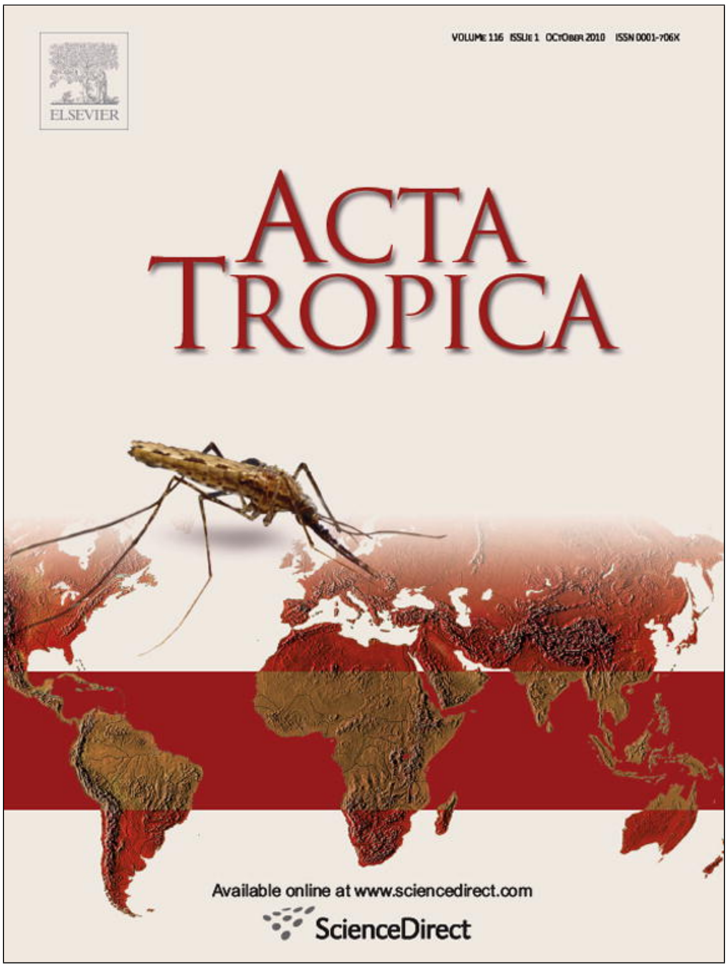
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Distribution of freshwater snails in family-based VAC ponds and associated waterbodies with special reference to intermediate hosts of fish-borne zoonotic trematodes in Nam Dinh Province, Vietnam

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ABSTRACT

Fish-borne zoonotic trematodes, such as *Clonorchis sinensis*, heterophyids and others, constitute a public health concern in parts of northern Vietnam and infections with these trematodes are often thought to be linked to fish culture. One common fish culture system is the integrated fish-livestock (VAC) ponds where individual households have 1 or more ponds. Fish fry, mainly of various carp species, produced in hatcheries, not necessarily local, are introduced into nursery ponds and after approximately 6 weeks, juvenile fishes are transferred to household ponds, referred to as grow-out ponds. Grow-out ponds are usually fertilized with organic debris, including animal excreta, to stimulate algal growth and subsequently fish growth. This paper describes the distribution of freshwater snails and occurrence of trematode infections in these in VAC ponds and associated habitats as part of a major study on risk factors of FZT infections in cultured fish in two communes, Nghia Lac and Nghia Phu, Nghia Hung District, Nam Dinh Province. The area is under intense rice cultivation with an extensive canal network supplying fields and also household VAC ponds. A total of 16 snail species was found and four were widely distributed i.e. *Angulyagra polyzonata*, *Melanoides tuberculata*, *Bithynia fuchsiana* and *Pomacea insularum*. Snail diversity and counts were higher in nursery ponds than in grow-out ponds. 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. Trematode infections were found in eight snail species and among these *M. tuberculata* had the highest overall prevalence of infection (13.28%). No trematode infections were found in species of the Viviparidae and Ampullaridae except for metacercariae. 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%). *Bithynia fuschiana* and *M. tuberculata* had the most diverse trematode fauna. *C. sinensis* was not recorded in this study. The VAC pond system in this area, is very important for transmission of minute intestinal trematodes while they play little role in transmission of *C. sinensis* as its intermediate hosts, bithynid snails, rarely occur in these ponds. From a public health perspective this is positive as the effects of infections with intestinal trematodes are considered mild. On the other hand it is possible that even such subtle effects could have importance in public health as transmission is very intense in the area. And this in combination with the aquaculture importance, reduced marketability of fishes with high metacercariae loads, warrants that control efforts against these trematodes are initiated to reduce transmission in this production system.

1. Introduction

Fish-borne zoonotic trematode (FZT) parasites are an increasing concern in both developing and developed countries (Verle et al., 2003; World Health Organization, 2004; Chai et al., 2005; Keiser and Utzinger, 2005, 2009). Diseases such as clonorchiasis, opisthorchiasis constitute important public health problems in several parts of Vietnam (De et al., 2003; Chai et al., 2005; Verle et al., 2003; Dung et al., 2007). In Nam Dinh, intestinal FZT have been reported from humans (Dung et al., 2007), domestic animals (Anh et al., 2009a) and freshwater farmed and wild fish (Phan et al., in press-a,b). Distribution and density of the first intermediate hosts,

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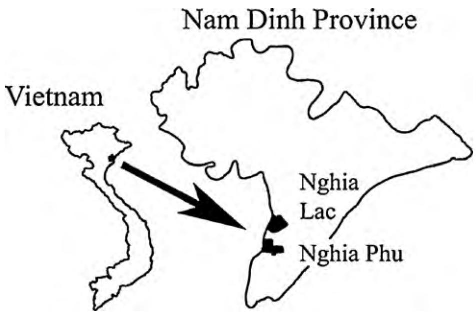

Fig. 1. Showing Nam Dinh Province and Nghia Lac and Nghia Phu communes.

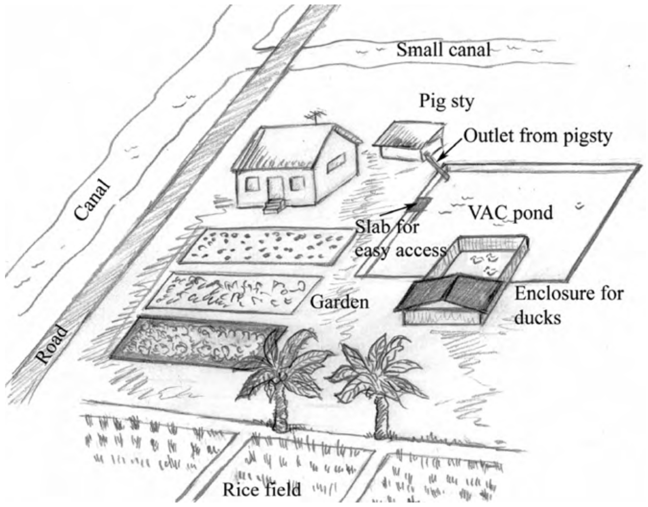

Fig. 2. Schematic representation of VAC pond.

species of freshwater snails, are important in determining transmission patterns of the trematodes (World Health Organization, 1995; Chai et al., 2005). Some studies on trematode infections in snails have been made in different parts of Vietnam. Thus, four snail species, *Lymnaea viridis*, *L. swinhoei*, *Parafossarulus striatulus* and *Melanoides tuberculata* were reported as intermediate hosts for trematodes infecting poultry (Le et al., 1990). *Lymnaea swinhoei* was found infected with six trematode species (Le and The, 1993; Le et al., 1995). Five cercariae types and 7 metacercaria types were found in *P. striatulus* (Le et al., 2000). In addition, snails of the family Viviparidae, which are often used for human consumption, may have high prevalence of metacercariae, i.e. 69.31% in *Angulyagra polyzonata*, 40.06% in *Cipangopaludina lecythoides* and 54.16% in *Sinotoia aeruginosa* (Le and The, 1993).

In Nghia Hung District, Nam Dinh Province, *C. sinensis* is present in people (Dung et al., 2007); the overall prevalence of small trematode eggs (*C. sinensis* and intestinal trematodes) in faecal samples was 61.5% and adult *C. sinensis* was expelled from 51.5% of 33 people with high (>1000 epg) faecal egg counts. Infections with these trematodes are often thought to be linked to fish culture (De et al., 2003). In this area, fish culture is based on the integrated fish-livestock (VAC) ponds where individual households have one or more ponds. Transmission of *C. sinensis* has been reported with *M. tuberculata* as the intermediate host in the Nam Dinh and Ninh Binh Provinces (Kino et al., 1998; De et al., 2003). This is rather unique as usually species of the Bithyniidae are intermediate hosts (Walker, 1927; Chung, 1984; Joo, 1980; World Health Organization, 1995; Rim, 2005; De et al., 2003; Zhang et al., 2007). In China, however, *M. tuberculata* is also recognized as intermediate host of *C. sinensis* (Lun et al., 2005) although Zhang et al. (2007) did not find it infected. The present study was undertaken in Nghia Lac and Nghia Phu communes to determine trematode infections in the freshwater snails, distribution and density of snails in VAC ponds as part of a major study on risk factors of FZT infections in cultured fish (Dung et al., 2007; Anh et al., 2009a; Phan et al., in press-a,b) and in other habitats in the area relation to selected environmental factors such as habitat and aquaculture practices.

2. Material and methods

2.1. Study area

Nam Dinh Province is a coastal province located at the south-east of the Red River delta (from 19°55′ to 20°16′ latitude north and from 106° to 106°33′ longitude east) and Nghia Lac and Nghia Phu communes are situated in the Nghia Hung District (Fig. 1). Each commune is subdivided into a number of hamlets (13 in Nghia Lac and 15 in Nghia Phu). In both communes the VAC system is very common. The VAC system is considered as an ecological way of using farm products in natural cycles (Tai et al., 2004). Since 1989, the VAC system has been encouraged by the Vietnamese government and has had remarkable results from economic, health and nutrition perspectives (Hop, 2003). In the traditional form, a garden (Vuon), a fish pond (Ao Ca) and a cattle shed (Chuong) constitute a functional unit (Fig. 2). However, V has been extended to include all kinds of land farming and C all husbandry activities, including raising cattle, pigs and poultry (Hop, 2003). In the study area pigs and poultry are the main husbandry activities. Manure from the husbandry is used to fertilize ponds, so as to stimulate algal growth and subsequently fish growth, and remnants from garden products and fish remains can be fed to the pigs. Mud from the VAC pond is used to fertilize gardens or fields. The traditional VAC system is widely practiced in Nam Dinh Province but since husbandry is considered as the main income source, the system has been modified such that many animals (mainly pigs, chicken and ducks) are maintained and ponds become overloaded with manure from the husbandry. In some households, domestic waste water and latrine flush enters into the ponds as well. In Nam Dinh, VAC ponds are commonly associated with rice growing and water can be exchanged between rice fields and ponds. A common practice in VAC ponds, is addition of floating aquatic macrophytes (for example duck weed) collected from other habitats as fish feed and this was identified as risk factor for infection in fish (Phan et al., in press-b). Similarly, ducks in VAC ponds may be fed snails, primarily viviparid or ampullarid snails, collected from outside the VAC pond and this was a risk factor for infection in ducks (Anh et al., 2010). Snails may be crushed before fed to the ducks.

Some ponds are nursery ponds where fry from hatcheries are kept for a period before introduced into grow-out ponds at household level. Nursery ponds resemble grow-out ponds but are usually less loaded with organic material. The main nursing season is from February to May and fish species are nursed in succession in the same pond for about 6 weeks per species and ponds are emptied and refilled between the different nursing cycles. The main water source in Nghia Lac is from two rivers, Day and Ninh Co rivers, forming the western and eastern boundaries, respectively and in Nghia Phu from only Day River (along its western boundary). The climate is wet-hot tropical monsoon and the annual average temperature range from 23 to 24 °C. The coldest months are December and January with average temperatures from 16 to 17 °C; and the hottest July with mean temperatures of about 29 °C. Mean annual rainfall is 1750–1800 mm.

This study was a cross-sectional study conducted from July to September 2006 to determine the occurrence of snails in VAC ponds as part of a major study on risk factors of FZT infections in cultured

Table 1
Proportion of sites with snails of different species by commune and habitat. *p*-values are from logistic regression analysis adjusting for clustering within hamlets and with both commune and habitat as predictors.

	Total	Nghia Lac	Nghia Phu	*p*-Value[a]	Large canal	Small canal	Rice field	Pond	*p*-Value[b]
Total sites	211	121	90		15	38	43	109	
Neritidae									
Neritina violacea	0.01	0.00	0.02		0.07	0.00	0.02	0.00	
Thiaridae									
Thiara scabra	0.10	0.07	0.14	ns	0.20	0.05	0.02	0.14	<0.05
Tarebia granifera	0.01	0.02	0.01	ns	0.07	0.00	0.00	0.02	ns
Melanoides tuberculata	0.41	0.45	0.36	ns	0.73	0.58	0.49	0.29	<0.001
Sermyla tornatela	0.04	0.04	0.03	ns	0.13	0.05	0.00	0.04	ns
Ampullaridae									
Pila polita	0.01	0.00	0.02		0.00	0.00	0.00	0.02	
Pomacea canaliculata	0.14	0.20	0.06	<0.05	0.07	0.34	0.19	0.06	<0.001
Pomacea bridgesi	0.26	0.36	0.12	<0.05	0.40	0.47	0.37	0.13	<0.001
Viviparidae									
Idiopoma umblicata	0.01	0.02	0.00		0.00	0.03	0.02	0.00	
Angulyagra polyzonata	0.54	0.56	0.51	ns	0.33	0.53	0.28	0.71	<0.001
Bithynidae									
Bithynia fuchsiana	0.21	0.28	0.11	ns	0.00	0.24	0.72	0.04	<0.001
Stenothyridae									
Stenothyra messageri	0.08	0.08	0.07	ns	0.00	0.24	0.12	0.02	<0.001
Planorbdae									
Polypylis haemispherula	0.05	0.07	0.01	ns	0.00	0.08	0.14	0.01	<0.001
Gyraulus convexiusculus	0.06	0.07	0.03	ns	0.00	0.13	0.14	0.01	<0.001
Lymnaeidae									
Lymnaea swinhoei	0.00	0.01	0.00		0.00	0.00	0.00	0.01	
Lymnaea viridis	0.04	0.02	0.06	ns	0.00	0.05	0.07	0.03	<0.001
No. of species	15	14	13		7	12		14	

[a] Comparison between communes.
[b] Comparison among habitats (river sites excluded).

fish (Dung et al., 2007; Phan et al., in press-a,b; Anh et al., 2009a,b) but additionally we selected sites in all available habitats (rivers, streams, canals of various sizes, drainage canals, ponds of various types and rice fields). Sites were as far as possible selected such that all hamlets were represented by one or more sites.

Snail sampling was conducted by the same person in all sites during the morning hours for 30 min per site using scooping (Madsen et al., 1987) and/or hand-picking. Other parameters recorded at each site were water level and degree of cover of vegetation such as floating plants (i.e. *Eichhornia*, *Pistia*, *Nymphaea*), emergent plants (i.e. water-taro, *Colocasia esculenta*, and various grasses). Degree of cover was estimated to the following classes, (0) not seen, (1) less than 5%, (2) 5–25%, (3) 25–50%, (4) 50–75%, (5) more than 75% (Madsen et al., 1987). For ponds, farmers were questioned about fish stocking (species of fish) and pond maintenance history, i.e. when ponds were last emptied, treated with lime and sometimes other chemicals (for example chlorine or cupper sulphate).

Snails were transported to a temporary laboratory in the area where they were identified according to keys by Brandt (1974) and Thanh (1980). Identification of *Pomacea* was based on Thanh et al. (2003), except that *P. bridgesi* should rightly be *P. insularum*. According to Hayes et al. (2008), *P. bridgesi* is not found in Asia and in Vietnam only *P. canaliculata* and *P. insularum* are found. *Sermyla tornatella* is used by Thanh (1980) but according to Brandt (1974) this is a junior synonym of *S. riquetii*. Snails were then examined for trematode infections using one or more of three methods depending on snail size, i.e. shedding, crushing and cutting. For the shedding method (Frandsen and Christensen, 1984) smaller snails were placed individually in small plastic containers with 5 ml of tap water and left for 24 h for shedding. This procedure was also used for larger snails except they were kept in larger containers. Description and identification of cercariae was always based on cercariae that had been retrieved by shedding. The crushing method involved crushing whole snails between two glass plates, while cutting (only done on large snails) involved cutting open the shell and transferring tissue parts and haemolymph to a glass slide. All samples were checked under stereomicroscope for cercariae, redia and/or sporocysts; metacercariae were occasionally observed but not specifically checked for. Identification of cercariae to major level was done using live and unstained cercariae specimens observed under microscope Olympus-CH40. Cercariae were identified according to the keys of Ginetsinskaya (1988) and Schell (1985).

2.2. Statistical analysis

Occurrence (present/not present) of the various snail species was analysed using logistic regression using commune, habitat, and vegetation cover as predictors after adjusting for possible clustering within hamlets. Similarly, infections in snails were analysed using logistic regression using snail species and habitat as predictors. Snail counts were compared between communes and habitats after adjusting for random effects within hamlets using count models (Hilbe, 2008). In these analyses, we first used Poisson regression which involves estimation of one parameter, the mean (μ). If the variance (V) was greater than μ (data overdispersed), we would try to model using negative binomial regression where the variance is modelled as $V = \mu + \alpha \times \mu^2$. The ancilliary parameter (α) was estimated for each species separately (only for the most abundant species) using full maximum likelihood estimation as described in Hilbe (2008). The ancillary parameter was then entered into a Generalized Linear Model and model fit was assessed using dispersion statistics to check for overdispersion and deviance and Anscombe

Table 2
Number of sites from where different snail species were collected (total number of sites sampled = 211), total number of snails collected, arithmetic mean counts in these sites and maximum snail number collected from a single site.

Species	Number of sites	Total snails collected	Mean	Maximum number in one site
Neritina violacea	2	5	2.5	4
Thiara scabra	21	805	38.3	324
Tarebia granifera	3	34	11.3	27
Melanoides tuberculata	86	3335	38.8	693
Sermyla riquetii	8	112	14.0	62
Pila polita	2	2	1.0	1
Pomacea canaliculata	29	198	6.8	30
Pomacea insularum	54	632	11.7	200
Idiopoma umbilicata	2	3	1.5	2
Angulyagra polyzonata	114	3472	30.5	400
Bithynia fuchsiana	44	1672	38.0	244
Stenothyra messageri	16	200	12.5	52
Polypylis hemisphaerula	10	182	18.2	53
Gyraulus convexiusculus	12	208	17.3	79
Lymnaea swinhoei	1	1	1.0	1
Lymnaea viridis	8	17	2.1	4

residuals to check for outliers (see Hilbe, 2008). If negative binomial count models showed signs of overdispersion (dispersion statistic > 1.00), which could arise if the data contain more zero counts than predicted by the negative binomial model, we would try zero-inflated negative binomial regression (Hilbe, 2008). In zero-inflated negative binomial regression zero counts and non-zero counts are modelled separately, and we used the Vuong test (Hilbe, 2008) to test whether the zero-inflated model was superior to the negative binomial regression. All analysis was done in Stata 10 and differences with probability value of $p < 0.05$ were considered significant.

3. Results

3.1. Diversity and distribution of snails

A total of 15 species were found (Table 1). Especially, *M. tuberculata*, *A. polyzonata*, *Bithynia fuchsiana*, and *Pomacea insularum*, were common. Some species such as *Thiara scabra*, *Sermyla riquetii*, *Tarebia granifera*, *Stenothyra messageri*, *Gyraulus convexiusculus*, were found sporadically. No snails were found in the river sites. The greatest number of species was found in ponds, but this could be a result of the larger representation of this habitat. A. polyzonata was particularly common in ponds and small canals, while *M. tuberculata* tended to be more common in canals than in ponds but not statistically significant. *B. fuchsiana* was especially common in rice fields. Pulmonate snails were more commonly found in small canals and rice fields than in other habitats.

Eichhornia crassipes and *Pistia stratiotes* were the most common floating aquatic macrophytes in the area. They were found in 64 and 51 sites, respectively and could be found in most habitats. Very few associations between presence of snails and these two macrophyte species were statistically significant after adjusting for habitat, commune and random effects within hamlets, i.e. *A. polyzonata* was positively associated with presence of *E. crassipes* and *T. scabra* with *P. stratiotis* ($p < 0.05$). Especially VAC ponds had high density of floating vegetation and *A. polyzonata* was associated with medium densities of floating vegetation (all types combined). Emergent vegetation included primarily marginal vegetation, such as grasses, *Typha* sp. and others, but none showed any association with snail occurrence.

Snail counts varied greatly among sites for all species (Table 2). Especially, *M. tuberculata*, *A. polyzonata*, *T. scabra*, *B. fuchsiana* and *P. insularum* were occasionally found in great numbers (Table 2). *T. scabra* was recorded in high numbers in large canal sites although this high count was caused by a single site. Comparison of counts of the most common snail species between areas and habitats using negative binomial regression produced models that still had evidence of over dispersion. Relatively few significant effects were found. For *M. tuberculata* there was no effect of habitat or commune and the best model was negative binomial; although there was some evidence of over dispersion, zero-inflated models did not work better. Zero-inflated negative binomial regression showed that *A. polyzonata* was found in higher numbers in ponds than in other habitats ($p < 0.001$). *B. fuchsiana* occurred in higher numbers in rice fields than in other habitats ($p < 0.001$) and there was no effect of commune. Zero inflation was primarily caused by habitat.

In total, 10 nursery ponds and 99 grow-out ponds were included. The total snail counts in nursery ponds were higher than in grow-out pond (Table 3). For *A. polyzonata* the counts were greater in grow-out ponds while *M. tuberculata* was found in much greater numbers in nursery ponds, but this was partly due to one site.

The grass carp (*Ctenopharyngodon idellus*), silver carp (*Hypophthalmichthys harmandi*) and mud carp (*Cirrhina molitorella*) were very common in these ponds. A few ponds (1 in Nghia Lac and 3 in Nghia Phu) were reported to have been stocked with specimens of the black carp, *Mylopharyngodon piceus*, which is known to prey on snails, and they were introduced for snail control. In the 3 ponds in Nghia Phu, 6 specimens of *A. polyzonata* were the only snails found while in the pond in Nghia Lac, 96 specimens of *A. polyzonata*, 40 *M. tuberculata*, 18 *T. scabra*, and 1 *B. fuchsiana* were found. Presence of the black carp in these ponds was not verified and size of the black carp specimens was not informed.

3.2. Trematode infection

A total of 10 cercariae types were recorded (Fig. 3) and some dimensions of these are summarised in Table 4 and their presence in snails is summarised in Table 5. Cercariae were only identified to major type and more specific identification was not made, so these groups could well comprise of more species. Furcocercous cercariae (Strigea) (Fig. 3a) were found only in *G. convexiusculus* in a small canal. Monostome cercariae (Fig. 3b) were found in 6 VAC

Table 3
Mean snail counts in 99 grow-out ponds and 10 nursery ponds.

	Average per pond	
	Grow-out	Nursery
Thiara scabra	2.8	17.9
Tarebia granifera		3.1
Melanoides tuberculata	5.3	99.6
Pomacea sp.	2.6	0.1
Angulyagra polyzonata	26.3	7.8
All species	37.7	132.1

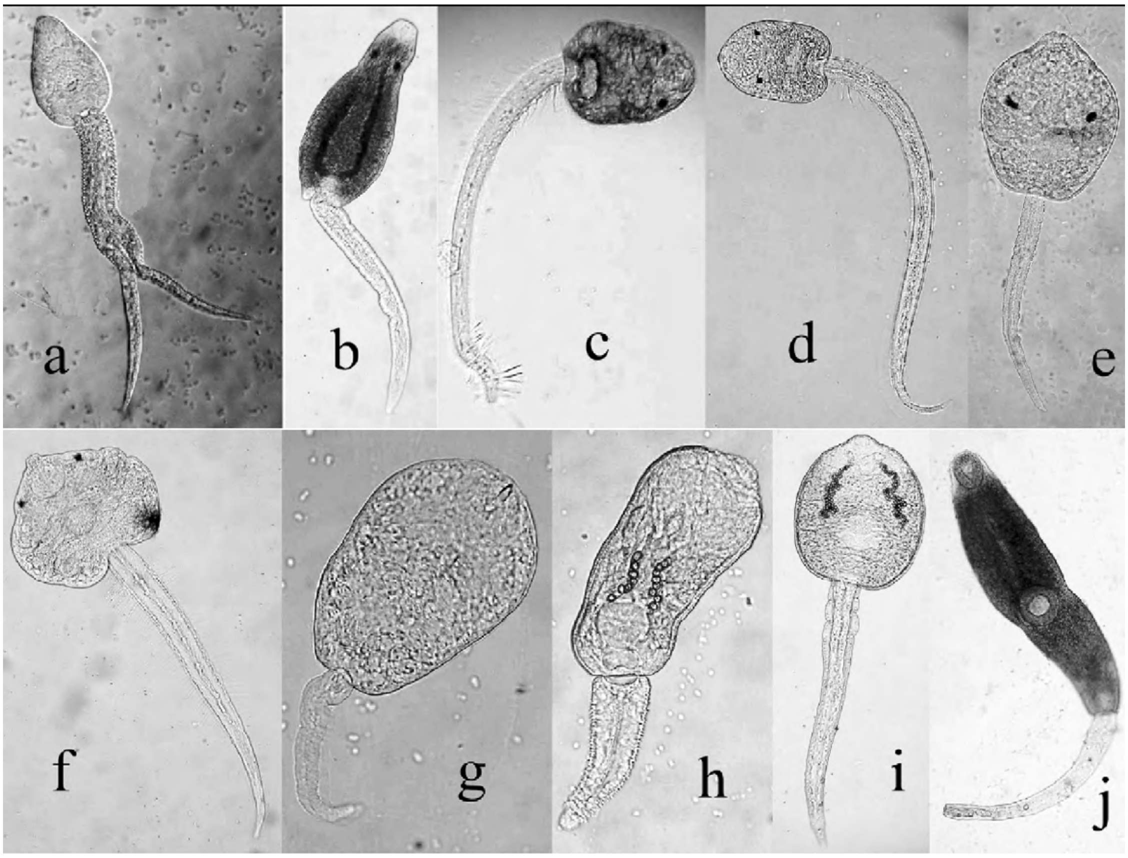

Fig. 3. Cercariae found in Nam Dinh Province, Vietnam, i.e. furcocercous (a), monostome (b), parapleurolophocercous (c, d and f), pleurolophocercous (e), xiphidiocercariae (g), echinostome (h and i) and gymnocephalous (j). Dimensions are given in Table 4.

ponds and 3 small canals in *M. tuberculata*, *T. granifera*, *S. messageri*, *G. convexiusculus*. Adult cercaria have three prominent eyespots. Free cercariae lost their tails and encysted. Three types of parapleurolophocercous cercariae (Fig. 3c, d, and f) were recorded. Type 1 was found occasionally in *B. fuchsiana* and *T. scabra* (1 specimen of each species), but *M. tuberculata* was the main intermediate host. The tail has long lateral fin folds until the tip of tail. The ventral sucker is vestigial. Type 1 was found in all habitats but it was less common in VAC ponds than in the three other habitats (OR = 0.31, $p < 0.001$). Type 2 was common in *M. tuberculata* and *S. riquetii* in all habitats but was occasionally found in *T. scabra* and *S. messageri*. It was more common in small canals than in other habitats (OR = 5.06, $p < 0.001$). This type is similar to type 1 except that it is smaller.

Table 4
Measurements of cercariae (μm) found in Nam Dinh Province. Letters in brackets refer to photographs in Fig. 2.

Cercariae	Body length	Body width	Tail length
Furcocercous (a)	160–180	72–73	200–240
Monostome (b)	312–376	144	416–432
Parapleurolophocercous			
Type 1 (c)	200–204	72–76	440–456
Type 2 (d)	112–136	52–68	288–304
Type 3 (f)	84–104	52–66	138–150
Pleurolophocercous (e)	188–192	112	nd
Xiphidio cercaria (g)	112–126	68–72	102–124
Echinostome			
Type 1 (h)	134–136	56–60	92–100
Type 2 (i)	272	204	320
Gymnocephalous (j)	472–480	128–144	nd

nd: not done.

Type 3 was found in *M. tuberculata* in only 3 sites, i.e. 2 rice fields and 1 VAC pond. The tail has long lateral fin folds until the tip of tail while the dorso-ventral fin folds are short. One type of pleurolophocercous cercariae was found in one small canal site in *B. fuchsiana* and *S. messageri*. Xiphidiocercariae (Fig. 3g) were common in all habitats and found primarily in *M. tuberculata* and *B. fuchsiana*, but were found also in *S. riquetii* and *S. messageri*. Differences between habitats were not significant, while this type of cercariae was more likely to be found in *B. fuchsiana* than in *M. tuberculata* (OR = 2.01, $p < 0.001$). Two types of echinostome cercariae, one large and one small (see Table 4), were found (Fig. 3h and i). Type 1 (small) was very common and was found in all habitats but was particularly common in large canals and least so in VAC ponds. Type 2 was found in *G. convexiusculus* and *L. swinhoei* and was found only in 1 VAC pond. Gymnocephalous cercariae (Fig. 3j) were found in *M. tuberculata* and *T. scabra*. Cyst glands fill the whole body and the bifurcation of the gut is clearly visible just anterior to the ventral sucker. This is similar to that described for *Philophthalmus* (Urabe, 2005).

The most common cercariae constituting more than 40% of all cercariae recorded were those that use fish as second intermediate host, i.e. the parapleurolophocercous (three types) and pleurolophocercous cercariae. The echinostomes was the second most common type (35%). The echinostome type 1 was found in seven snail species and *M. tuberculata* was the snail species having the most diverse trematode fauna (Table 5). Parapleurolophocercous and pleurolophocercous cercariae also have multiple hosts (as a group) within the families Thiaridae, Bithynidae and Stenothyridae and cercariae are produced in all habitats (Table 6). Thiarid snails (*Thiara* and *Melanoides*) were more common in nursery ponds than in grow-out ponds and were the only species found with infec-

Table 5
Number of sites where cercariae of various types were found by snail species.

Species	Total sites with snail species	No. of snails collected	No. of snails infected	Echino-stome 1	Echino-stome 2	Furco-cercous	Gymnoce-phalous	Monos-tome	Parapleurolo-phocercaria 1	Parapleurolo-phocercaria 2	Pleurolopho-cercaria	Parapleurolo-phocercaria 3	Xiphidiocer-cariae	No of trematode species found
Thiara scabra	21	805	74	3	0	0	1	1	1	1	0	0	0	5
Tarebia granifera	3	34	2	1	0	0	0	1	0	0	0	0	0	2
Sermyla tornatella	8	112	23	2	0	0	0	0	0	3	0	0	1	3
Melanoides tuberculata	86	3335	441	29	0	0	3	5	24	25	0	3	12	7
Bithynia fuchsiana	44	1672	74	7	0	0	0	0	1	0	1	0	14	4
Stenothyra messageri	16	200	13	0	0	0	0	1	0	2	1	0	1	4
Gyraulus convexiusculus	12	208	12	1	1	1	0	1	0	0	0	0	0	4
Lymnaea swinhoei	1	1	1	1	0	0	0	0	0	0	0	0	0	1
Total		6367	640	222	2	1	4	40	121	133	2	4	111	
Relative contribution (%)				34.7	0.3	0.2	0.6	6.3	18.9	20.8	0.3	0.6	17.3	
No. of sites and species				44	1	1	4	9	26	31	2	3	28	
No. of intermediate hosts				7	1	1	2	5	3	4	2	1	4	

tions by parapleurolophocercous cercariae in these ponds (Table 7). The prevalence of infections with these trematodes did not differ significantly between nursery and grow-out ponds.

4. Discussion

Two snail species, *A. polyzonata* and *M. tuberculata* are the most frequent species in the area, and both species are very common in VAC ponds. Snail distribution and density did not correlate with aquatic vegetation and this is probably because these habitats are highly dynamic and pond or canal cleaning can cause great reductions in snail density. Sometimes sites contained plenty of empty shells which would suggest that the site might be a good habitat, but snail populations had been reduced temporarily. The main factors affecting snail density would appear to be related to the agricultural/aquacultural practices including use of different chemicals, some of which could have some molluscicidal activity, for example lime.

The golden apple snails, *P. insularum* and *P. canaliculata* are not very abundant in this area which is in contrast to the findings by Thanh et al. (2003), that these snails are very abundant in rural areas of Vietnam. In the study area, *Pomacea* snails are manually collected from the rice fields as part of care for young rice plants. The snails may be killed or fed to ducks.

Snail density is considerably higher in nursery ponds than in grow-out ponds. In nursery pond, the water is clearer than in grow-out ponds, where the feeding activities of the larger fish could contribute to the turbidity but also the organic loading will increase plankton growth. Fish in nursery ponds are probably too small to cause any physical effect on snails in the ponds, while in grow-out ponds some of the larger fish species, even if they do not feed on snails, could cause damage or disturbance when probing various items (including snails) for suitability as food. Nursery ponds are cleaned more often than grow-out ponds that are usually not cleaned more than once per year. The clearer water in nursery ponds could be favourable to the snails, but the larger exchange of water in nursery ponds (during the main nursing period, nursery ponds are emptied and refilled up to three times) could also secure greater contamination of ponds with snails and cercariae from the sites from where water is supplied. It has been shown in different areas that fishes receive a major portion of their metacercarial load during the nursery period (Thien et al., 2009; Phan et al., in press-a,b).

In grow-out ponds, presence of the black carp seems to reduce snail density although the evidence collected from this study is only circumstantial. The black carp is a well known predator of snails (Rothbard and Rubinshtein, 1999; Venable et al., 2000; Ben-Ami and Heller, 2001) and it is introduced by some farmers in VAC ponds for snail control. The actual presence of the black carp in these VAC ponds was not confirmed, just based on interviewing farmers. Further studies to evaluate the effect of the black carp are recommended.

The most common trematode species recorded as metacercariae in fish from aquaculture systems in Vietnam include *Haplochis pumilio*, *H. taichui*, *H. yokogawai*, *Centrocestus formosanus*, *Stellanchasmus falcatus*, *Echinochasmus japonicus* (Thien et al., 2007; Thu et al., 2007; Chi et al., 2008; Phan et al., in press-a). All these parasite species, except *E. japonicus*, produce cercariae of the parapleurolophocercous or pleurolophocercous type. We did not identify the cercariae to species and Skov et al. (2008) also found three types of paraplerolophocercous cercariae in Nam Dinh but could not relate them to metacercariae found in fish based on morphology alone. PCR analysis on cercariae similar to our type 1 showed it to be *H. pumilio* (Skov et al., 2008). Van et al. (2009) showed that *H. taichui* and *H. pumilio* can be identified unambiguously using PCR

Table 6
Contribution of various snail families to transmission of parapleurolophocercous cercariae in different habitats.

Family	Large canal (n = 15)			Small canal (n = 38)			Rice field (n = 43)			Pond (n = 109)		
	No. of sites[a]	No. of snails[b]	Prevalence (%)	No. of sites	No. of snails	Prevalence (%)	No. of sites	No. of snails	Prevalence (%)	No. of sites	No. of snails	Prevalence (%)
Thiaridae	17	31.6	2.4	27	30.0	15.4	22	41.3	6.2	53	38.6	2.6
Bithyniidae	0			9	11.1	2.0	31	50.1	0	4	5	0
Stenothyridae	0			9	20.6	5.9	5	1.8	0	2	3	0

[a] No. of sites where snails were found and checked for cercariae.
[b] No. of snails per standard sample in sites where found.

Table 7
Total number of host snails sampled per 30 min search per site, infected snails and prevalence of infection in 99 grow-out ponds and 10 nursery ponds.

	Total no. host snails collected (in all sites)		Prevalence (%) Parapleurolophocercous cercariae		Prevalence (%) other cercariae	
	Grow-out	Nursery	Grow-out	Nursery	Grow-out	Nursery
Thiaridae	8.2	123.5	2.7	2.5	2.3	5.3
Bithynidae	0.2	0	0		20	
Ampullaridae	2.8	0.1	0	0	0	0
Viviparidae	5.3	99.6	0	0	0	0
Other	0.3	0.1	0	0	7.4	0

from any life-stage, including the cercarial stage that is difficult to identify using morphology. Although the liver fluke *C. sinensis* is found in people in Nam Dinh (Dung et al., 2007), we did not find its cercariae during this study. This suggests that *C. sinensis* is less associated with aquaculture in this area than believed (De et al., 2003).

Parapleurolophocercous cercariae were recorded from snail species belonging to three families, i.e. Thiaridae (*T. scabra*, *S. riquetii* and *M. tuberculata*), Bithynidae (*B. fuchsiana*) and Stenothyridae (*S. messageri*). The most important host, however, is *M. tuberculata* due to its great abundance in the area. *T. granifera* was not found infected, but this species was only found in 3 sites. Heterophyid infections have been reported from this species elsewhere (Abbott, 1952). Although *B. fuchsiana* and *S. messageri* were found in rice fields and ponds, we only found them infected with heterophyid trematodes in small canals. *B. fuchsiana* is widely distributed in the area and this species can function as intermediate host of *C. sinensis* (Müller et al., 2007). In this study, we did not report *P. striatulus*, which is known as host of *C. sinensis*, but later surveys have shown it to be present in the area and occasionally at high density. Previous reports of very high prevalence of infection by *C. sinensis* in *M. tuberculata* (De et al., 2003) probably are based on misidentification of cercariae. According to Schell (1985) only pleurolophocercous cercariae are produced within the families Opistorchidae and the Cryptogonimidae, while both parapleurolophocercous and pleurolophocercous cercariae are found within the Heterophyidae (intestinal trematodes). We found that parapleurolophocercous constituted 40.3% of all infections found in snails while pleurolophocercous only constituted 0.3%, but these could thus potentially belong to three different families. Those that we have found resemble that described for *C. formasanus* (Lo and Lee, 1996). One species, however, belonging to the Cryptogonimidae, *Exorchis* sp. (not zoonotic), has been found as metacercariae in fish in this area (unpublished). Clearly, further studies using molecular techniques for identifying cercariae are required to elucidate the host–parasite relationships in this area. Although cercariae of *C. sinensis* resemble those of the intestinal trematodes, it is possible to morphologically distinguish them; according to Ngo (2003) cercariae of *C. sinensis* have clear transverse bands at anterior part of the tail. Usually, prevalence of infection by *C. sinensis* in snails is quite low, i.e. less than a few percent (Choi et al., 1975; Joo, 1980; Chung et al., 1980; Chen, 1991). Other members of the Bithynidae such as *Bithynia misella* (Soh, 1978) and *Aloncinma longicornis* (Lun et al., 2005) may also play a role. Chung (1984), however, failed to infect *B. misella* with *C. sinensis* experimentally.

Cercariae of FZT species are produced in small and large canals, rice fields and VAC ponds and hence fish originating from any of these habitats could be infected. Since most ponds receive water from canals, there is a possibility that cercariae of FZT species can enter with water but how important this source of cercariae is for infection of fishes in ponds, remains to be evaluated. The VAC system, however, often allows exchange of water between the VAC pond and an associated rice field and this might be another important source of cercariae. Hung et al. (2009) reported that density of heterophyid infected snails was a poor predictor of infections with these trematodes in juvenile fishes in nursery ponds. Infection level in fish could be very high even when density of infected snails was low (Hung et al., 2009). This means that control measures against the intermediate host snails should not be limited to the VAC ponds but should include neighboring habitats and further density of the intermediate hosts should be reduced to very low levels before an effect on transmission can be anticipated.

We did not find any cercariae shedding from species of the Viviparidae and Ampullaridae, but we found metacercariae in some species of the Viviparidae. Le and The (1993) also found metacercariae in these two families. In other areas of Asia viviparid species have been reported as hosts for species of the Echinostomatidae (Burch and Lohachit, 1983; Tropmed Technical Group, 1986). Also species of the Echinostomatidae are classified as FZT (Graczyk and Fried, 1998) and people can become infected by eating insufficiently cooked snails. Therefore, supposedly these snail species, at least in some areas, should be included as targets for control. According to Thanh et al. (2004), species of the Viviparidae are widespread in Vietnam.

Pulmonate snail species are not commonly found in VAC ponds in Nam Dinh. VAC ponds are often heavily loaded by organic material and the bottom sediment very soft and unsuitable for pulmonate snails although they could attach themselves to floating plants, the fish probing the plants for food items or feeding on the plants (grass carp) may prevent pulmonate snails from establishing. In conclusion, the VAC ponds undoubtedly are transmission sites for FZTs, but the VAC system is a very dynamic system where surrounding habitats can contribute significantly to infection in fishes in these ponds. We think that studies into the quantitative aspects of the allochthonous sources of cercariae should be conducted as they are relevant for interventions against the FZT.

In general, the clinical symptoms due to heterophyid infections in people are mild and transient; unless hosts are heavily infected or immunocompromised, the most prominent symptoms are malabsorption and diarrhea (see review in Toledo et al., 2006). This is in marked contrast to the severe pathology caused by the liver flukes, *Opistchorchis vivirini*, *O. felinus* and *C. sinensis* (Sripa et al., 2007). From a public health point of view this is a positive finding, but even these minor effects might be important as transmission of the intestinal trematodes is very intense. The presence of intestinal trematodes also complicates diagnosis of infections with *C. sinensis* by faecal examination as the eggs of the heterophyids ressemble those of the liver flukes. From an aquaculture perspective, the intense transmission of intestinal trematodes is important as it reduces marketability of the fishes, especially for export. This in combination with the potential effects of infections (even if subtle) in people warrant that the intestinal trematodes should be controlled in the VAC culture system.

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References

Abbott, R.T., 1952. A study of an intermediate snail host (*Thiara granifera*) of the oriental lung fluke (*Paragonimus*). Proc. U.S. Nat. Mus. 102, 71–116.

Anh, N.T.L., Madsen, H., Dalsgaard, A., Phuong, N.T., Thanh, D.T.H., Murrell, K.D., 2010. Poultry as reservoir hosts for fishborne zoonotic trematodes in Vietnamese fish farms. Vet. Parasitol. 169, 391–394.

Anh, N.T.L., Phuong, N.T., Johansen, M.V., Murrell, K.D., Van, P.T., Dalsgaard, A., Thu, L.T., Thamsborg, S.M., 2009a. Prevalence and risks for fishborne zoonotic trematode infections in domestic animals in a highly endemic area of North Vietnam. Acta Trop. 112, 198–203.

Anh, N.T.L., Phuong, N.T., Murrell, K.D., Johansen, M.V., Dalsgaard, A., Thu, L.T., Chi, T.T.K., Thamsborg, S.M., 2009b. Animal reservoir hosts and fish-borne zoonotic trematode infections on fish farms, Vietnam. Emerg. Infect. Dis. 15, 540–546.

Ben-Ami, F., Heller, J., 2001. Biological control of aquatic pest snails by the black carp *Mylopharyngodon piceus*. Biol. Control 22, 131–138.

Brandt, A.M.R., 1974. The non-marine aquatic Mollusca of Thailand. Ach. Molluskenk. 105, 1–247.

Burch, J.B., Lohachit, C., 1983. Snails of medical importance in Thailand. Walkerana. Trans. POETS Soc. 1, 395–398.

Chai, J., Murrell, K.D., Lymbery, A.J., 2005. Fish-borne parasitic zoonoses: status and issue. Int. J. Parasitol. 35, 1233–1254.

Chen, E.R., 1991. Clonorchiasis in Taiwan. Southeast Asian J. Trop. Med. Public Health 22 (Suppl.), 184–185.

Chi, T.T.K., Dalsgaard, A., Turnbull, J.F., Tuan, P.A., Murrell, K.D., 2008. Prevalence of zoonotic trematodes in fish from a Vietnamese fish farming community. J. Parasitol. 94, 423–428.

Choi, D.W., Chung, B.J., Ahn, D.H., Lee, D.M., 1975. *Clonorchis sinensis* in Kyungpook Province, Korea. 1. Distribution and demonstration of the cercaria of *Clonorchis sinensis* from snail. Parafossarulus manchouricus Bourgingat. Korean J. Parasitol. 13, 133–138.

Chung, P.-R., 1984. A. comparative study of three species of Bithyniidae (Mollusca: Prosobranchia): *Parafossarulus manchouricus*, *Gabbia misella* and *Bithynia tentaculata*. Malacol. Rev. 17, 1–66.

Chung, B.J., Joo, C.Y., Choi, D.W., 1980. Seasonal variation of snail population of *Parafossarulus manchouricus* and larval trematode infection in River Kumho, Kyungpook Province, Korea. Korean J. Parasitol. 18, 54–64.

De, N.V., Murrell, K.D., Cong, L.D., Cam, P.D., Chau, L.V., Toan, N.D., Dalsgaard, A., 2003. The food-borne trematode zoonoses of Vietnam. Southeast Asian J. Trop. Med. Public Health 34, 12–34.

Dung, D.T., De, N.V., Waikagul, J., Dalsgaard, A., Chai, J.-Y., Sohn, W.-M., Murrell, K.D., 2007. Fishborne zoonotic intestinal Trematodes, Vietnam. Emerg. Infect. Dis. 13, 1828–1833.

Frandsen, F., Christensen, N.Ø., 1984. An introductiontory guide to the identification of cercariae from African freshwater snails with special reference to cercariae of trematode species of medical and veterinary importance. Acta Trop. 41, 181–202.

Ginetsinskaya, T.A., 1988. Trematodes, their Life Cycles, Biology and Evolution. Amerind Publishing Company, Pvt, New Delhi (translation of the original Russian edition, 1968).

Graczyk, T.K., Fried, B., 1998. Echinostomiasis: a common but forgotten food-borne disease. Am. J. Trop. Med. Hyg. 58, 501–504.

Hayes, K.A., Joshi, R.C., Thiengo, S.C., Cowie, R.H., 2008. Out of South America: multiple origins of non-native apple snails in Asia. Divers. Distrib. 14, 701–712.

Hilbe, J.M., 2008. Negative Binomial Regression. Cambridge University Press, Cambridge, UK.

Hop, L.T., 2003. Programs to improve production and consumption of animal source foods and malnutrition in Vietnam. J. Nutr., Suppl., 4006S–4009S.

Hung, N.M., Khue, N.V., Clausen, J., Murrell, K.D., Van, P.T., Dalsgaard, A., The, D.T., Madsen, H., 2009. Density of snails infected with heterophyids as a predictor of such infections in fish cultured in nursery ponds in northern Vietnam. In: 6th Seminar on Food- and Water-borne Parasitic Zoonoses (FBPZ6) Incorporating the 3rd International Meeting on Gnathostomiasis, 2–4 December, Bangkok, Thailand, p. 27.

Joo, C.-Y., 1980. Epidemiological studies of *Clonorchis sinensis* in vicinity of River Taewha, Kyungnam Province, Korea. Korean J. Parasitol. 18, 199–214.

Keiser, J., Utzinger, J., 2005. Emerging foodborne trematodiasis. Emerg. Infect. Dis. 11, 1507–1514.

Keiser, J., Utzinger, J., 2009. Food-borne trematodiases. Clin. Microbiol. Rev. 22, 466–483.

Kino, H., Inaba, H., De, N.V., Chau, L.V., Son, D.T., Hao, H.T., Toan, N.D., Cong, L.D., Sano, M., 1998. Epidemiology of clonorchiasis in Ninh Binh Province, Vietnam. Southeast Asian J. Trop. Med. Public Health 29, 250–254.

Le, N.T., Doanh, P.N., The, D.T., 2000. Trematode larvae in the snail *Parafossarulus striatulus* (Bithyniidae) collected from Ba Vi and Ngoc Tao areas of Ha Tay. J. Biol. 22, 15–21 (in Vietnamese with English abstract).

Le, N.T., The, D.T., 1993. The larvae of trematodes in the species of snail belonging to families Pilidae and Viviparidae in Ha Tay and Hoa Binh Provinces. In: Research work collection on Ecology and Biological Resources (1990–1992). Science and Technology Publishing House, Ha Noi, pp. 318–323 (in Vietnamese with English abstract).

Le, N.T., The, D.T., Ngai, D.D., 1990. Examination of freshwater snails as intermediate hosts of trematodes, causing trematodosis in poultry. In: Research work collection on Ecology and Biological Resources. Science and Technology Publishing House, Ha Noi, pp. 164–169 (in Vietnamese with English abstract).

Le, N.T., The, D.T., Ngo, H.D., 1995. The larvae of trematodes and cestodes in snails, *Lymnaea* (Lymnaeidae). J. Biol. 17, 11–18 (in Vietnamese with English abstract).

Lo, C.-T., Lee, K.-M., 1996. Infectivity of cercariae of *Centrocestus formasanus* and *Haplorchis pumilio* (Digenea: Heterophyidae) in *Cyprinus carpio*. Zool. Stud. 35, 305–309.

Lun, Z.R., Gasser, R.B., Lai, D.H., Li, A.X., Zhu, X.Q., Yu, X.B., Fang, Y.Y., 2005. Clonorchiasis: a key foodborne zoonosis in China. Lancet Infect. Dis. 5, 31–41.

Ngo, H.D., 2003. Research on distribution of small liver fluke (*Clonorchis sinensis*) in two provinces, Nam Dinh and Ninh Binh, and suggestions of some methods to reduce *C. sinensis* transmission. Project report. Association of Science and Technology, Vietnam (in Vietnamese).

Madsen, H., Coulibaly, G., Furu, P., 1987. Distribution of freshwater snails in the river Niger basin in Mali with special reference to the intermediate host of Schistosomes. Hydrobiology 146, 77–88.

Müller, B., Schmidt, J., Mehlhorn, H., 2007. Sensitive and species-specific detection of *Clonorchis sinensis* by PCR in infected snails and fishes. Parasitol. Res. 100, 911–914.

Phan, V.T., Ersbøll, A., Bui, T., Nguyen, H., Murrell, D., Dalsgaard, A., in press-a. Fish-borne zoonotic trematodes in cultured and wild-caught freshwater fish from the Red River Delta, Vietnam. Vector Borne Zoonotic Dis. doi:10.1089/vbz.2009.0134.

Phan V, Ersbøll AK, Nguyen K, Madsen H, Dalsgaard A., in press-b. Farm-level risk factors for fish-borne zoonotic trematode infection in integrated small-scale fish farms in Northern Vietnam. PLoS Negl. Trop. Dis.

Rim, H.J., 2005. Clonorchiasis: an update. J. Helminthol. 79, 269–281.

Rothbard, S., Rubinshtein, I., 1999. The black carp, a potential biocontrol of molluscs. Isr. J. Aquacult. Bamidgeh 51, 74–83.

Schell, S.C., 1985. Trematodes of North America (north of Mexico). University Press of Idaho.

Skov, J., Kania, P.W., Dalsgaard, A., Jørgensen, T.R., Buchmann, K., 2008. Life cycle stages of heterophyid trematodes in Vietnamese freshwater fishes traced by molecular and morphometric methods. Vet. Parasitol. 160, 66–75.

Soh, C.T., 1978. Snail-mediated parasites in Korea. Yonsei Rep. Trop. Med. 9, 1–10.

Sripa, B., Kaewkes, S., Sithithaworn, P., Mairiang, E., Laha, T., Smout, M., Pairojkul, C., Bhudhisawasdi, V., Tesana, S., Thinkamrop, B., Bethony, J.M., Loukas, A., Brindley, P.J., 2007. Liver Fluke induces cholangiocarcinoma. PLoS Med. 4, 1148–1155.

Tai, M.V., Thuyet, B.D., Nam, T.H., Binh, N.D., Chuong, N.Q., 2004. Aquaculture practices in NamDinh province. Report from Fibozopa Project. Research Institute of Aquaculture No. 1, TuSon Bach Ninh.

Thanh, D.N., 1980. Identification of Freshwater Invertebrates in Northern Vietnam. Science and Technology Publisher, pp. 440–490 (in Vietnamese).

Thanh, D.N., Hai, H.T., Cuong, D.N., 2003. Species components of the family Ampullariidae Gray, 1824 in Vietnam. J. Biol. 25, 1–5 (in Vietnamese with English abstract).

Thanh, D.N., Hai, H.T., Cuong, D.N., 2004. Viviparidae-Gastropoda in Vietnam. J. Biol. 26, 1–5 (in Vietnamese with English abstract).

Thien, P.C., Dalsgaard, A., Bui, N.T., Olsen, A., Murrell, K.D., 2007. Prevalence of fishborne zoonotic parasites in important cultured fish species in the Mekong Delta, Vietnam. Parasitol. Res. 101, 1277–1284.

Thien, C.P., Dalsgaard, A., Nhan, N.T., Olsen, A., Murrell, K.D., 2009. Prevalence of zoonotic trematode parasites in fish fry and juveniles in fish farms of the Mekong Delta, Vietnam. Aquaculture 295, 1–5.

Thu, N.D., Loan, T.T., Dalsgaard, A., Murrell, K.D., 2007. Survey for zoonotic liver and intestinal trematode metacercariae in cultured and wild fish in An Giang Province, Vietnam. Korean J. Parasitol. 45, 45–54.

Toledo, J.R., Esteban, G., Fried, B., 2006. Immunology and pathology of intestinal trematodes in their definitive hosts. Adv. Parasitol. 63, 285–365.

Tropmed Technical Group, 1986. Snails of medical importance in Southeast Asia. Southeast Asian J. Trop. Med. Public Health 17, 282–322.

Urabe, M., 2005. Cercaia of species of *Philophthalmus* detected in a freshwater snail. Semiculcospira libertine, in Japan. Parasitol. Int. 54, 55–77.

Van, K.V., Dalsgaard, A., Blair, D., Le, T.H., 2009. *Haplorchis pumilio* and *H. taichui* in Vietnam discriminated using ITS-2 DNA sequence data from adults and larvae. Exp. Parasitol. 123, 146–151.

Venable, D., Gaude, A.P., Klerks, P., 2000. Control of the trematode *Bolbophorus confusus* in channel catfish ponds using salinity manipulation and polyculture with black carp *Mylopharyngodon piceus*. J. World Aquacult. Soc. 31, 158–166.

Verle, P., Kongs, A., De, N.V., Thieu, N.Q., Depraetere, K., Kim, H.T., Dorny, P., 2003. Prevalence of intestinal parasitic infections in northern Vietnam. Trop. Med. Int. Health 8, 961–964.

Walker, B., 1927. Studies on *Clonorchis sinensis* (Cobbold). VI. The molluscan hosts of *Clonorchis sinensis* (Cobbold) in Japan, China and Southeastern Asia, and other species of molluscs closely related to them. Am. J. Hyg. 8, 208–284.

World Health Organization, 1995. Control of foodborne trematode infections. WHO Tech. Rep. Ser. 849. Geneva, Switzerland, World Health Organization.

World Health Organization, 2004. Report of Joint WHO/FAO Workshop on Food-borne Trematode Infections in Asia. Ha Noi, Vietnam, 26–28 November, WHO Rept. Ser. no. RS/2002/GE/40(VTN).

Zhang, R., Gao, S., Geng, Y., Huang, D., Yu, L., Zhang, S., Cheng, J., Fu, Y., 2007. Epidemiological study on *Clonorchis sinensis* infection in Shenzhen area of Zhujiang delta in China. Parasitol. Res. 101, 179–183.