

Research Note

Cercarial fauna of freshwater snails in selected agricultural areas in Laguna, Philippines

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Summary

Freshwater snails serve as one of trematodes' intermediate hosts. Previous studies on trematode larval stages in the Philippines have largely focused on species with public health importance. This study sought to investigate the prevalence of cercarial morphotypes in several freshwater snail species found in different habitat types (rice field, irrigation canals, and residential area) in selected agricultural areas in Los Baños and Bay in Laguna. Cercarial emergence was induced through exposure to artificial light. A total of 2,720 freshwater snails were collected and were represented by seven species, namely, *Melanoides tuberculata* Muller 1774 (n = 1229), *Radix quadrasi* von Moellendorf (n = 630), *Tarebia granifera* Lamarck, 1816 (n = 417), *Pomacea canaliculata* Lamarck 1819 (n = 257), *Vivipara angularis philippinensis* Nevill (n = 18), *Stenomelania* sp. (n = 104), *Thiara scabra* Muller 1774 (n = 65). A 2.57 % over-all prevalence was recorded; the infected snail species were *M. tuberculata* (2.21 %), *R. quadrasi* (0.21 %), *T. granifera* (0.11 %). Four cercarial morphotypes, namely, Parapleurolophocercous cercaria (1.80 %), Virgulate xiphidiocercaria (0.26 %), Megaluroous cercaria (0.29 %), and Echinostome cercaria (0.22 %) were recovered from the infected snail species. Prevalence of cercarial infection was significantly different ($p < 0.05$) among habitat types.
Keywords: Trematodes; cercarial infection; freshwater snails; Laguna, Philippines

Introduction

Digenetic trematodes, commonly known as flukes, demonstrate a heteroxenous life cycle which includes various intermediate hosts for their development (Roberts & Janovy, 2009). Different species of freshwater snails have received considerable attention as they harbor larval stages of trematodes known to have both public health and veterinary importance (Mohammed *et al.*, 2016). Since the larval development in snails is obligatory, the distribution of these freshwater snail species dictates the occurrence of different trematode taxa in a locale (Hechinger & Lafferty, 2005). For instance, the freshwater snail, *Oncomelania hupensis quadrasi* Möllendorf, 1895, has been studied for infection of the strain of *Schistosoma japonicum* in the Philippines (Pesigan *et al.*, 1958a;

Madsen *et al.*, 2008). Likewise, attention has been given to the control of these snail host species (Pesigan, *et al.*, 1958b; Ohmae, *et al.*, 2003). However, there is a dearth of recent information on cercarial fauna of other freshwater snail species in the country. Researches on cercarial prevalence are deemed important to survey other snail species that may serve as intermediate hosts of other trematodes. Hence, this study generally sought to investigate the prevalence of cercarial types in freshwater snail species in selected agricultural areas in Laguna, Philippines: Los Baños and Bay. Specifically, it aimed to examine the snail species present for cercarial infection, to morphologically identify the cercarial species that will be recovered from the snail samples, and to compare their prevalence among the different habitat types.

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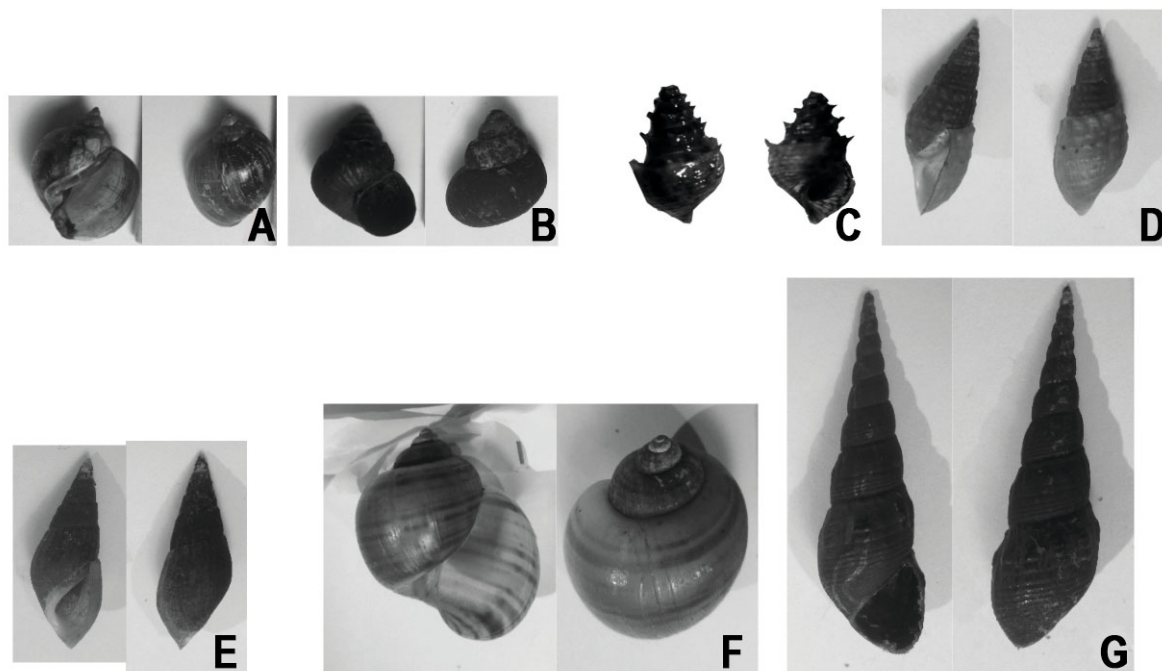


Fig. 1. Freshwater snail species collected in Los Baños and Bay, Laguna: (A) *Radix quadrasi* von Moellendorf, (B) *Vivipara angularis philippinensis* Nevill, (C) *Thiara scabra* Muller, 1774, (D) *Tarebia granifera* Lamarck, 1816, (E) *Stenomelania* sp., (F) *Pomacea canaliculata* Lamarck, 1819, (G) *Melanoides tuberculata* Muller 1774. (Scale bar = 1 cm)

Materials and Methods

Study site

This study was conducted in two municipalities in Laguna, Philippines: Los Baños and Bay. Los Baños (14° 09' 53.1" N, 121° 15' 21.8" E) is classified as a first-class urban municipality, while Bay (14° 10' 58" N, 121° 17' 5" E) is a second-class coastal municipality. Both municipalities generate income from agriculture and fishery. Moreover, both municipalities are part of the Mount Makiling Forest Reserve that has a wide range of ecosystems serving as habitats to diverse flora and fauna.

Sample collection

Semi-purposive sampling was done based on the following criteria: (1) having or being situated near an agricultural land, (2) presence of snails, and (3) having consent from owners. Selected sampling sites were of different habitat types – (1) rice fields, (2) irrigation canals, and (3) residential areas/ houses near the rice fields. A total of six (6) collection sites were selected representing each of the habitat types. Collection was conducted from 06:00 to 09:00 AM by hand picking and representatives were sent to UPLB Museum of Natural History for species identification. The snail samples were brought to the laboratory for processing.

Sample processing

Snail samples were individually placed in 50mL glass containers filled to half with dechlorinated tap water and were exposed to artificial light for six hours during daytime (08:00 AM to 02:00 PM)

at room temperature. The water in each container was checked for the presence of cercaria every two hours. Snails that did not shed cercaria on the first exposure were subjected to the same procedure for second trial on the following day. The snails that were negative for cercarial emergence after two trials of light exposure were subjected to crushing method. Briefly, the snails were crushed and the hepatopancreas of each snail was isolated and squashed onto a glass slide. A drop of 0.95 % physiological saline solution was added to the sample. The samples were viewed under a compound microscope to check for the presence of cercaria. Cercarial morphotypes were identified using the classification key of Frandsen and Christensen (1984) for cercarial species emerging from African snails and the illustrations provided by Chontanarith and Wongsawad (2013) for cercarial species emerging from snails collected in Thailand.

Data Analysis

Prevalence was computed using the formula:

$$\% \text{ Prevalence} = \frac{\text{number of infected snails}}{\text{total number of snails}} \times 100$$

To compare cercarial prevalence among habitat types, Chi-square test was employed using Quantitative Parasitology version 3 (Rózsa, *et al.*, 2000). Values were considered statistically significant if $p < 0.05$.

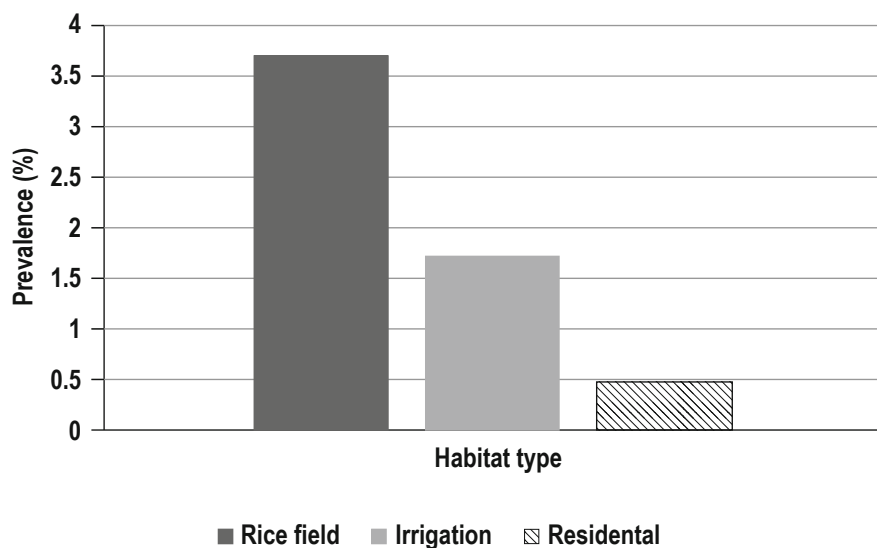


Fig. 2. Cercarial prevalence among habitat types

Ethical Approval and/or Informed Consent

The conducted research included animal subjects that are not covered by the institutional guidelines for the care and use of animals; hence, ethics clearance is not required.

Results and Discussion

A total of 2,720 freshwater snails represented by seven species, namely, *Melanoides tuberculata* Muller 1774 (n = 1229), *Radix quadrasi* von Moellendorf (n = 630), *Tarebia granifera* Lamarck, 1816 (n = 417), *Pomacea canaliculata* Lamarck 1819 (n = 257), *Vivipara angularis philippinensis* Nevill (n = 18), *Stenomelania* sp. (n = 104), *Thiara scabra* Muller 1774 (n = 65) were collected (Fig. 1).

Of the total samples, 1541 snails were collected in rice fields, 579 in irrigations, and 600 in residential areas near the rice fields. Overall, only 70 (1.56 %) of the snails collected were found positive for cercarial infection. Highest cercarial infection was recorded in rice fields, accounting to 3.70 % of the total collected snails in all rice fields surveyed. On the other hand, snails from irrigation canals and residential areas had 1.73 % and 0.5 % cercarial prevalence, respectively (Fig. 2). Chi-square test revealed that the prevalence of cercarial infection differed significantly ($p = 0.0001$). The differences in the cercarial prevalence among the three habitat types surveyed may be due to various possible reasons. Although environmental factors were not measured in this study, parameters such as water temperature (Studer & Poulin, 2013), pH (Candia *et al.*, 2015), vegetation cover (Koprivnikar *et al.*, 2007), and leaf litter (Luth *et al.*, 2016) were found to influence snail community and trematode prevalence in an area. It is also important to note that the presence of other animals that may serve as final hosts for these trematodes may also be one reason for prevalence of

infection. Interestingly, more rodents, amphibians, and some species of migratory birds have been observed in the rice fields than in the irrigation canals and household areas surveyed in this study. In general, freshwater snail species have been extensively studied due to their role as intermediate hosts of several trematode species known to cause diseases to humans and domestic animals. Members of the *Melanoides* genus have been recorded as hosts of various trematodes of medical and veterinary importance. For instance, numerous researches on *M. tuberculata* have been conducted as it harbors different trematode parasites such as *Philophthalmus gralli* (Pinto & Melo, 2010), *Centrocestus formosanus* (Paula-Andrade *et al.*, 2012; Dos Santos *et al.*, 2013; Najet *et al.*, 2014; Yousif *et al.*, 2016), *Haplorchis taichui* and *H. pumilio* (Krailas *et al.*, 2011), and various types of cercarial species (Devkota *et al.*, 2011; Duangduen *et al.*, 2014).

In this study, highest cercarial infection was observed in *M. tuberculata* (2.21 %), followed by *R. quadrasi* (0.21 %), and *T. granifera* (0.11 %). Cercariae were putatively classified into four morphotypes, namely, Virgulate xiphidocercaria, Parapleurolophocercous cercaria, Echinostome cercaria, and Megalurous cercaria (Fig. 3) based on the taxonomic classification key for cercariae by Frandsen and Christensen (1984) and illustrations provided by Chontanarith and Wongsawad (2013). Among the infected snails, *Melanoides tuberculata* was found to harbor three types of cercariae: Parapleurolophocercous cercaria (3.74 %), Megalurous cercaria (0.65 %), and Echinostome cercaria (0.49 %). However, no co-infection per individual was noted among the *M. tuberculata* snails examined. On the other hand, *T. granifera* was found infected with Parapleurolophocercous cercaria while Virgulate xiphidocercaria was recovered from *R. rubiginosa*.

Prevalence of the cercarial types in the infected snail species is summarized in Table 1. Virgulate xiphidocercaria exhibits unique

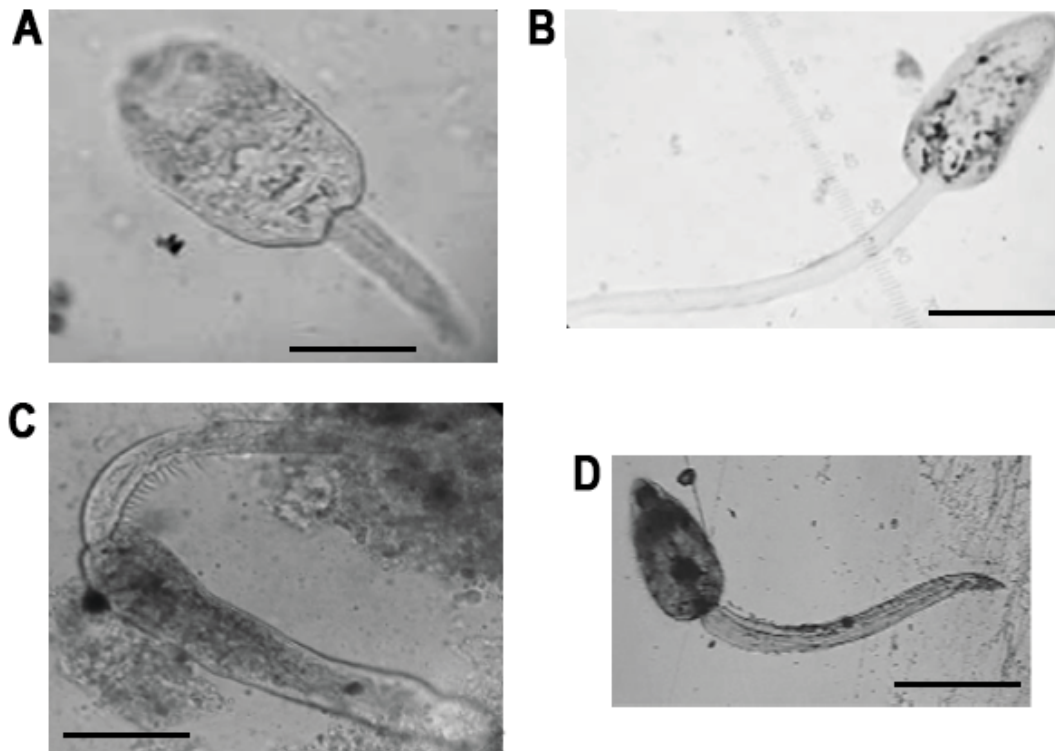


Fig. 3. Cercarial types found in snail species: (A) Virgulate xiphidocercaria, (B) Parapleurolophocercous cercaria, (C) Megalurous cercaria, and (D) Echinostome cercaria. (Scale bar = 20 μ m)

features such as its small size, a tail shorter than the body and has no dorsoventral finfold, a bilobed or pyriform virgula organ in the oral sucker region, and a ventral sucker smaller than the oral sucker. Parapleurolophocercous cercaria has distinct features including unforked tail with well-developed finfolds, absence of ventral sucker, presence of eyespots, absence of adhesive organs at posterior end of the body, and few cystogenous glands. Echinostome cercaria can be distinguished by its unforked tail, ventral sucker on mid-ventral surface of the body, oral sucker without stylet but surrounded by a spiny collar, and numerous cystogenous glands in the body. Megalurous cercaria is characterized by an elongated body with yellowish-brown granules, minute spines covering the posterior half of the body, bifurcated esophagus located in the middle of pharynx and ventral sucker, ventral sucker slightly larger

than the oral sucker and located medially on the body, sub-terminal oral sucker with complex muscular apparatus, and long, elastic, and slender tail with adhesive gland cells at the tip. These cercarial morphotypes are characteristic larval stages of various trematode families. Virgulate xiphidocercariae can develop into intestinal trematodes in the family Lecithodendriidae parasitizing bats, birds, and amphibians. Parapleurolophocercous cercaria is commonly produced by members of the family Heterophyidae which include species of intestinal trematodes known to infect birds and mammals. Echinostome cercariae are produced by species belonging to the family Echinostomatidae which is comprised by various species of intestinal parasites of birds, reptiles, and mammals. Megalurous cercaria is the characteristic larval stage of the avian eye trematodes in the family Philophthalmidae (Frand-

Table 1. Prevalence of the total cercarial types among infected snail species.

Snail species	No. of snail examined	No. of infected snails (% prevalence)			
		Virgulate xiphidocercaria	Parapleurolophocercous cercaria	Megalurous cercaria	Echinostome cercaria
<i>M. tuberculata</i>	1229	–	46 (3.74)	8 (0.65)	6 (0.49)
<i>R. rubiginosa</i>	630	7 (1.11)	–	–	–
<i>T. granifera</i>	417	–	3 (0.72)	–	–

sen & Christensen, 1984; Chontanarath & Wongsawad, 2013). The present study provides baseline data for cercarial morphotypes infecting freshwater snails in selected sampling sites in Los Baños and Bay in Laguna, Philippines. The result of the study revealed 2.57 % over-all prevalence of cercarial infection among the snail species collected in different habitat types. Interestingly, prevalence was significantly different among habitat types, with most of the infected snails recovered in rice fields. The snail species namely, *M. tuberculata*, *R. quadrasi*, and *T. granifera*, were found to be infected with one or more cercarial morphotypes. The cercariae were putatively identified as Virgulate xiphidiocercaria, Parapleurolophocercous cercaria, Echinostome cercaria, and Megalourous cercaria. These are known to be characteristic larval stages of diverse trematode parasites known to infect a wide range of vertebrates including humans. Although this revealed a low cercarial prevalence, monitoring and survey should still be done to determine the presence of trematodes to raise awareness regarding the potential public health and veterinary importance. Due to the lack of recent published information on the cercarial prevalence in the Philippines, surveillance in other parts of the country is recommended to document the cercarial types infecting freshwater snail species inhabiting the different locales. Moreover, other identification protocols, such as the use of scanning electron microscopy and molecular biological methods, should be employed to further characterize the cercariae and to identify the possible adult trematode species that may develop from them.

Conflict of Interest

Authors state no conflict of interest.

Acknowledgments

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