

Research Article

**FRESHWATER SNAIL DIVERSITY IN GROWOUT PONDS
OF GIANT GOURAMI REGARDING INTERMEDIATE HOSTS
OF TREMATODE LARVAE (CERCARIAE STAGE) IN TIEN GIANG PROVINCE***Pham Cu Thien^{1*}, Tran Thi Thu Thuy²*¹*Ho Chi Minh City University of Education, Vietnam*²*Le Quy Don Secondary School, Thu Duc City, Vietnam***Corresponding author: Pham Cu Thien – Email: thienpc@hcmue.edu.vn**Received: September 01, 2021; Revised: September 07, 2021; Accepted: September 12, 2021***ABSTRACT**

*Research on the composition of freshwater snails and trematode larvae (cercariae stage) in giant gourami ponds (N=20) was carried out in Tien Giang province in the wet season of 2020 and in the dry season of 2021. A total of 8 snail species from 4,548 samples was identified by the morphological method. The results showed that *Melanoides tuberculata* was infected *Xiphidio cercariae* and *Furcocercous cercariae* whereas *Xiphidio cercariae* and *Parapleurolophocercous cercariae* were recovered from *Bithynia sp.* No cercariae were found in *Filopaludina sumatrensis*, *Pomacea sp.*, *Sermyla tornatella*, *Sinotaia lithophaga*, *Tarebia granifera* and *Thiara scabra*. The trematode pond prevalence in the dry season was higher than in the wet season ($P>0.05$). Further research on the trematode prevalence in snails in ponds of giant gourami should be done in different months to contribute to the prevention of the trematode infection in snails and giant gourami cultured in ponds.*

Keywords: cercariae; giant gourami; snail; trematode

1. Introduction*Culture of giant gourami*

Fish culture systems are more diverse, with most freshwater fish species raised in monoculture or polyculture, including cage culture, fish rice culture, pond monoculture, and pond polyculture, and stocked at different levels of intensification. Commercial fish culture in the Mekong Delta began with the production of river catfish for export. In addition, other common grow-out systems have been developed to mainly supply fish for domestic consumption, including giant gourami. The pond area for giant gourami monoculture ranged from 200-800 m² with a stocking density of 5-7 fish/m² (Nguyen, 2005). Giant gourami typically gained only 0.4-0.6 kg after 10-12 months' culture and reached 1.0-1.5 kg after two years of pond culture (Pham, 2006). In grow-out ponds, the

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main feed for giant gourami were vegetables, aquatic plants, household waste, agricultural by-products, and commercial pelleted feed (Duong, 2003; Nguyen, 2005).

Freshwater snail distribution

Snail composition and distribution have been studied in recent years. Dang Ngoc Thanh et al. published a book in 1980 stating that there were 47 freshwater snail species in the North of Vietnam. Bui et al. (2010) researched the distribution of snails in VAC ponds and associated water bodies and found that there were 16 snail species in two communes of Nghia Phu district, Nam Dinh province. Nguyen Phuoc Bao Ngoc et al. (2014) collected 11 snail species including *Melanoides tuberculata*, *Sermyla tornatella*, *Tarebia grannifera*, *Filopaludia sumatensis*, *Pomacea* sp., *Sinotaia lithophaga*, *Gyraulus* sp., *Lymnaea* sp., *Bithynia* sp., *Indoplanorbis exustus*, and *Thiara scabra* in An My and An Hoa communes, Tuy An district, Phu Yen province. Ha Huynh Hong Vu et al. (2014) surveyed the snail composition in Vinh Long and Dong Thap provinces and 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 martensi*.

Snail as the second intermediate host

Foodborne trematodiasis is an emerging public health problem particularly in Southeast Asia (Keiser, & Utzinger, 2005). In aquaculture systems, the main risk factors for trematode infection and transmission include contamination of pond environments with trematode eggs from infected hosts. Factors that promote the diversity and population growth of snail intermediate hosts also increase risk (Bui et al., 2010) as snail is the first intermediate host of trematodes (Thai, 2010).

In a research by Bui et al. (2010), no cercariae were found in species of Viviparidae and Ampullaridae, but *Melanoides tuberculata* belonging to the genus of Thiaridae had the highest prevalence (Bui et al., 2010). Among the seven snail species, only *Melanoides tuberculata* and *Bithynia fuchsiana* were infected cercariae (Nguyen et al., 2015). It seemed that two of these snail species were common infected cercariae. The snail intermediate hosts for the heterophyid trematode species were primarily species of the Thiaridae and Bithynidae (Madsen & Nguyen, 2014). *M. tuberculata*, *Thiara* and *Terabia granifera* were the first intermediate hosts of heterophyids (Waikagul, & Radomyos, 2005). *M. tuberculata* was the host of *Haplorchis pumilio* (Khalifa et al. 1977; Wang et al. 2002; Dechruksa et al. 2007) and *Centrocestus formosanus* (Scholz & Salgado-Maldonado, 1999). *Melania newcombi* was the host of *Stellantchasmus falcatus* (Lee & Cheng, 1970). *Thiara granifera* was found commonly infected with *H. pumilio* in Taiwan (Wang et al. 2002), *C. formosanus* in Thailand (Dechruksa et al. 2007), and *S. falcatus* in Hawaii (Lee & Cheng, 1970). *T. riquetti* has been found to be the host of *Procerovum*

calderoni in the Philippines (Velasquez, 1973). Ten species of snails belonging to four families have shown to be the first intermediate host for *Clonorchis sinensis*. The first intermediate snail species of *C. sinensis* in China, Taiwan, Korea, and Japan was *Parafossarulus manchouricus* (Yoshida, 2005). Dubey et al. (2006) reported that *Parafossarulus*, *Bythynia*, and *Alocinma* spp. were the first intermediate snail host of *C. sinensis*, and *Bythynia* spp. was also the host of *Opisthorchis viverrini* and *Codiella* spp. for *O. feliensis*. Two species of *Bithynia* snails were found to be the first intermediate host of *Opisthorchis viverrini* in Thailand (Tesana, 2005). Species of the families Thiaridae and Viviparidae were more abundant than other species in VAC ponds while species of the Bithyniidae, Stenothyridae, and Planorbidae dominated in rice fields and small canals (Bui et al. 2010).

Trematode infection (cercariae stage)

Bui Thi Dung et al. (2010) researched the distribution of freshwater snails and the occurrence of trematode infections in VAC ponds and associated habitats in cultured fish in two communes, Nghia Lac and Nghia Phu, Nghia Hung District, Nam Dinh Province. Trematode infections were found in eight snail species. Parapleurolophocercous and pleurolophocercous cercariae constituted the most common type of cercariae recovered, contributing 40.6% of all infections followed by echinostome cercariae (35.0%) and xiphidiocercariae (17.3%). In 2013, Besprozvannykh et al. studied the presence of cercariae in three snails species of *Bithynia fuchsiana*, *Parafossarulus striatulus* (Bithyniidae), and *Melanoides tuberculata* (Thiaridae) in Nam Dinh province and recorded 12 cercariae species of 8 families including Cyathocotyliidae, Pleurogenidae, Lecithodendriidae, Notocotyliidae, Heterophyidae, Paramphistomidae, Psilostomidae, and Echinostomatidae. Nguyen Phuoc Bao Ngoc et al. (2014) 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. Krailas et al. (2014) obtained nine types of cercariae from *Melanoides tuberculata* from 120 locations in Thailand including Parapleurolophocercous cercariae, Pleurolophocercous cercariae, Xiphidiocercariae, Megalourous cercariae, Furcocercous cercariae, Echinostome cercariae, Amphistome cercariae, Rencolid cercariae and Cotylomicrocercous cercariae. Nguyen Manh Hung et al. (2015) recovered Xiphidiocercariae, Xiphidiocercariae, Furcocercariae, Parapleurolophocercous from seven snail species in Gia Vien district, Ninh Binh province, in which *Melanoides tuberculata* had the highest prevalence. Pham Ngoc Doanh et al. (2019) carried out a study in Kim Son district of Ninh Binh province and Ba Vi district of Ha Noi City and got nine snail species and cercariae of Echinostome, Monostome, Parapleurolophocercous, Xiphidiocercariae, Furcocercariae, Gymnocephalous, and Megalourous.

The results show that there are a lot of studies on snail composition and trematode larvae (cercariae stage) in Vietnam and other countries. However, there is no information about the snail composition and trematode in snails in the grow-out pond of giant gourami, so research on snails and cercariae in giant gourami ponds in Chau Thanh district, Tien Giang Province, Vietnam was implemented.

2. Materials and methods

Study areas

According to the data from the Department of Agriculture of Chau Thanh district, Tien Giang Province in 2020, giant gourami was cultured mostly in Chau Thanh district, especially in two communes namely Than Cuu Nghia and Long An. Therefore, snails in grow-out ponds of giant gourami from these two communes were chosen for research in October 2020 and April 2021 (Table 1).

Table 1. Total sampled ponds of grow-out giant gourami in Chau Thanh district (N=20)

No	Name of communes	Sampled ponds in October 2020	Sampled ponds in April 2021
1	Than Cuu Nghia	18	18
2	Long An	2	2

Sampling of snails

Two cross-sectional studies on snails were carried out in October 2020 (the wet season) and in April 2021 (the dry season). A total of 20 grow-out ponds of giant gourami was randomly selected for snail examination. Snail sampling was done using a 25-cm wide dredge to scrape the pond bottom from 1.5 m out from the pond bank. A total of five such samples were taken at different locations in each giant gourami pond. A separate dredge and scoop were used at each farm to avoid the possible spread of fish disease-causing pathogens. Each sample was washed in the pond water and collected snails were transferred to cloth bags and transported to the laboratory where they were analyzed within 24 h of collection. Snails were identified as species following the keys of Dang et al. (1980), Madsen and Nguyen (2014).

Examination of snails for cercariae

Snails were examined for trematode infection (cercariae stage) by shedding method (Frandsen, & Christensen, 1984; Bui et al., 2010) in different sized containers from 5mL to 100 mL small plastic beakers, depending on snail size, and left for 24 hours for shedding. Recognition of cercariae was made by using systematic key references (Frandsen, & Christensen, 1984; Schell, 1985).

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-square test was used to compare the difference of trematode pond prevalence (cercariae stage) between seasons. A value of $P < 0.05$ is considered significant.

3. Results and discussion

3.1. Snail composition in grow-out ponds of giant gourami

Eight snail species belonging to eight genera, four families in Order of Mesogastropoda, Class of Gastropoda, Phylum of Mollusca were collected and identified by the morphological method. The snail species were *Sermyla tornatella*, *Melanoides tuberculata*, *Tarebia granifera*, *Thiara scabra*, *Filopaludina sumatrensis*, *Sinotaia lithophaga*, *Pomacea* sp., and *Bithynia* sp. (Table 2).

Table 2. Snail composition in grow-out ponds of giant gourami in Tien Giang Province (N=20)

Family	Genus	Species
Thiaridae	<i>Sermyla</i>	<i>Sermyla tornatella</i>
	<i>Melanoides</i>	<i>Melanoides tuberculata</i>
	<i>Tarebia</i>	<i>Tarebia granifera</i>
	<i>Thiara</i>	<i>Thiara scabra</i>
Viviparidae	<i>Filopaludina</i>	<i>Filopaludina sumatrensis</i>
	<i>Sinotaia</i>	<i>Sinotaia lithophaga</i>
Ampulariidae	<i>Pomacea</i>	<i>Pomacea</i> sp.
Bithyniidae	<i>Bithynia</i>	<i>Bithynia</i> sp.

A total of 4,548 samples of snails was collected in both the wet season (October 2020) and the dry season (April 2021). The snail species had high numbers of occurrence such as *Sermyla tornatella* (55.5%), *Filopaludina sumatrensis* (16.1%), *Sinotaia lithophaga* (15.6%), *Pomacea* sp. (4.0%), *Melanoides tuberculata* (3.9%), and *Bithynia* sp. (3.5%). The snails had low numbers such as *Thiara scabra* (0.8%) and *Tarebia granifera* (0.7%). (Table 3).

Table 3. Total sampled snails in the wet season and the dry season (N=20)

Snail species	Number of snails			Percentage (%)
	In the wet season (October 2020)	In the dry season (April 2021)	In two seasons	
<i>Bithynia</i> sp.	21	136	157	3.5
<i>Filopaludina sumatrensis</i>	360	372	732	16.1
<i>Melanoides tuberculata</i>	119	57	176	3.9
<i>Pomacea</i> sp.	123	58	181	4.0
<i>Sermyla tornatella</i>	2043	480	2523	55.5
<i>Sinotaia lithophaga</i>	380	328	708	15.6
<i>Tarebia granifera</i>	3	31	34	0.7
<i>Thiara scabra</i>	18	19	37	0.8
Total	3,067	1,481	4,548	100

The research result of snail diversity in giant gourami cultured in ponds provides more information about snails in aquaculture in Vietnam. The dominant families of snails in the study were Thiariidae and Viviparidae. This result is similar to the findings by Pham et al. (2015) that most snails in the juvenile giant gourami nurseries belonged to the families of Viviparidae and Thiariidae. The explanation may be that the two study areas are located in the districts of Chau Thanh (grow-out ponds) and Cai Lay (nursing ponds) but in the same province of Tien Giang, so the snails' species are not different. When comparing to the research on snails in VAC ponds in Nam Dinh province of Vietnam (Bui et al., 2010), a total number of snail species and families in giant gourami ponds (8 snail species in 4 families) were much smaller than that in the VAC ponds with 14 snail species in 8 families. The main reason is that VAC ponds had water from canals and rivers while giant gourami ponds were supplied by well water; therefore, there was less chance for new snails to invade the giant gourami ponds. Moreover, the soil structure in ponds and study areas might make the difference in snail diversity.

In the fish ponds from the research by Madsen et al. (2015), *Melanoides tuberculata* was much presented but only some of them were found in giant gourami ponds in this paper because of the location. However, the presence of *Bithynia* sp. in both of these fish ponds and giant gourami ponds are alike as they were rarely found. *Sermyla tornatella*, *Filopaludina sumatrensis*, and *Sinotaia lithophaga* were particularly abundant in the giant gourami ponds, but all of them had cercariae free. This result is similar to the research by Nguyen et al. (2014) that *Filopaludina sumatrensis* and *Sinotaia lithophaga* had no cercariae. The two snail species in giant gourami ponds infected with cercariae were *Bithynia* sp. and *Melanoides tuberculata* with the prevalence of 11.5% and 6.8%, respectively. This finding agrees with the previous research that *Melanoides tuberculata* and *Bithynia* sp. were infected with the high prevalence in ponds and canals (Bui et al., 2010; Nguyen et al., 2014), in natural water bodies (Nguyen et al., 2015), and in Chao-Phraya Basin in Thailand (Anucherngchai et al., 2016). Therefore, the result in this research confirms that *Melanoides tuberculata*, the most important host species for trematode belonging to the Heterophyidae (Madsen et al., 2015), and *Bithynia* sp., a potential host for both Heterophyidae and Opisthorchiidae, were easier to be infected with cercariae than the other sampled snails.

3.2. Cercariae morphotypes infected in snails

Two of eight snail species were infected with cercariae including *Bithynia* sp. and *Melanoides tuberculata*. *Xiphidiocercariae* was recovered from both of two snails species of *Bithynia* sp. and *Melanoides tuberculata* while *Parapleurolophocercous cercariae* were recovered only from *Bithynia* sp., and *Furcocercous cercariae* were found only in *Melanoides tuberculata* (Table 4). The research result showed that the trematode pond prevalence (cercariae stage) in the dry season (30.0%) was higher than in the wet season

(20.0%). However, there was no significant difference from the prevalence between the two seasons ($P > 0.05$). For the overall prevalence in both of the two seasons for each snail species, *Bithynia* sp. had the highest prevalence at 11.5% and *Melanooides tuberculata* at 6.8%.

Table 4. Number of snails infected with different cercariae morphotypes

No	Snail species	Infected snails/ Total samples	Prevalence (%)	Morphotypes of cercariae	
				Wet season (October 2010)	Dry season (April 2021)
1	<i>Bithynia</i> sp.	18/157	11.5	<i>Xiphidio cercariae</i>	<i>Xiphidio cercariae</i> , <i>Parapleurolophocercous cercariae</i>
2	<i>Filopaludina sumatrensis</i>	0/732	0	X	X
3	<i>Melanooides tuberculata</i>	12/176	6.8	<i>Xiphidio cercariae</i>	<i>Furcocercous cercariae</i>
4	<i>Pomacea</i> sp.	0/181	0	X	X
5	<i>Sermyla tornatella</i>	0/2523	0	X	X
6	<i>Sinotaia lithophaga</i>	0/708	0	X	X
7	<i>Tarebia granifera</i>	0/34	0	X	X
8	<i>Thiara scabra</i>	0/37	0	X	X

Only two morphotypes of cercariae were recovered from each snail species of *Melanooides tuberculata* (*Xiphidio cercariae* and *Furcocercous cercariae*) and *Bithynia* sp. (*Xiphidio cercariae* and *Parapleurolophocercous cercariae*) in giant gourami ponds in the research. *Xiphidio cercariae* seemed to have more frequent occurrence than the others as it was recovered from eight snails while *Furcocercous cercariae* had in four snails and *Parapleurolophocercous cercariae* were in two snails. This is similar to the findings by Nkwengulila and Kigadye (2005) that xiphidiocercariae was the most prevalent in snails in ponds and streams. However, it is different from the result by Bui et al. (2010) researched the VAC pond that parapleurolophocercous and pleurolophocercous cercariae constituted the most common types of cercariae recovered, followed by echinostome cercariae and then xiphidiocercariae. The type of cercariae in VAC ponds was more various than giant gourami ponds in this paper. It can be explained that water supply to VAC ponds was from canals and rivers, various types of feed including animal manure were put in the ponds; therefore, more types of cercariae must be presented. On the contrary, all the giant gourami ponds were supplied by well water, and feed for fish were vegetables and pelleted feed, so only three morphotypes of cercariae were found. Comparing to the other research on snails in natural water bodies, morphotypes of cercariae were much higher than in the giant gourami ponds (Nguyen et al., 2014; Nguyen et al., 2015; Anucherngchai et al., 2016).

Based on the total numbers of snail samples, snail populations were typically more abundant in the rainy season than in the dry season. This is similar to the result by Brockelman et al. (1986) which provided good conditions for the multiplication of snails. However, the trematode pond prevalence in snails (cercariae stage) in Tien Giang province in the dry season was higher than in the wet season, but there was no significant difference ($P > 0.05$). The seasonality of trematode prevalence observed in this study is in agreement with the data reported earlier by Nguyen et al. (2014) that the occurrence of cercariae was different among the months of the year and the prevalence was higher in the dry season because of the temperature. This also agreed with the publish by Nkwengulila and Kigadye (2005) that the prevalence of cercariae was fluctuated by season, it was high in the dry season and decreased in the wet season. Back to the paper by Pham et al. (2007), the sampling of grow-out giant gourami in Tien Giang province showed that the trematode prevalence (metacercariae stage) in the flooding season (most of the months in this season belongs to the wet season) was significantly higher than in the non-flooding season ($P < 0.05$). How season affects snail and fish infections is not known yet. Therefore, more research should be done to know the relationship between the prevalence of cercariae in snails and metacercariae in giant gourami cultured in ponds.

4. Conclusions

The trematode pond prevalence (cercariae stage) in the dry season was higher than in the wet season ($P > 0.05$). *Melanoides tuberculata* infected Xiphidio cercariae and Furcocercous cercariae while *Bithynia* sp. infected Xiphidio cercariae and Parapleurolophocercous cercariae. More research on the trematode prevalence in snails from ponds of giant gourami should be done in different months to identify the infected snails to contribute to clean aquaculture development in Tien Giang province and Mekong Delta.

❖ **Conflict of Interest:** Authors have no conflict of interest to declare.

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**THÀNH PHẦN LOÀI ỐC NƯỚC NGỌT VÀ TỈ LỆ NHIỄM SÁN LÁ SONG CHỦ
TRÊN ỐC (GIAI ĐOẠN CERCARIAE)**

THU ĐƯỢC TRONG AO NUÔI CÁ THỊT TẠI TƯỢNG Ở TỈNH TIỀN GIANG

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TÓM TẮT

Nghiên cứu thành phần loài ốc nước ngọt và tỉ lệ nhiễm sán lá song chủ (giai đoạn cercariae) trong ao nuôi cá tại tượng (N=20) được thực hiện ở tỉnh Tiền Giang vào mùa mưa năm 2020 và mùa khô năm 2021. Tổng số 8 loài ốc từ 4548 mẫu ốc được phân loại dựa theo đặc điểm hình thái. Kết quả cho thấy, ốc *Melanoides tuberculata* bị nhiễm *Xiphidio cercariae* và *Furcocercous cercariae* trong khi ốc *Bithynia* sp. nhiễm *Xiphidio cercariae* và *Parapleurolophocercous cercariae*. Sáu loài ốc còn lại không nhiễm cercariae bao gồm *Filopaludina sumatrensis*, *Pomacea* sp., *Sermyla tornatella*, *Sinotaia lithophaga*, *Tarebia granifera* và *Thiara scabra*. Tỉ lệ ao nhiễm cercariae trong mùa khô cao hơn trong mùa mưa ($P > 0.05$). Cần tiếp tục nghiên cứu tỉ lệ nhiễm sán lá song chủ trên ốc trong ao nuôi cá tại tượng ở những tháng khác nhau nhằm góp phần phòng ngừa nhiễm sán lá song chủ trên ốc và cá tại tượng nuôi trong ao.

Từ khóa: ốc; sán lá song chủ; cercariae; cá tại tượng