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Original Research Articles

Trematodes obtained from snails in giant gourami ponds supplied by well water in Tien Giang province, Vietnam

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A study on trematode obtained from freshwater snails in 30 grow-out giant gourami ponds supplied by well water was conducted in Chau Thanh district, Tien Giang province, Vietnam. The snails were sampled in the flooding season (October 2020), the intermediary season (January 2021) and the non-flooding season (April 2021). Eight snail species belonging to 8 genera, 4 families were found including *Sermyla tornatella* (48.6%), *Sinotaia lithophaga* (19.3%), *Filopaludina sumatrensis* (15.4%), *Melanoides tuberculata* (6.4%), *Pomacea sp.* (4.7%), *Bithynia siamensis* (4.2%), *Thiara scabra* (0.8%) and *Tarebia granifera* (0.6%). Cercariae were recovered from only two snail species of *Melanoides tuberculata* with *Xiphidio cercariae* and *Furcocercous cercariae* with a prevalence of 7.4%, and *Bithynia siamensis* with *Xiphidio cercariae* and *Pleurolophocercous cercariae* with the infection rate of 6.9%. The trematodes were found in 8 ponds (26.7%), 7 ponds (23.3%), and 3 ponds (10.0%) in the intermediary season, the non-flooding season, and the flooding season, respectively (P>0.05). More research on the epidemiology of trematodes in snails in fishponds should be done to contribute to food safety and sustainable aquaculture.

INTRODUCTION

Tien Giang is a province where giant gourami is often raised the most in monoculture in Vietnam. Farmers mainly used vegetables, aquatic plants, leftover feed from households, and agricultural by-products to feed giant gourami in grow-out ponds.[1,2] Pham et al.[3] found that giant gourami was infected with fish borne zoonotic parasites and the flooding season was a very suitable period for developing snails in the ponds. Studies on snail composition and distribution have been done in Vietnam. Dang et al.[4] stated that 47 freshwater snail species existed in the North of Vietnam. Bui et al.[5] researched the distribution of snails in Nam Dinh province, a northern province, and found that there were 16 snail species in two communes of the Nghia Phu district of this province. Species of the Bithyniidae, Stenothyridae and Planorbidae dominated in rice fields and small canals, and species of the families Thiaridae and Viviparidae were common in VAC ponds. In An My and An Hoa communes, Tuy An district, Phu Yen province in the Central of Vietnam, Nguyen et al.[6] found 11 snail species including *Melanoides tuberculata*, *Sermyla tornatella*, *Tarebia grannifera*, *Filopaludia sumatensis*, *Pomacea* sp., *Sinotaia lithophaga*, *Gyraulus* sp., *Lymnaea* sp., *Bithynia* sp., *Indoplanrbis exustus* and *Thiara scabra*. Ha et al.[7] found 14 freshwater snails including *Lymnaea swinhoei*, *Lymnaea viridis*, *Indoplanorbis exustus*, *Clea* sp., *Bithynia siamensis*, *Mekongia* sp., *Eyriesia* sp., *Adamietta* sp., *Melanoides tuberculata*, *Sermyla* sp., *Tarebia granifera*, *Pomacea canaliculata*, *Trochotaia* sp., and *Filopaludina martensi*.

Snails (or sometimes bivalves) are the first intermediate hosts everywhere where trematodes are transmitted.[8] The diversity and population growth of intermediate snail hosts also increase the risk of foodborne trematodiasis,[5] an emerging public health problem, particularly in Southeast Asia.[9] Bui et al.[5] found that *Melanoides tuberculata* had the highest prevalence of trematodes, and two of seven snail species, *Melanoides tuberculata* and *Bithynia fuchsiana* were infected cercariae.[10] The snail intermediate hosts for the heterophyid trematode species were mainly species of the Thiaridae and Bithynidae (Madsen and Nguyen, 2014) such as *M. tuberculata*, *Thiara* and *Terabia granifera*.[11] *M. tuberculata* was the host of *Haplorchis pumilio*[12] and of *Centrocestus formosanus*.[13] *Thiara granifera* was found commonly infected with *H. pumilio* in Taiwan,[14] with *C. formosanus* in Thailand[12] and with *Stellantchasmus falcatus* in Hawai.[15] The first intermediate snail species of *Clonorchis sinensis* was *Parafossarulus manchouricus*[16,17] and *Bithynia* and

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Table 1. Total sampled ponds of grow-out giant gourami in three communes of Chau Thanh district, Tien Giang province, Vietnam (N=30)

No	*Name of communes*	*Sampled ponds in flooding season (October 2020)*	*Sampled ponds in intermediary season (January 2021)*	*Sampled ponds in non-flooding season (April 2021)*
1	Than Cuu Nghia	18	18	18
2	Long An	7	7	7
3	Tam Hiep	5	5	5

Alocinma spp.[17] Bithynia snails were found to be first intermediate host of *Opisthorchis viverrini* in Thailand.[18]

In Vietnam, cercariae were found in eight snail species in Nam Dinh province in the North of Vietnam.[5] Pleurolophocercous cercariae were the most common type of cercariae (40.6% of all infections), followed by echinostome cercariae (35.0%) and xiphidiocercariae (17.3%). Besprozvannykh et al.[19] found the occurrence of 12 cercariae species in 8 families including Cyathocotylidae, Pleurogenidae, Lecithodendriidae, Notocotylidae, Heterophyidae, Paramphistomidae, Psilostomidae and Echinostomatidae. Nguyen et al.[6] found Pleurolophocercariae, Xiphidiocercariae, Echinostome, Monostome, and Gymnocephalus from 11 snail species in two communes of An My and An Hoa, Tuy An district, Phu Yen province in the Central of Vietnam. In 2015, Nguyen et al. recovered Xiphidiocercariae, Furcocercariae, and Pleurolophocercous from 7 snail species in Ninh Binh province. In 2019, Pham et al. implemented the research in Ninh Binh province and Ha Noi city and found 9 snail species and cercariae of Echinostome, Monostome, pleurolophocercous, Xiphidiocercariae, Furcocercariae, Gymnocephalous, Megalurous.

Giant gourami is a popular fish species cultured in ponds in Tien Giang province of Vietnam and found infected metacercariae.[3] However, there is no information about the presence of snails and trematode infections in these. A study on snail distribution in these ponds and cercariae infection can contribute to the food safety and the development of stable aquaculture.

MATERIALS AND METHODS

STUDY SITES

The study site of Chau Thanh district, Tien Giang province, is located in the Mekong Delta of Vietnam, where giant gourami culture is common. Based on the data from the Department of Agriculture and Rural Development of Tien Giang province in 2020, giant gourami was cultured mainly in Than Cuu Nghia, Long An, and Tam Hiep communes of Chau Thanh district. Therefore, snails in grow-out ponds of giant gourami from these three communes were chosen for research from October 2020 to April 2021 (**Table 1**).

SAMPLING OF SNAILS

According to Vietnam Meteorological and Hydrological Administration,[20] the flooding season in 2020 in the Mekong Delta of Vietnam peaked in October and then went down faster than the previous years. To implement the research on different seasons, three cross-sectional studies on snails were carried out in October 2020 (the flooding season), January 2021 (the intermediary season), and April 2021 (the non-flooding season). Thirty fishponds of grow-out giant gourami were randomly selected in the study. A 25-cm wide dredge was used to scrape the pond bottom 10cm deep and from 1.5 m out from the pond bank to collect snails. A total of five such samples were taken at different locations in each giant gourami pond. Each sample was washed, and the snails collected were transferred to cloth bags and transported to the laboratory for analysis. Snails were identified as species following the keys of Dang et al.[4]

EXAMINATION OF SNAILS FOR CERCARIAE

Snails were examined for trematode infection (cercariae stage) by shedding method[5,21] in containers of 100 mL, and left for 24 hours for shedding. Cercariae were identified to morphotype using key from Schell.[22] Specimens of snails and cercariae were preserved in 70% ethanol and 4% formalin, respectively, such that identifications could later be verified.

DATA ANALYSIS

Microsoft Excel 2010 was used for data entry, and SPSS (Statistical Package for Social Sciences version 20; SPSS Inc., Chicago, Illinois) was applied for data analysis. The Chi-squared test was used to compare the difference in trematode pond prevalence proportion of ponds where trematodes were found among sampling times. A value of $P<0.05$ was considered significant.

RESULTS

SNAIL COMPOSITION AND DISTRIBUTION IN GROW-OUT PONDS OF GIANT GOURAMI

Eight snail species belonging to 8 genera and 4 families were collected and identified using the morphological method (**Table 2**). Six snail species were found in the sampling ponds, except for two. *Tarebia granifera* was not found in Long An and Tam Hiep communes, whereas *Thiara scabra* was not in Tam Hiep commune.

A total of 7,643 samples of snails were collected in the flooding season in October 2020 (3,635 snails), in the intermediary season in January 2021 (2,313 snails) and the non-flooding season in April 2021 (1,695 snails). The snail species had high numbers of occurrence including *Sermyla*

Table 2. Snail composition and distribution in grow-out ponds of giant gourami in three communes of Chau Thanh district, Tien Giang province, Vietnam (N=30).

Family	*Genus*	*Species*	*In three sampling times**		
			Than Cuu Nghia (N=18 x 3= 54)	*Long An (N=7 x 3 = 21)*	*Tam Hiep (N=5 x 3 =15)*
Thiaridae	Sermyla	*Sermyla tornatella*	45	13	7
	Melanoides	*Melanoides tuberculata*	22	8	5
	Tarebia	*Tarebia granifera*	5	0	0
	Thiara	*Thiara scabra*	5	1	0
Viviparidae	Filopaludina	*Filopaludina sumatrensis*	37	17	9
	Sinotaia	*Sinotaia lithophaga*	44	20	9
Ampulariidae	Pomacea	*Pomacea* sp.	43	18	7
Bithyniidae	Bithynia	*Bithynia siamensis*	31	10	9

* Showed total ponds having snails in three sampling times.

Table 3. Percentage contribution of each snail species in 30 giant gourami ponds.

Snail species	*In flooding season (Oct 2020) (n=30)*		*In intermediary season (Jan 2021) (n=30)*		*In non-flooding season (Apr 2021) (n=30)*		*In three sampling times*	
	Total	*(%)*	*Total*	*(%)*	*Total*	*(%)*	*Total*	*(%)*
Sermyla tornatella	2190	60.2	1024	44.3	503	29.7	3717	48.6
Melanoides tuberculata	138	3.8	287	12.4	64	3.8	489	6.4
Tarebia granifera	3	0.1	14	0.6	31	1.8	48	0.6
Thiara scabra	18	0.5	23	1.0	19	1.1	60	0.8
Filopaludina sumatrensis	365	10.0	370	16.0	438	25.8	1173	15.4
Sinotaia lithophaga	692	19.0	392	16.9	393	23.2	1477	19.3
Pomacea sp.	159	4.4	101	4.4	99	5.8	359	4.7
Bithynia siamensis	70	1.9	102	4.4	148	8.7	320	4.2
Grand total	3,635	100	2,313	100	1,695	100	7,643	100

tornatella (48.6%), *Sinotaia lithophaga* (19.3%), *Filopaludina sumatrensis* (15.4%), *Melanoides tuberculata* (6.4%), *Pomacea* sp. (4.7%), and *Bithynia siamensis* (4.2%), and the two snail species had low percentage such as *Thiara scabra* (0.8%) and *Tarebia granifera* (0.6%) (**Table 3**).

CERCARIAE MORPHOTYPES INFECTED IN SNAILS

The prevalence of cercariae infection was 26.7% (N=8/30), 23.3% (N=7/30) and 10.0% (N=3/30) in the intermediary season, the non-flooding season and the flooding season, respectively, however, there was no significantly different from the prevalence among the three seasons (P>0.05). All infected snails were *Melanoides tuberculata*, the most important host species for trematode belonging to the Heterophyidae,[23] and *Bithynia siamensis*, a potential host for both Heterophyidae and Opisthorchiidae.

For the overall prevalence in three seasons for each snail species, *Melanoides tuberculata* had the highest prevalence at 7.4% (N=36/489), and *Bithynia siamensis* had the prevalence of 6.9% (N=22/320) (**Table 4**).

The result showed that pleurolophocercous cercariae was found in two snails whereas xiphidiocercariae and furcocercous cercariae were more abundant, especially in the non-flooding season and the intermediary season. *Melanoides tuberculata* was infected xiphidio cercariae and furcocercous cercariae while *Bithynia siamensis* infected xiphidio cercariae and pleurolophocercous cercariae (**Table 5**).

Table 4. Number of infected snails in 30 giant gourami ponds (N=30).

Snail species	*In flooding season (Oct 2020) (n=30)*		*In intermediary season (Jan 2021) (n=30)*		*In non-flooding season (Apr 2021) (n=30)*	
	Collected snails	*Infected snails*	*Collected snails*	*Infected snails*	*Collected snails*	*Infected snails*
Sermyla tornatella	2190	0	1024	0	503	0
Melanoides tuberculata	138	4	287	22	64	10
Tarebia granifera	3	0	14	0	31	0
Thiara scabra	18	0	23	0	19	0
Filopaludina sumatrensis	365	0	370	0	438	0
Sinotaia lithophaga	692	0	392	0	393	0
Pomacea sp.	159	0	101	0	99	0
Bithynia siamensis	70	2	102	4	148	16
Grand total	3,635		2,313		1,695	

Table 5. Cercariae groups found in infected snails in 30 giant gourami ponds (N=30)

Cercariae groups	*In flooding season (Oct 2020) (n=30)*		*In intermediary season (Jan 2021) (n=30)*		*In non-flooding season (Apr 2021) (n=30)*	
	M[1]	*B*[2]	*M*[1]	*B*[2]	*M*[1]	*B*[2]
Xiphidio cercariae	4	2	11	4	0	15
Furcocercous cercariae	0	0	11	0	10	0
Pleurolophocercous cercariae	0	0	0	0	0	2

[1] M: means *Melanoides tuberculata*;
[2] B: means *Bithynia siamensis*

DISCUSSION

This study on snail diversity and trematode larvae in grow-out giant gourami in earthen ponds provided more information about snails in aquaculture and the occurrence of cercariae obtained from snails in fish ponds in Vietnam. The study's most dominant family of snails was Thiaridae, with the highest number of *Sermyla tornatella* species, and then the family of Viviparidae. This finding was similar to the research by Pham et al.[24] that most snails in the juvenile giant gourami nurseries in Cai Lay district, Tien Giang province, belonged to the families of Viviparidae and Thiaridae. However, the numbers of snail species were completely different when comparing the diversity of snails in the fish ponds integrated with cash crops and livestock (VAC ponds). The research result by Bui et al.[5] in Nam Dinh province in the north of Vietnam showed that the total number of snail families in VAC ponds, with 14 snail species in 8 families, was double that obtained from giant gourami ponds with 8 snail species in 4 families. The explanation was that VAC ponds were supplied water from canals and rivers containing almost snail species from the natural water sources, while all giant gourami ponds in this research were supplied by well water only; water was pumped directly from the well into fish ponds; therefore, the snails were mainly available in the ponds already, and it had less chance for new snails to invade into the giant gourami ponds. One more reason was that giant gourami was fed by vegetables and clearly if they were collected from other habitats in the area having snails that could be one way that snails entered these ponds. The use of well water will affect the natural resources of ground water in the long term, but it was meaningful to the current situation of giant gourami culture in Chau Thanh district, Tien Giang province, where the main water source is water from the wells. Moreover, the layout and structure of the ponds could allow the invasion of snails and eggs of fishborne zoonotic parasite in run-off water entering the ponds.

Madsen et al.[23] researched on snail distribution in fish ponds and found a lot of *Melanoides tuberculata*. In the current study, there was only 6.4% of *Melanoides tuberculata* in giant gourami ponds because of the location of the ponds, the source of well water supplying to the ponds because no snails in well, and also the snail distribution in different areas. For the presence of *Bithynia*, it is interesting that both of these fish ponds and giant gourami ponds were similar as it was rarely found. Three snail species were abundant in the giant gourami ponds including *Sermyla tornatella*, *Filopaludina sumatrensis* and *Sinotaia lithophaga* with no

cercariae. This finding agreed with the research result by Nguyen et al.[6] that *Filopaludina sumatrensis* and *Sinotaia lithophaga* had cercariae free.

This research confirmed again that *Melanoides tuberculate* and *Bithynia* snails were easier to infect cercariae than the other sampled snails. This finding was similar to the previous research that *Melanoides tuberculate,* and *Bithynia fuchsiana* was infected with a high prevalence in ponds and canals[5,6] in natural water bodies[10] and in Chao-Phraya Basin in Thailand.[25]

Three morphotypes of cercariae were recovered from *Melanoides tuberculata* and *Bithynia siamensis*, two from *Melanoides tuberculata* (Xiphidio cercariae and Furcocercous cercariae), and two from *Bithynia siamensis* (xiphidio cercariae and pleurolophocercous cercariae). Xiphidio cercariae were more frequent in this research. These findings were different from the research result in VAC ponds by Bui et al.,[5] that pleurolophocercous cercariae were the most common type of cercariae recovered, followed by echinostome cercariae and then xiphidiocercariae. Moreover, the critical finding in this research was that snails were infected with cercariae, so fish species of giant gourami cultured in ponds in the same research area were found infected metacercariae as the report by Pham et al.[3]

When comparing to the other research on snails in natural water bodies by Nguyen et al.,[6] Nguyen et al.,[10] and Anucherngchai et al.,[25] morphotypes of cercariae in snails from the wild water were much higher than in the pond culture of giant gourami. It can be explained that the natural water bodies were open, giving various types of cercariae more chances to get into snails. In VAC ponds, cercariae morphotypes were also more varied than in giant gourami ponds. The reason was that feed for fish in VAC ponds, including animal manure, by-products from agriculture, and vegetables, was put in the ponds; therefore, more cercariae must be presented. On the contrary, all the giant gourami ponds in this paper were supplied by well water, and the main feed for fish was vegetables and pelleted feed, so only three morphotypes of cercariae were found.

According to the data of total numbers of snails collected in each season in all research ponds, snail populations were typically more abundant in the flooding season, and total snails were higher than in the intermediary season and lowest in the non-flooding season. This finding was similar to the research result by Brockelman et al.[26] that the flooding season, which most of the months in this season belongs to the rainy season, provided good conditions for the multiplication of snails. In this research, the trematode pond prevalence in snails (cercariae stage) was high in the intermediary season and also high in the non-flooding season; however, it was low in the flooding season, but there was no significantly different from the trematode pond prevalence among the three seasons (P>0.05).

The occurrence of cercariae was different among the flooding season, the intermediary season and the non-flooding season, but there was no significantly different (P>0.05). This result agreed with the data reported earlier by Nguyen et al.[6] that the occurrence of cercariae was different among the months of the year, and the prevalence was higher in the dry season from November to April because of the high temperature. The samplings in January and April in this research were in the intermediary season and the non-flooding season, respectively; therefore, the prevalence was higher than in October, belonging to the non-flooding season or in the wet season from May to October in the research by Nguyen et al.[6] How season affects snail and cercariae infection is not known yet, so more research should be done to know the relationship between snail distribution and the occurrence of cercariae in snails in giant gourami cultured in ponds.

CONCLUSIONS

The trematode pond prevalence in the grow-out pond of giant gourami was high in the intermediary season (26.7%) and in the non-flooding season (23.3%), but it was low in the flooding season (10.0%). Xiphidiocercariae was recovered from two snail species of *Melanoides tuberculata* and *Bithynia siamensis*. *At the same time,* Furcocercous cercariae was found only in *Melanoides tuberculata* and pleurolophocercous cercariae was recovered only from *Bithynia siamensis*. No cercariae were found in the other six snail species including *Sermyla tornatella*, *Sinotaia lithophaga*, *Filopaludina sumatrensis*, *Pomacea* sp., *Thiara scabra,* and *Tarebia granifera*. Further research on trematode infection of the snails in fish cultured in ponds should be done to contribute to sustainable aquaculture development and food safety.

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