morphotype based on the genus classification. Thus, for this study the important characters that proved to be very useful in differentiating the 5 morphs were mainly on the suture, last or body whorl width, color, basal lip, shell size, body whorl banding patterns, spire coloration and the presence of spines.

Accordingly, shell characters could be used to discriminate between species. As to the ecological adaptations of the variations in shapes, it is argued that species with high spire are able to burrow at soft substrate and could survive periods of droughts for months because they are hidden in the bottom of the mud. Characters of the shells of freshwater gastropods are very important in species recognition and usually for generic and familial placement as well. Especially, useful are the size and general form of the shell. Among the many species, the shell may take various shapes, yet, for any one species, the shell shape is usually quite constant (excepting, of course, minor clinal, populational and ecophenotypic variations exhibited by some species).

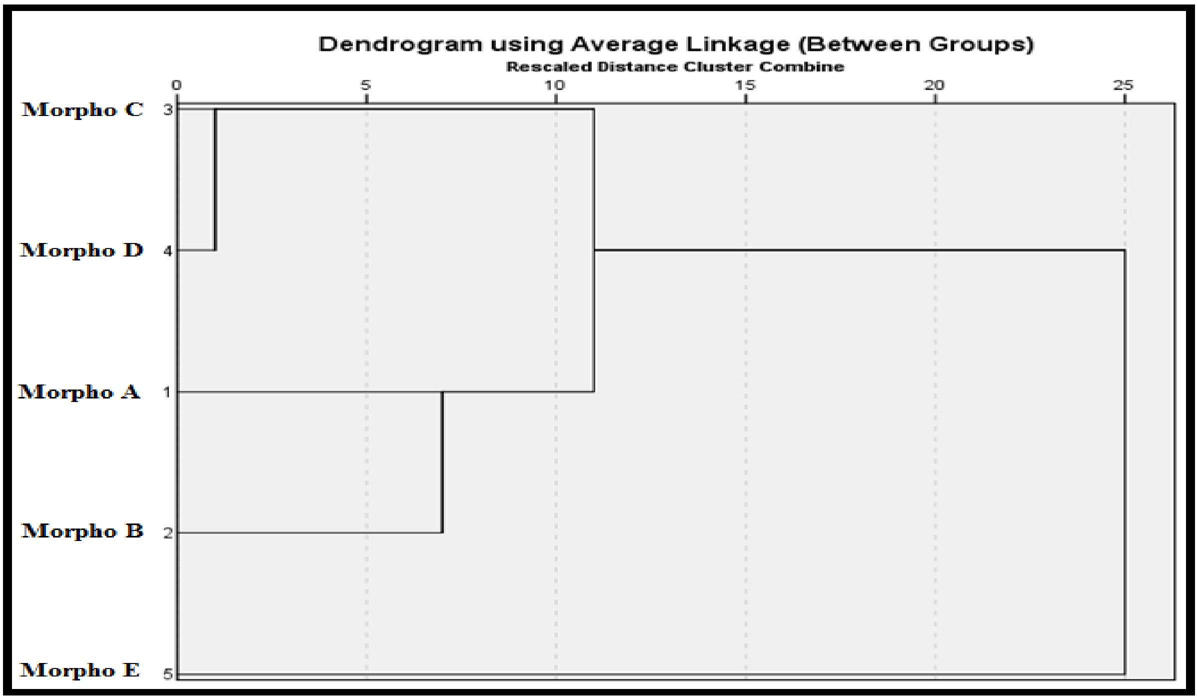

Fig. 2. Phenogram of intermediate host snails based on the Squared Euclidean Distance using 42 characters (Average Linkage between groups) showing two clusters based on existing morphs.

The shells among the different species may vary from very elongate to nearly globose, depressed and discoidal. The shell may be longer than wide, or wider than long [the columella determining the antero-posterior shell axis. The shell's coils (whorls) may turn either to the left or to the right, be round angular, shouldered or flattened and have shallow or impressed sutures. The shell may have few or many whorls, but all the shells of adults of any particular species will have approximately the same number of whorls. The shell may lack an opening (umbilicus) at its base, or may have either a narrow or wide opening. The columella or central axial column of the shell may be either twisted or straight and may or may not end abruptly. The outer lip of the shell may be either straight or variously curved and is sometimes turned back or reflected. The surface of the shell may be marked in various ways, differentially colored or sculptured, or may be simply unicolored and smooth. The outline of the shell aperture ("mouth") may take many forms due to the shape and relation of the whorls to each other. The aperture may or may not be closed by an operculum, which itself has important recognition characters. The operculum may be round, oval or spindle-shaped, and concentric, paucispiral or multispiral, depending on the way it is formed (Burch, 1984; Moneva *et al.,* 2012). In this respect, utilizing a combination of characters aide in correct assignment and delineation process thus, delineating species based on overall phenotypic similarity is appealing.

Conclusion

Results revealed the presence of five (5) morphotypes belonging to three genera: *Oncomelania, Tricula* and *Thiara,* which was contrary to local knowledge that a single genus was the sole intermediate host snail. Phenetic analysis proved to be efficient in describing and delineating morphology of snails. A total of 42 characters were used to construct the character matrix. The important characters in species delineation were mainly on the suture, last or body whorl width, color, basal lip, shell size, body whorl banding patterns, spire coloration and the presence of spines. The generated phenogram revealed the existence of two possible clusters based on overall phenotypic similarity. Noteworthy, an important character that helped in species delineation was body whorl banding patterns. Shell size was evidently reduced in infected populations. Parasite infected snails has narrow body whorl, arch-shaped apex and pronounced narrow apertural opening. Herewith, parasitism influenced the phenotype. Hence, this study provides knowledge and information on the variation and nature of species of intermediate hosts snails of *Schistosoma* that caused Schistosomiasis. Information obtained may also aide in the development of tailor-fit preventive and health programs.

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References

Bethel WM, Holmes JC. 1973. Altered evasive behavior and responses to light in amphipods harboring acanthocephalan cystacanths. The journal of parasitology **59,** 945-956. http://dx.doi.org/10.2307/3278623

Burch JB. 1984. Freshwater Snails of the Philippines. Walkerana Trans. POETS Soc. **2,** 81-112.

Caldeira RL, Jannotti-Passos LK, Lira PM, Carvalho OS. 2004. Diagnostic of Biomphalaria snails and Schistosoma mansoni: DNA obtained from traces of shell organic materials. Memorias do Instituto Oswaldo Cruz **99,** 499-502. http://dx.doi.org/10.1590/S00702762004000500007

Davis GM, Wu WP, Xu XJ. 2006. Ecogenetics of shell sculpture in Oncomelania (Gastropoda) in canals of Hubei, China, and relevance for schistosome transmission. Malacologia **48**, 253-264.

Duncombe J, Clements A, Hu W, Weinstein P, Ritchie S, Espino FE. 2012. Geographical information systems for dengue surveillance. The American journal of tropical medicine and hygiene **86,** 753-755. https://doi.org/10.4269/ajtmh.2012.11-0650

Gutierrez PM, Torres MAJ, Demayo CG. 2011. Thin-Plate Spline (TPS) and Correlation Based on Distances (CORIANDIS) Analyses as Tools for the analysis of Morphological Differences in Dragonfly Wings. In 2011 2nd International Conference on Environmental Science and Technology (ICEST 2011) **2,** 375-379.

Kim SB, Oh HS, Kim WT, Tadauchi O. 2009. Phenetic Analysis of the Anisoptera (Insecta: Odonata) in Jeju Island, Korea, based on Morphological Characters. Journal of the Faculty of Agriculture, Kyushu University **54,** 123-132.

Köhler F. 2003. Brotia in space and time: phylogeny and evolution of Southeast Asian freshwater gastropods of the family Pachychilidae (Caenogastropoda, Cerithioidea). na.

Lefevre T, Thomas F. 2008. Behind the scene, something else is pulling the strings: emphasizing parasitic manipulation in vector-borne diseases. Infection, Genetics and Evolution **8,** 504-519. https://doi.org/10.1016/j.meegid.2007.05.008

Mayr E, Ashlock P. 1991. Principles of Systematic Zoology (2nd edition). McGraw-Hill Inc, Singapore.

Moneva CSO, Torres MAJ, Wada T, Joshi R, Demayo CG. 2012. Relative warp and correlation analysis based on distances of the morphological shell shape patterns of Pomacea canaliculata Lamarck from Japan and the Philippines. Advances in Environmental Sciences **4,** 12-21.

Oetinger DF, Nickol BB. 1981. Effects of acanthocephalans on pigmentation of freshwater isopods. The Journal of Parasitology **67,** 672-684. http://dx.doi.org/10.2307/3280441

Olveda DU, Li Y, Olveda RM, Lam AK, Chau TN, Harn DA, Williams GM, Gray DJ, Ross AG. 2013. Bilharzia: pathology, diagnosis, management and control. Tropical medicine & surgery **1,** 1-19. http://dx.doi.org/10.4172/2329-9088.1000135

Rumpus AE, Kennedy CR. 1974. The effect of the acanthocephalan Pomphorhynchus laevis upon the respiration of its intermediate host, Gammarus pulex. Parasitology **68**, 271-284. http://dx.doi.org/10.1017/S0031182000045789

Skorping A. 1985. Parasite–induced reduction in host survival and fecundity: the effect of the nematode Elaphostrongylus rangiferi on the snail intermediate host. Parasitology **91,** 555-562. http://dx.doi.org/10.1017/S003118200006279X

Tabugo SRM, Torres MAJ, Demayo CG. 2013. Describing species of Neurothemis Dragonflies found in Iligan City, Philippines. Natura **17,** 3-14.

Thieltges DW, Jensen KT, Poulin R. 2008. The role of biotic factors in the transmission of free-living endohelminth stages. Parasitology **135,** 407-426. http://dx.doi.org/10.1017/S0031182007000248

Vasallo Jr EGR, Gorospe JM, Torres MAJ, Amparado Jr RF, Demayo CG. 2015. Relative Warp Analysis of Parasite–Induced Plasticity in the Shell Shape of Bithynia sp.(Bithyniidae). Journal of Medical and Bioengineering **4,** 159.

Webster JP, Shrivastava J, Johnson PJ, Blair L. 2007. Is host-schistosome coevolution going anywhere? BMC evolutionary biology **7**, 91 http://dx.doi.org/10.1186/1471-2148-7-91

Zhao QP, Jiang MS, Littlewood DTJ, Nie P. 2010. Distinct genetic diversity of Oncomelania hupensis, intermediate host of Schistosoma japonicum in mainland China as revealed by ITS sequences. PLoS neglected tropical diseases **4,** e611. http://dx.doi.org/10.1371/journal.pntd.0000611