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Research article

Digenean infections in snails (cercariae stage) in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam

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ABSTRACT

Foodborne zoonotic trematodiasis is a public health problem in many countries. A study on digenean larvae (cercariae stage) obtained from snails in all ten main small canals and two rice fields was carried out in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh city, Vietnam to identify the snail composition and digenean prevalence in snails. The snails were sampled in the dry season (January 2022) and the wet season (June 2022). In the ten canals, 23 snail species belonging to 16 genera, 10 families from the samplings were found; however, there were no cercariae in these 23 snail species. In the two rice fields, 11 snail species in 11 genera and 7 families were collected. In which, Bithynia siamensis in Ly Nhon rice field and Thiara scabra in Binh Khanh rice field infected xiphidio cercariae. For Bithynia siamensis, the prevalence in the dry season (5.6%) was higher than in the wet season (4.6%) ($P > 0.05$). In Binh Khanh rice field, only one Thiara scabra infected cercariae in the wet season. Further research on digenean infection in different months and in other water bodies in Can Gio Mangrove Biosphere Reserve should be done to contribute to the control of digeneans and reserve the biodiversity.

1. Introduction

Can Gio Mangrove Biosphere Reserve is located in Can Gio, the coastal district southeast of Ho Chi Minh City, Vietnam. The Biosphere Reserve was divided into three zones: the core zone (4720 ha) was for conservation, research, monitoring, and human activities were prohibited in this zone; the buffer zone (37,340 ha) was as a protective buffer for the core zone, and tourists could come to visit; the transition zone (29,310 ha) which surrounds the core and buffer zones was for socio-economic development (1). Therefore, Can Gio is a very different area from other places in Vietnam. The research in 2019 in this area finds that the digenean prevalence of wild fish in canal in Ho Chi Minh City is 80% [1], and Sea bass (*Lates calcarifer)* cultured in ponds in Can Gio Mangrove Biosphere infects digenean (metacercariae stage) with the prevalence at 0.8% [2]. However, no research on snails and cercariae in snails in this area is carried out.

The study on trematode is needed as foodborne zoonotic trematodiasis are a public health problem in many countries in the world [3], especially in Southeast Asia [4]. Humans are often infected by helminth parasites transmitted from fish [5] and crustaceans [6]. Freshwater snails play a role as the first intermediate host in the life cycle of digeneans. The distribution of foodborne trematodes depends on the relationship between the hosts, parasite, environment and eating habits [6]. For example, large liver flukes can cause

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disease in humans and livestocks [7]; common symptoms when infected with *Fasciola hepatica* are epigastric pain, upper abdominal pain, and malaise, even having fever and arthralgia [8], and fascioliasis can be associated with long-term complications such as anemia and malnutrition [7]. There is not much information about the clinical importance of infections with minute intestinal flukes, but heavy infections can cause serious gastrointestinal symptoms [9] while small liver flukes cause significant human-health impact and substantial clinical or sub-clinical disease [10]. For lung flukes, adult worms can cause focal haemorrhagic pneumonia when they migrate in the tissue [6].

Snails are the first intermediate hosts everywhere where trematodes are transmitted [11]. Snails belonging to the Lymnaeidae family are the first intermediate host of large liver fluke [7]. *Lymnaea viridis* and *Lymnaea swinhoei* are the intermediate hosts for *Fasciola gigantica* in Vietnam [12]. The natural snail host of *Paragonimus siamensis* in Thailand is *Filoparudina martensi* [13]. Two species of snails including *Oncomelania* sp.1 and sp.2 are the first intermediate hosts of *Paragonimus* in Vietnam [14]. Ten species of snails belonging to four families is the first intermediate host for *Clonorchis sinensis* [15]. The first intermediate snail species of *C. sinensis* in China, Taiwan, Korea and Japan is *Parafossarulus manchouricus* [16]. *Parafossarulus*, *Bythynia* and *Alocinma* spp. are the first intermediate snail host of *C. sinensis*, and *Bythynia* spp. is the host of *O. viverrini* and *Codiella* spp. for *Opisthorchis feliensis* [17]. Two species of *Bithynia* snails is found to be the first intermediate host of *Opisthorchis viverrini* in Thailand [18]. *Melanoides tuberculata, P. manchouricus* and *Maningilla* sp. are the first intermediate hosts of liver flukes in Vietnam, in which *M. tuberculata* is the most common species [19]. However, *M. tuberculata* and *Tarebia granifera* are not suitable first intermediate hosts of *C. sinensis* in Northern Vietnam [20]. *M. tuberculata, Thiara* and *Terabia granifera* are the first intermediate hosts of heterophyids [21]. *M. tuberculata* is the host of *Haplorchis pumilio* [22–24] and the host of *Centrocestus formosanus* [25]. *Thiara granifera* is found commonly infected with *H. pumilio* in Taiwan [23], *C. formosanus* in Thailand [24] and *Stellantchasmus falcatus* in Hawai [26]. *Pirenella* spp. of snails is the first intermediate host for *H. heterophyes* and *Semisulcospira* spp. provides the snail hosts for *M. yokogawai* [17]. Species of the Bithyniidae, the Stenothyridae and the Planorbidae dominated in rice fields and small canals, and *M. tuberculata* are found to be highest FZT prevalence among eight snail species [27].

In Vietnam, snail composition and distribution has been studied for a long time. There are 47 freshwater snail species in the North of Vietnam [28]; and sixteen snail species distributes in ponds, rivers, streams, canals and rice fields in two communes of Nghia Phu district, Nam Dinh province [27]. In the Central of Vietnam, 11 snail species is collected in An My and An Hoa communes, Tuy An district, Phu Yen province [29]. In the Mekong Delta of Vietnam, 14 freshwater snails is found in Vinh Long and Dong Thap provinces [30]. In 2015, 137 freshwater snail species have been recorded from Vietnam and 94 of them is assessed in IUCN list [31]. In Can Gio Mangrove Biosphere Reserve, 19 snail species belonging to 16 genera and 13 families are collected from 4 research points in the wet season 2010 and the dry season 2011. In which, total snail species in the dry season in Can Gio mangrove forest is higher than in the

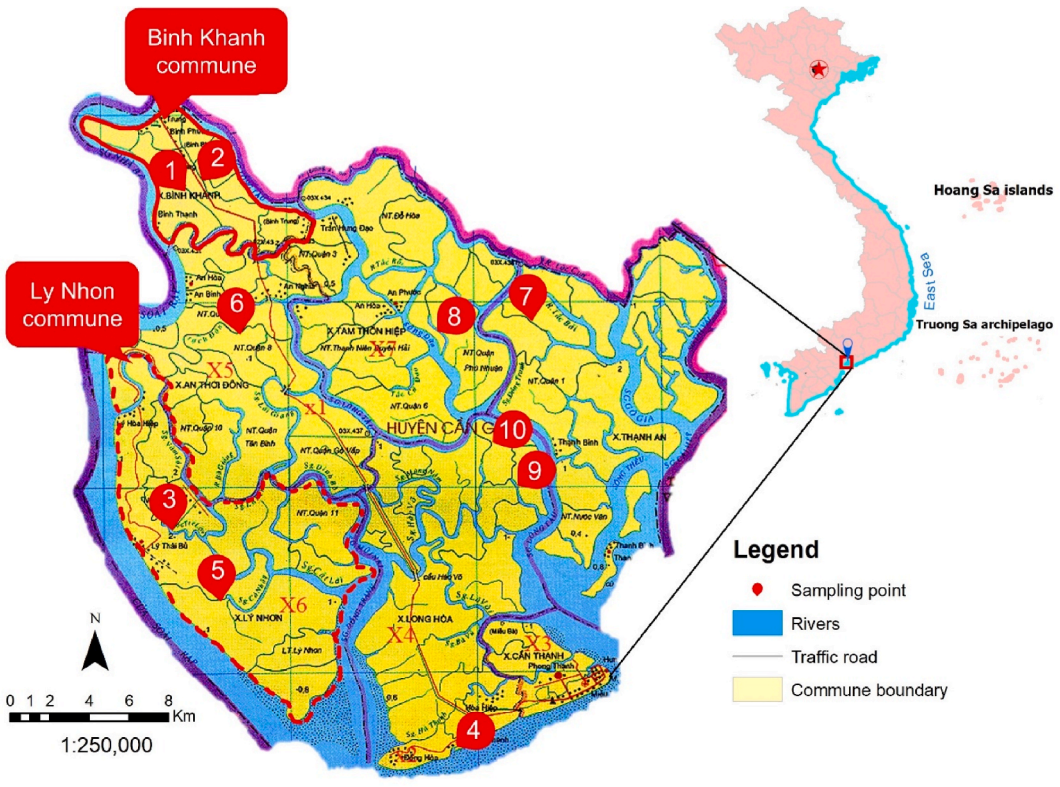

Fig. 1. Map showing location of the study area of Can Gio Mangrove Biosphere Reserve.

wet season with the highest number of snail species in the canal [32].

Trematode infections are found in eight snail species in snails in Nam Dinh province in the North of Vietnam [27]. The study in 2013 shows that the presence of cercariae in three snail species of *Bithynia fuchsiana*, *Parafossarulus striatulus* (the Bithyniidae) and *Melanoides tuberculata* (the Thiaridae) and records 12 cercariae species of 8 families [33]. Five groups of cercariae from 11 snail species were found in Phu Yen province in 2014 [29]. In 2015, three cercariae groups are recovered from 7 snail species, in which *Melanoides tuberculata* has the highest prevalence [34]. Nine snail species infected with cercariae in Ninh Binh province and Ha Noi city [35].

The above information showed that there were a lot of studies on snail composition and cercariae from snails in Vietnam. However, all this research was done in the agriculture and aquaculture areas in Phu Yen, Nam Dinh, Ninh Binh and Ha Noi, and there was no research in the Mangrove Biosphere Reserve in Can Gio, the first Mangrove Biosphere Reserve in Vietnam, where are rich in mangrove fauna comprising 210 species of planktonic and benthic organisms, 19 species of mammals, 133 species of birds, 283 species of fishes [36], 46 species of reptiles and amphibians [37], 204 species of crustaceans, gastropoda, bivalves, and 89 species of insects [38]. Therefore, a study to investigate the digenean infections in snails and snail composition in canals and rice fields in Can Gio Mangrove Biosphere, Ho Chi Minh City is implemented to contribute to the control of digeneans and conserve the biodiversity. The results can use to find the strategy to reduce the trematode prevalence in fish and the harmful snaisl for aquaculture and agriculture in Can Gio area.

2. Materials and methods

2.1. Study sites

The study site of Can Gio Mangrove Biosphere Reserve is in Can Gio district of Ho Chi Minh City, Vietnam (Fig. 1).

Snails were mainly distributed in canals, fishponds and rice fields with the highest trematode prevalence in small canals [39], so rice fields and canals were considering choosing for the study because most of ponds in Can Gio were for shrimp culture. According to Decision No 2580 by Ho Chi Minh city People's Committee dated July 20, 2021 [40], there were 10 sixth-level canals in Can Gio Mangrove Biosphere Reserve. The sixth-level canal was the lowest range in the waterway system in Ho Chi Minh city which was connected directly to the smaller canals linking with rice fields, fish farms, residential areas and wetlands. The water in all 10 sixth-level canals in Can Gio Mangrove Biosphere Reserve was brackish. For the rice fields, using completely rainwater, Decision No 2816 by Ho Chi Minh city People's Committee dated July 01, 2019 [41] showed that three communes and one town cultivated rice including Binh Khanh (69.58 ha), Ly Nhon (62.85 ha), An Thoi Dong (0.15 ha) and Can Thanh town (0.11 ha) in Can Gio. Therefore, ten sixth-level canals and two large rice fields of Binh Khanh and Ly Nhon were chosen for the research in January and June 2022 (Table 1).

2.2. Sampling of snails

Two cross-sectional studies on snails were carried out in January 2022 (the dry season) and in June 2022 (the wet season). Four samples from four different habitats, the natural environment where snails lived, were done in each canal and 15 samples were collected in each rice field. Water environment like temperature and pH at the sampling points in each season were measured.

In canals, quantitative snail sampling was done by using a 25-cm wide dredge to scrape the canal bottom from 1.0 m out from the bank and back to the edge. Each point was sampled once in the dry season and once in the wet season. Each sample was transferred to cloth bags and transported to the laboratory where they were analyzed within 24 h of collection. Four habitats for samling are different from each other [Fig. 2(a-d)]: the first point has no grass, no trees, and no aquatic plants (Fig. 2a); the second point has grass and aquatic plants but no trees (Fig. 2b); the third point has aquatic plants and trees on the canal bank but no grass (Fig. 2c); the fourth point has grass on the side of canal bank but no trees and no aquatic plants (Fig. 2d).

In rice fields, snails were collected by hand along the rice field bank in the area of 0.4 m wide × 10 m long, and each sampling point is 500 m far from each other. The first sampling point at the beginning of the main path at the edge of the rice field and the next

Table 1
Total sampled canals and rice fields in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam.

No	Name of canals/Rice fields	Zone	Sampling in the dry season (January 2022)	Sampling in the wet season (June 2022)
A	The sixth-level canals			
1	Tan canal - Bong Gieng Lon canal	Transition zone	4	4
2	Duoc canal - Sau Quan canal -Lap Doi canal	Transition zone	4	4
3	Goc Tre canal	Transition zone	4	4
4	Ca Nham Lon canal	Transition zone	4	4
5	Long Thanh canal	Transition zone	4	4
6	Mong Ga canal - Tac Moc Mu	Core zone	4	4
7	Don canal	Transition zone	4	4
8	Duoi Ca canal	Buffer zone	4	4
9	Tac An Tet - Tac Cau Kho canal	Buffer zone	4	4
10	Tac Cong canal	Core zone	4	4
B	Rice fields			
11	Binh Khanh	Transition zone	15	15
12	Ly Nhon	Transition zone	15	15

(a) (b) (c) (d)

Fig. 2. Examples of four different habitats for sampling.

sampling points were continued along this path.

2.3. Examination of snails for cecariae

Snails were examined for digenean infection (cercariae stage) by shedding method [27,42] in the containers of 250 mL beakers, and left for 24 h for shedding, and then checked in three days for cercariae at 8:00AM and 2:00PM each day. Each snail was kept separated in the different beakers filled with water and kept at the room temperature. All beakers were covered by a tray to prevent snails to escape. Water in each beaker was poured into the Petri dish and checked cercariae under the stereo miroscope and then optical microscope. Morphological methods were used to identify snails following the key by Dang et al. (1980) [28] and cercariae was identified following the keys by Frandsen and Christensen (1984) [42] and Shell (1985) [43]. Specimens of each snail species were preserved separately in the small plastic bottles filled with 70 percent ethanol and all cercariae were kept in the small bottles filled with 4 percent formalin, such that identifications could later be verified. All of these plastic bottles with specimens were kept in the laboratory of Department of Biology.

Table 2
Snail samples in canals and rice fields in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam (January and June 2022).

Family	Genus	Species	Total snails in canals		Total snails in rice fields	
			Jan	Jun	Jan	Jun
Pisaniidae	Cantharus	*Cantharus spiralis* (Gray, 1839)	7	0		
Ellobiidae	Cassidula	*Cassidula aurisfelis* (Bruguière, 1789)	2	9		
	Ellobium	*Ellobium chinense* (L. Pfeiffer, 1854)	0	36		
	Laemodonta	*Laemodonta punctatostriata* (H. Adams & A. Adams, 1854)	0	10		
	Pythia	*Pythia plicata* (Férussac, 1821)	0	7		
Potamididae	*Cerithidea*	*Cerithidea djadjariensis* (K. Martin, 1899)	4	2		
		Cerithidea cingulata (Gmelin, 1791)	6	9		
		Cerithidea obtusa (Lamarck, 1822)	0	46		
Cymatiidae	*Cymatium*	*Cymatium pileare* (Linnaeus, 1758)	10	14		
Muricidae	*Chicoreus*	*Chicoreus capucinus* (Lamarck, 1822)	1	58		
	Thais	*Thais gradata* (Jonas, 1846)	3	42		
Littorinidae	*Littoraria*	*Littoraria intermedia* (Philippi, 1846)	16	85		
		Littoraria mauritiana (Lamarck, 1822)	75	28		
		Littoraria melanostoma (Gray, 1839)	7	52		
		Littoraria scabra (Linnaeus, 1758)	79	77		
Assimineidae	*Omphalotropis*	*Omphalotropis rubens* (Quoy & Gaimard, 1832)	18	1		
Neritidae	*Neripteron*	*Neripteron pileolus* (Récluz, 1850)	15	14		
		Neripteron violaceum (Gmelin, 1791)	34	2		
	Neritina	*Neritina undata* (Lamarck, 1822)	11	10		
		Neritina canalis (G.B. Sowerby I, 1825)	19	71		
	Clithon	*Clithon oualaniense* (Lesson, 1831)	0	1		
Melongenidae	*Pugilina*	*Pugilina cochlidium* (Linnaeus, 1758)	19	0		
Assimineidae	*Sphaerassiminea*	*Sphaerassiminea miniata* (Habe, 1942)	0	16		
Bithyniidae	*Bithynia*	*Bithynia siamensis* (I. Lea, 1856)			162	87
Viviparidae	*Filopaludina*	*Filopaludina sumatrensis* (Dunker, 1852)			23	0
Lymnaeidae	*Lymnaea*	*Lymnaea viridis* Quoy & Gaimard, 1832			10	0
Planorbidae	*Gyraulus*	*Gyraulus convexiusculus* (T. Hutton, 1849)			4	0
Thiaridae	*Melanoides*	*Melanoides tuberculata* (O.F. Müller, 1774)			226	9
	Sermyla	*Sermyla tornatella* (I. Lea & H.C. Lea, 1851)			109	506
	Thiara	*Thiara scabra* (O.F. Müller, 1774)			98	27
Ampullariidae	*Pomacea*	*Pomacea canaliculata* (Lamarck, 1822)			381	477
	Pila	*Pila conica* (W. Wood, 1828)			484	19
Stenothyridae	*Stenothyra*	*Stenothyra messageri* (Bavay & Dautzenberg, 1900)			28	17
		Stenothyra sp.			37	12

2.4. 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 of digenean prevalence (cercariae stage) between two sampling times. A value of $P < 0.05$ was considered significant.

3. Results

Snail composition and distribution in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam.

The result from two samplings of snails in three zones of Can Gio Mangrove Biosphere Reserve showed that 34 snail species belonging to 27 genus and 17 families was collected and identified by morphological methods. In which, 23 snail species in 16 genus and 10 families was collected from 10 canals, and 11 snail species in 11 genus and 7 families was got from the two rice fields (Table 2). Total snail species were found differently in the three research zones with 11, 14 and 30 snail species in Core zone, Buffer zone and Transition zone, respectively.

A total of 3632 snails were collected in both of the dry season (January 2022) and the wet season (June 2022). The snail species had high numbers of occurrences such as *Pomacea canaliculata* (23.6%), *Sermyla tornatella* (16.9%), *Pila conica* (13.8%), *Bithynia siamensis* (6.9%) and *Melanoides tuberculata* (6.5%). The percentage of the other snails was less than 5.0 percent (Table 3).

3.1. Cercariae morphotypes infected in snails

Xiphidio cercariae (Fig. 3) was the only cercariae group recovered from both of two snail species of *Bithynia siamensis* (Fig. 4a) and *Thiara scabra* (Fig. 4b) in Ly Nhon and Binh Khanh rice fields. For *Bithynia siamensis* in Ly Nhon rice field, the prevalence in the dry season (5.6%) was higher than the wet season (4.6%); however, there was no significantly different from the prevalence between two seasons ($P > 0.05$) (Table 5). In Binh Khanh rice field, only one *Thiara scabra* was infected in the wet season. For the overall prevalence

Table 3
Percentage contribution of each snail species in canals and rice fields in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam in January and June 2022.

Snail species	Dry season (January 2022)		Wet season (June 2022)		Two seasons	
	Total	%	Total	%	Total	%
Cantharus spiralis	7	0.37	0	0.00	7	0.19
Cassidula aurisfelis	2	0.11	9	0.52	11	0.30
Ellobium chinense	0	0.00	36	2.06	36	0.99
Laemodonta punctatostriata	0	0.00	10	0.57	10	0.28
Pythia plicata	0	0.00	7	0.40	7	0.19
Cerithidea djadjariensis	4	0.21	2	0.11	6	0.17
Cerithidea cingulata	6	0.32	9	0.52	15	0.41
Cerithidea obtusa	0	0.00	46	2.64	46	1.27
Cymatium pileare	10	0.53	14	0.80	24	0.66
Chicoreus Capucinus	1	0.05	58	3.33	59	1.62
Thais gradata	3	0.16	42	2.41	45	1.24
Littoraria intermedia	16	0.85	85	4.87	101	2.78
Littoraria mauritiana	75	3.97	28	1.61	103	2.84
Littoraria melanostoma	7	0.37	52	2.98	59	1.62
Littoraria scabra	79	4.18	77	4.42	156	4.30
Omphalotropis rubens	18	0.95	1	0.06	19	0.52
Neripteron pileolus	15	0.79	14	0.80	29	0.80
Neripteron violaceum	34	1.80	2	0.11	36	0.99
Neritina undata	11	0.58	10	0.57	21	0.58
Neritina canalis	19	1.01	71	4.07	90	2.48
Clithon oualaniense	0	0.00	1	0.06	1	0.03
Pugilina cochlidium	19	1.01	0	0.00	19	0.52
Sphaerassiminea miniate	0	0.00	16	0.92	16	0.44
Bithynia siamensis	162	8.58	87	4.99	249	6.86
Filopaludina sumatrensis	23	1.22	0	0.00	23	0.63
Lymnaea viridis	10	0.53	0	0.00	10	0.28
Gyraulus convexiusculus	4	0.21	0	0.00	4	0.11
Melanoides tuberculate	226	11.97	9	0.52	235	6.47
Sermyla tornatella	109	5.77	506	29.01	615	16.93
Thiara scabra	98	5.19	27	1.55	125	3.44
Pomacea canaliculate	381	20.18	477	27.35	858	23.62
Pila conica	484	25.64	19	1.09	503	13.85
Stenothyra messageri	28	1.48	17	0.97	45	1.24
Stenothyra sp.	37	1.96	12	0.69	49	1.35
Total	1888	100	1744	100	3632	100

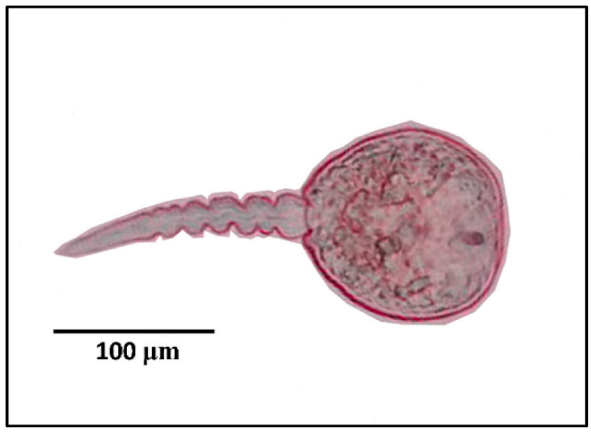

Fig. 3. Xiphidio cercariae.

a. **b.**

Fig. 4. Infected snails in the research. a. *Bithynia siamensis*. b. *Thiara scabra*.

in both two seasons for each snail species, *Bithynia siamensis* had the prevalence at 5.2 percent and *Thiara scabra* had the lower prevalence at 0.8 percent (Table 4).

pH of water at the sampling time in canals in the dry season was slightly higher than in the wet season. For the temperature in the dry season, it was also a little higher than in the wet season (Table 6).

4. Discussion

The research result provides more information about snail diversity in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh city, Vietnam. It is interesting that 23 snail species in the research canals are completely different from 11 snail species in rice fields. It can be explained that the research canals and rice fields do not link directly to each other. Therefore, the linked canals which connect the

Table 4
Number of infected snails in canals and rice fields in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam.

Species	In the dry season (January 2022)		In the wet season (June 2022)		In two seasons	
	Collected snails	Infected snails/ %	Collected snails	Infected snails/ %	Collected snails	Infected snails/ %
Bithynia siamensis (in Ly Nhon rice field)	162	9/5.6	87	4/4.6	249	13/5.2
Thiara scabra (in Binh Khanh rice field)	98	0	27	1/3.7	125	1/0.8

Table 5
Statistical analysis for the difference of prevalence for Bithynia siamensis between the dry and the wet seasons.

Chi-Square Tests					
	Value	df	Asymp. Sig. (2-sided)	Exact Sig. (2-sided)	Exact Sig. (1-sided)
Pearson Chi-Square	0.105[a]	1	0.746		
Continuity Correction[b]	0.001	1	0.980		
Likelihood Ratio	0.107	1	0.744		
Fisher's Exact Test				1.000	0.501
Linear-by-Linear Association	0.105	1	0.746		
N of Valid Cases	249				

[a] . 1 cells (25.0%) have expected count less than 5. The minimum expected count is 4.54.
[b] . Computed only for a 2 × 2 table.

Table 6
Environmental data measured at the sampling points in canals in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam.

Species	In the dry season (January 2022)		In the wet season (June 2022)	
	Min	Max	Min	Max
pH	6.5	7.0	6.0	7.0
Temperature (^{0}C)	27.5	29.0	27.0	29.0

research canals, and the rice fields should be studied in the future to find if these 34 snail species exist. In addition, water in canals is brackish while rice fields use complete rainwater, so snail species must be different. Total snail species in this research (34 snail species belonging to 28 genus and 17 families) is higher than the study in Can Gio Mangrove Biosphere Reserve in 2012 [32] with 19 snail species (16 snail species from the canal) in 16 genera and 13 families. The main reason for the difference is that the area in this study with 10 canals and two rice fields is larger than 4 research points in 2012 [32]. Moreover, the number of snail species might increase after the period of ten years. However, this can conclude only if the two studies are applied the same research method.

The dominant families of snails in the study are the Ampullariidae, the Thiaridae and the Bithyniidae. This result is similar to the finding by the previous researchers in Vietnam [39] that most snails (5629 snails of total 25,000 snails) are collected from small canals and rice fields belonging to the Thiaridae, and the Bithyniidae had the highest density of distribution in canals, rice fields and marslands [29]. However, snail species of *Pomacea canaliculata* belonging to the Ampullariidae which was not abundant in the previous study in rice fields [39] has the highest percentage in this research. The reason maybe that the rice fields in Can Gio Mangrove Biosphere Reserve are suitable for this species or the residents in Can Gio, the lowest population density in Ho Chi Minh city, are not interested in them; therefore, they do not get them for eating.

The collected snails from canals in this research have cercariae free whereas *Bithynia siamensis* and *Thiara scabra* from rice fields are infected with digeneans. No infected snails are found in the canals because water speed in all canals is high, so there is less chance for snails to eat digenean eggs like Heterophyidae or no opportunity for Sporocyst such as Echinostomatidae to get into snails if there are any. Moreover, snail species in the canals might not be suitable hosts for digeneans. Furthermore, there are less risks of digenean infections in the canal areas of Can Gio Mangrove Biosphere, so snails have no cercariae. In contrast, standing water in rice fields provides good environments for infection in snails if digenean eggs are available.

Pomacea canaliculata, Sermyla tornatella and *Pila conica* are particularly abundant in rice fields in this study, but all of them do not have any cercariae. Cercariae were not found in *Pomacea* sp. in Phu Yen province [29], but it was infected in Nam Dinh province [27]. *Pila polita* had cercariae free [27], but *Sermyla tornatella* infected Gymnocephalus (1,46%) and pleurolophocercariae (2,92%) [29]. *Thiara scabra* was infected digenean with the low prevalence at 0.8 percent, this number was much less than the research in Nam Dinh province [27] with the prevalence of 20.7 percent in fishponds, and the prevalence of 6.9 percent in Phu Yen province [29]. *Melanoides tuberculata*, the most important host for fishborne zoonotic parasites belonging to Heterophyidae, was plenty in small canals and *Bithynia siamensis*, a potential host for Heterophyidae and Opisthorchiidae, was found a lot in rice fields in the research in 2015 [39], but they occur less than 7.0 percent for each species in this research. *Melanoides tuberculata* and *Bithynia fuchsiana* was infected with the high prevalence in ponds and canals [27,29], in natural water bodies [34]; however, *Melanoides tuberculata* has cercariae free in this research and *Bithynia siamensis* has the highest overall prevalence at 5.2 percent. Therefore, the result in this research confirms again that *Bithynia* is easier to infect cercariae than the other sampled snails, and *Melanoides tuberculata* can infect digenean in most of the water bodies but it can have cercariae free in certain areas.

Only xiphidio cercariae are recovered from snail species of *Bithynia siamensis* (N = 13) and *Thiara scabra* (N = 1) in this study with the low numbers of cercariae per snail. Although this finding supports the result in 2005 [44] that xiphidio cercariae was the most prevalent in their research, and xiphidio cercariae was found only in *Thiara scabra* [29], this makes surprising because more than one morphotype of cercariae were found in the other studies in Vietnam in the previous years [27,30,34]. The explanation can be that Can Gio Mangrove Biosphere Reserve is separated from the other areas as it is like an island surrounded by water; therefore, digeneans from the other areas have not invaded into this Mangrove Biosphere Reserve yet. One more reason is that all water bodies are not examined, so other groups of cercariae in this area have not been recognized.

Some water environment factors were done during the research period. However, the collected data were not very different

between the dry and the wet seasons in the research canals. How pH and temperature in this study affect the distribution of snails and the digenean prevalence between the dry and the wet seasons can not be identified yet. Therefore, more research on the effects of water environment should be done more carefully and in more detailed from different months to find the clear answers for this issue.

Regarding the seasonality, snail populations were typically more abundant in the rainy season than in the dry season thanks to the good conditions for the multiplication of snails [45]. This is correct with the collected snails from the canals in this research. However, total numbers of snail samples in rice fields in the dry season in this research (1559 snails) is much higher than in the wet seasons (1145 snails). It seems that the seasonality does not affect the snail breeding in rice fields, especially the snail species of *Pila conica, Melanoides tuberculata, Bithynia siamensis* and so on in this study. More research should be done to get the answer to support this finding. The season also affects the digenean prevalence in snails in this research. Only one *Thiara scabra* is found Type 1 of x*iphidio cercariae* in the wet season; however, 13 snails of *Bithynia siamensis* is infected Type 2 of x*iphidio cercariae* in both of the two seasons, and the prevalence in the dry season is higher than in the wet season, but there is no significantly different ($P > 0.05$). The different digenean prevalence in this study is like the report in 2014 [29] that the occurrence of cercariae in snails was different among the months of the year and the prevalence in the dry season was higher than the wet season due to the high temperature. The seasonality of digenean prevalence observed in this study also agrees with the research results in 2005 [44] that the prevalence of cercariae was high in the dry season and decreased in the wet season. The findings of digenean prevalence in this area have not been very serious yet based on the research result of the low digenean prevalence in snails in this research and in cultured fish [1]. However, how many snails and fishes are infected digeneans in the other water bodies and how season affects the occurrence of digenean in snails and fish are not known clearly yet. Therefore, more research should be done in Can Gio Mangrove Biosphere Reserve to know the prevalence of cercariae in snails and in fish in different months of the year. Afterwards, the control strategy of digenean such as managing the infected snails and dangerous cercariae can be carried out in the area to contribute to food safety and hygiene, and human' s health in the future.

5. Conclusions

No cercariae were found in 23 collected snail species from ten canals in three research zones of Can Gio Mangrove Biosphere Reserve. In total 11 snail species collected from two rice fields, only *Bithynia siamensis* in Ly Nhon rice field and *Thiara scabra* in Binh Khanh rice field infected x*iphidio cercariae.* For *Bithynia siamensis*, the prevalence in the dry season (5.6%) was higher than in the wet season (4.6%) ($P > 0.05$). In Binh Khanh rice field, only one *Thiara scabra* infected cercariae in the wet season. Further research on digenean infection in different months and in other water bodies in Can Gio Mangrove Biosphere Reserve should be done to find the diversity of snails and their prevalence in the whole year to contribute to the control of digeneans and reserve the biodiversity.

The research identified the digenean prevalence and snail species composition collected in rice fields and small canals in Can Gio Mangrove Biosphere Reserve in January (the dry season) and June (the wet season) of the year 2022. However, this research still has some limitations. The research was conducted in two months of the two seasons, which provides a snapshot, but it can not fully capture the annual variation in snail populations and infection prevalence. Moreover, the study focused on small canals and rice fields may not capture the full diversity of snail species and digenean infections in the entire Mangrove Biosphere Reserve. Other habitats within the reserve may present different ecological dynamics. In addition, the study might not account for all environmental variables that could affect snail diversity and infection rates, such as water quality parameters, presence of predators, or human activities. For the identification, the morphological methods were used for snail and cercariae identification, but there were no applications of molecular techniques, which could be considered a limitation given that morphological identification can sometimes be less precise. Future research should consider all of these above limitations to find better results and carry out deeper statistical analysis when the results show more outputs.

Data availability statement

Data will be made available on request.

CRediT authorship contribution statement

Pham Cu Thien: Writing – review & editing, Writing – original draft, Validation, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Thi Duong Nguyen:** Writing – original draft, Formal analysis, Data curation.

Declaration of competing interest

Hereby the authors of the manuscript entitled "Digenean infections in snails (cercariae stage) in Can Gio Mangrove Biosphere Reserve, Ho Chi Minh City, Vietnam" would like to confirm that the authors declare no conflict of interest regarding the publication of this article.

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