TREMATODE INFECTION OF FRESHWATER SNAIL, FAMILY BITHYNIIDAE IN THAILAND

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Abstract. *Opisthorchis viverrini* is restricted to and requires for its aquatic life cycle only *Bithynia* snail as first intermediate host but many species of cyprinid fish as second intermediate hosts. A survey in Thailand of trematode infection in freshwater snails of the family Bithyniidae carried out during October 2008 - July 2009 found a total of 5,492 snails, classified into ten species distributed in various geographic areas. *Bithynia funiculata* and *Gabbia pygmaea* were localized to the north, *B. s. goniomphalos*, *Wattebledia siamensis* and *W. crosseana* to northeast and *B. s. siamensis*, *Hydrobioides nassa* and *G. wykoffi* to central region. *W. baschi* and *G. erawanensis* was found only in the south and Erawan waterfall, Kanchanaburi Province, respectively. Trematode infection rate was 3.15%. Cercariae were identified as belonging to six types, namely, amartae , monostome, mutabile, *O. viverrini*, virgulate, and unknown. The prevalence of cercarial infection in *B. s. goniomphalos* of amartae, mutabile, *O. viverrini*, virgulate, and unknown type cercaria was 0.55%, 0.74%, 1.07%, 2.87%, and 0.37%, respectively, and in *B. s. siamensis* monostome (1.10%) and virgulate (0.55%). Only virgulate cercariae were shed from *W. crosseana* (3.85%) and *W. siamensis* (5.19%). Cercariae of the unknown type were found in *G. wykoffi* (1.69%). No infection of *O. viverrini* cercariae was detected in the other species.

Keywords: *Opisthorchis viverrini*, cercarial type, family Bithyniidae, snail, Thailand

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INTRODUCTION

Freshwater snails of the family Bithyniidae serve as intermediate hosts for the multiplication of intramolluscan stages of many types of trematodes. Snails in this family were first described for native snails in Europe and Asia in the early

1870s (Brandt, 1974; Mills *et al*, 1994). A number of species have been reported as intermediate hosts for trematode parasites (Wykoff *et al*, 1965; Chitramvong and Upatham, 1989; Ellen *et al*, 2008).

In Thailand, three genera of Bithyniidae were classified by Brandt (1974), viz. *Bithynia* (2 subgenera of *Digoniostoma* and *Gabbia*), *Hydrobioides* and *Wattebledia*. Chitramvong and Upatham (1989) classified *Gabbia* as a valid genus. According to Chitramvong (1991, 1992), 10 species/subspecies, namely, *Bithynia funiculata, B. siamensis goniomphalos*, *B. s. siamensis*, *Gabbia wykoffi*, *G. pygmaea*, *G. erawanensis*, *Wattebledia crosseana*, *W. siamensis, W. baschi* and *Hydrobioides nassa* make up the family Bithyniidae as identified by morphological characteristics of shells, internal organs and radula cusp patterns. Their ecological habitats have been reported in various types of water bodies and a number of species share the same ecological niches. Within Bithyniidae, only the genus *Bithynia* has been identified as being of medical importance (Wykoff *et al*, 1965; Harinasuta *et al*, 1984; WHO, 1995; Waikagul, 1998; Sri-aroon *et al*, 2005).

In Thailand, two species of snails, *B. funiculata* and *B. siamensis* (2 subspecies of *B. s. goniomphalos* and *B. s. siamensis*, respectively) have been reported to serve as the first intermediate hosts of human liver fluke, *Opisthorchis viverrini* (Wykoff *et al*, 1965; Brockelman *et al*, 1986; Sri-aroon *et al*, 2005). Opisthorchiasis is believed to predispose the onset of cholangiocarcinoma , a cancer of the bile duct (Thamavit *et al*, 1978; Haswell-Elkins *et al*, 1992; IARC, 1994; Sithithaworn *et al*, 1994; Vatanasapt *et al*, 2000; Watanapa and Watanapa, 2002; Honjo *et al*, 2005; IARC, 2011). *O. viverrini* is a medically important parasite of humans in the Greater Mekong subregion (Cambodia, Lao People's Democratic Republic, Thailand and Vietnam) (IARC, 1994; Keiser and Utzinger, 2005; IARC, 2011). The aquatic life cycle of *O. viverrini* requires *Bithynia* snail as the first intermediate host and many species of cyprinid fish as second intermediate hosts (Wykoff *et al*, 1965; Harinasuta *et al*, 1984; WHO, 1995; Waikagul, 1998). In the endemic regions of northeast Thailand, the prevalence of *O. viverrini* infection in humans and cyprinid ranges from 2.1% to 97% (Brockelman *et al*, 1986; Haswell-Elkins *et al*, 1992; Sriamporn *et al*, 2004), but is lower in snail hosts (0.083%-1.6%) (Wykoff *et al*, 1965; Upatham and Sukhapanth, 1980; Upatham *et al*, 1983). Moreover, epidemiological surveys during 1981-1991 indicated that there is an increase in incidence of human *O. viverrini* infection in the north and central regions of the country, whilst in the northeastern region the incidence is decreasing due in part to the implementation of a drug control strategy (Jongsuksuntigul and Imsomboon, 1998).

Experimental infection studies showed that *B. funiculata* and *B. s. siamensis* have higher susceptibility to *O. viverrini* than *B. s. goniomphalos*, and within the same taxon immature laboratory bred snails are more susceptible to *O. viverrini* infection than mature field snails (Chanawong and Waikagul, 1991). Trematode infection in *B. s. goniomphalos* showed that it could serve as an intermediate host for 7 types of cercariae (amartae, amphistome, furcocercariae, monostome, *O. viverrini,* virgulate and xiphidiocercaria) when shedding is induced by illumination (Adam *et al*, 1993; Nithiuthai *et al*, 2002; Sri-aroon *et al*, 2005). Four types of cercariae (lophocercous, monostome, pleurolophocercous and virgulate) were found in *B. funiculata* in the north but no *O. viverrini* infection was detected (Ngern-klun *et al*, 2006).

There is no report of human liver

fluke, *O. viverrini*, or other cercarial infections in the other species of family Bithyniidae apart from the genus *Bithynia* even though they are in the same family and share the same ecological niche. The present study examined the prevalence of trematode infections particularly *O. viverrini*, in snail species of the family Bithyniidae in various localities in Thailand.

MATERIALS AND METHODS

Snail collection

The study localities for snail collection were in the north, south, west, northeast and central areas of Thailand. The locations were selected based on information from previous reports (Brandt, 1974; Chitramvong, 1989, 1992; Nithiuthai *et al*, 2002; Ngern-klun *et al*, 2006). The snail survey was conducted during October 2008 to July 2009. The locations of snail collection were recorded using the Global Positioning System (Garmin model Nuvi 203, Taiwan) and plotted on a map (Fig 1). All snails were collected by wire-mesh scoop or by hand, and each specimen was cleaned, air-dried, labeled and kept in porous plastic bags. The snail samples were brought back to the laboratory to be examined for trematode infection. Snails were identified based on shell morphology for species following available keys and descriptions (Brandt, 1974; Upatham *et al*, 1983; Chitramvong, 1992).

Examination for trematode infection

Trematode infection of snails were conducted using a cercarial shedding method (day time and night time shedding) as follows. Snails were placed in plastic containers with 50 ml of dechlorinated tap-water, exposed to electric light for 2 hours for day time shedding, or were covered with a black plastic sheet overnight at room temperature for night time shedding. Cercariae were preserved in 10% formalin and then observed under a stereo microscope (40x magnification). Snail specimens were observed for emerging cercariae at least 2 times within one month. Morphology study of cercariae was undertaken using both unstained and stained [with 0.1% (w/v) Fast Red B or 0.1% (w/v) Malachite Green dye] under a light microscope and photographed. Cercaria types were identified based on available morphological description (Wykoff *et al*, 1965; Schell, 1970; Frandsen and Christensen, 1984). For confirmation of *O. viverrini* cercariae, species specific PCR was used (Wongratanacheewin *et al*, 2001).

RESULTS

A total of 5,492 snails were collected from 17 locations of Thailand: Nong Khai, Nong Bua Lam Phu, Khon Kaen, Maha Sarakham, Yasothon, and Roi Et in the northeast; Chiang Mai, Lamphun in the north; Phitsanulok, Phichit, Nakhon Sawan, Sing Buri, Saraburi, Suphan Buri, Bangkok in central; Kanchanaburi in the west; and Surat Thani in the south (Fig 1). Snails were classified into ten species/subspecies in family Bithyniidae (Fig 2). The distribution of the snails were as follows: *B. funiculata* ($n = 120$) and *G. pygmaea* (120) from the north; *B. s. goniomphalos* (2,159), *W. siamensis* (154) and *W. crosseana* (104) from the northeast; *B. s. siamensis* (1,813), *H. nassa* (160) and *G. wykoffi* (592) from central; *W. baschi* (150) from the south and *G. erawanensis* (120) from Erawan waterfall, Kanchanaburi Province in the west (Table 1).

No night time cercarial shedding was observed. *B. s. goniomphalos*, *B. s. siamensis*, *G. wykoffi*, *W. crosseana* and *W. siamensis* shed cercariae in day time, with 6 types of cercariae, namely, amartae, monostome,

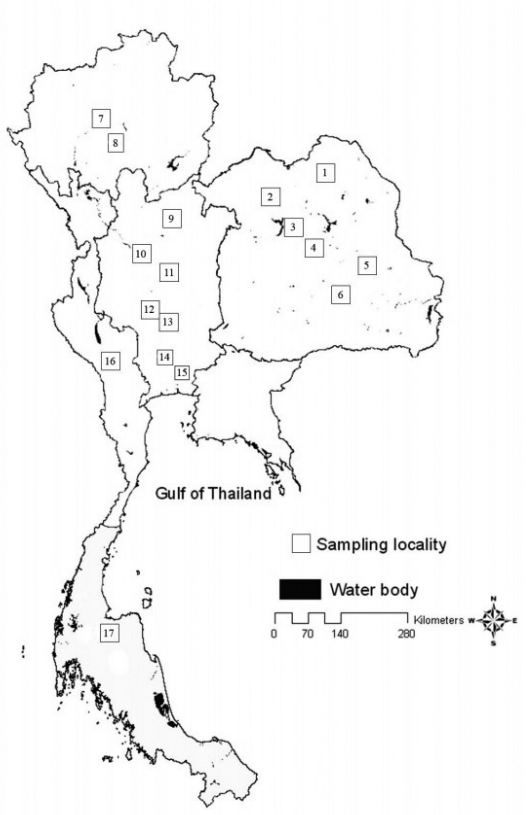

Fig 1–Map of Thailand showing collection localities in five regions of Thailand. 1, Nong Khai; 2, Nong Bua Lam Phu; 3, Khon Kaen; 4, Maha Sarakham; 5, Yasothon; 6, Roi Et; 7, Chiang Mai; 8, Lamphun; 9, Phitsanulok; 10, Phichit; 11, Nakhon Sawan; 12, Sing Buri; 13, Saraburi; 14, Suphan Buri; 15, Bangkok; 16, Kanchanaburi; 17, Surat Thani.

mutabile, *O. viverrini*, virgulate, and unknown type cercaria, being identified (Fig 3). Identification of *O. viverrini* cercariae was verified by PCR amplification of a 330 bp fragment (data not shown) (Wongratanacheewin *et al*, 2001).

The morphological characteristics of the cercaria types are as follows. Amartae cercaria has oral and ventral suckers of equal sizes or ventral sucker that is larger than the oral sucker, a tail without a dorsoventral fin fold, and no virgule organ. Monostome cercaria presents distinct three-eye spots, and with the tail having no fin fold. Mutabile cercaria has oral and ventral suckers of equal sizes and no tail. *O. viverrini* cercaria has a pair of eye spots and fin fold. Virgulate cercaria has virgule organs, ventral suckers smaller than oral suckers, and tail without a dorsoventral fin fold. The unknown type cercaria has a pair of eye spots located laterally to the oral sucker and the tail with a fin-fold.

Cercarial emergence was observed in 173 (3.15%) snails, the most frequent being in *B. s. goniomphalos* (5.60%), followed by *W. siamensis* (5.19%), *W. crosseana* (3.85%), *G. wykoffi* (1.69%) and *B. s. siamensis* (1.65%). The cercarial types found in *B. s. goniomphalos* were amartae (0.55%), mutabile (0.74%), *O. viverrini* (1.07%), virgulate (2.87%), and unknown type cercaria (0.37%). Only monostome (1.10%) and virgulate (0.55%) cercariae were found in *B. s. siamensis*, virgulate cercariae (3.85% and 5.19%) in *W. crosseana* and *W. siamensis,* respectively, and unknown type cercariae (1.69%) in *G. wykoffi* (Table 2).

DISCUSSION

The distribution of snail species is mostly limited to certain geographical areas and along river wetlands. In Thailand, *B. funiculata* distribution is restricted to the north of the country but *B. s. siamensis* is found not only in the north but also distributed throughout the central region (Brandt, 1974), similar to the findings of the present study. *B. s. goniomphalos* is limited to the northeast and no other *Bithynia* species are found as determined by DNA analysis (Kulsanthiwong *et al*, unpublished data). In this study *H. nassa* was

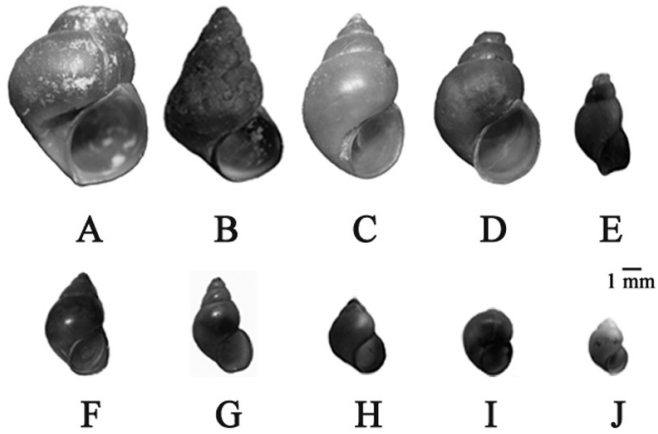

Fig 2–Shell morphology of ten snail species of the family Bithyniidae. (A) *B. funiculata*; (B) *B. s. goniomphalos*; (C) *B. s. siamensis*; (D) *H. nassa*; (E) *W. baschi*; (F) *W. crosseana*; (G) *W. siamensis*; (H) *G. wykoffi*; (I) *G. pygmaea*; (J) *G. erawanensis*. Scale bars = 1 mm.

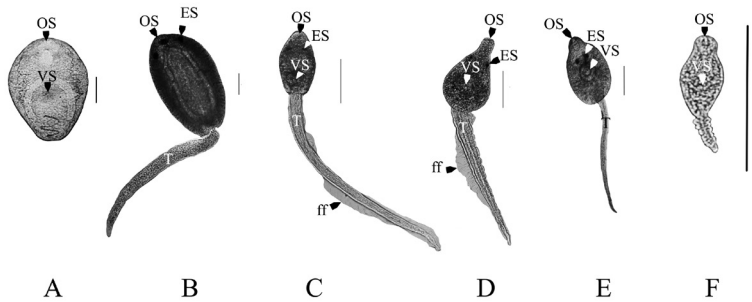

Fig 3–Morphology of cercariae found in snails collected in Thailand. (A) mutabile; (B) monostome; (C) *O. viverrini*; (D) amartae; (E) unknown; (F) virgulate. ES, eye-spot; ff, fin fold; OS, oral sucker; VS, ventral sucker. Scale bar = 1 μm.

discovered downstream of Chao Phraya River and its tributaries, in Sing Buri Province and in the northern provinces as previously reported (Brandt 1974; Chitramvong, 1991, 1992). *G. wykoffi* was present in central, northern and northeastern Thailand, which is different from previous reports (Brandt, 1974; Chitramvong, 1991, 1992), but only *G. pygmaea* was found in the north. *W. siamensis* and *W. crosseana* were found only in the northeast, but in previous reports they have been detected in the north, central and northeast(Brandt, 1974; Chitramvong, 1991, 1992). As for *W. baschi* and *G. erawanensis*, their distribution was limited to the south and the Erawan waterfall, Kanchanaburi Province as has been previously reported (Brandt, 1974; Chitramvong and Upatham, 1989).

Field investigation in this study indicated that Bithyniidae snails were infected with a number of trematodes, namely, amartae, monostome, mutabile, *O. viverrini*, virgulate, and unknown type cercaria. The species of snails infected were *B. s. goniomphalos*, *B. s. siamensis*, *G. wykoffi*, *W. crosseana*, and *W. siamensis*. To date, reports of natural infection have only been available for *B. s. goniomphalos*. In the present study six cercarial types (amartae, monostome, mutabile, *O. viverrini*, virgulate, and unknown) were found in *B. s. goniomphalos*. In previous reports, 9 cercarial types (amartae, amphistome, furcocercariae, lophocercous, monostome, *O. viverrini*, pleurolophocercous, virgulate, and xiphidiocercaria) were found in *B. s. goniomphalos* (Adam *et al*, 1993; Nithiuthai *et al*, 2002; Sri-aroon *et al*, 2005). Mutabile and unknown cercariae were found in our study but no furcocercariae, lophocercous, pleurolophocercous, or xiphidiocercaria were detected.

Table 1
Snails collected from five regions of Thailand during October 2008 - July 2009.

Species	Region [number of sites(s)]																	
	Northeast						North		Central							West	South	Total
	1[a] (3)	2 (3)	3 (30)	4 (4)	5 (1)	6 (1)	7 (12)	8 (3)	9 (7)	10 (1)	11 (2)	12 (4)	13 (10)	14 (12)	15 (9)	16 (12)	17 (1)	
B. funiculata	0	0	0	0	0	0	120	0	0	0	0	0	0	0	0	0	0	120
B. s. siamensis	0	0	0	0	0	0	317	178	34	0	65	50	302	482	135	250	0	1,813
B. s. goniomphalos	126	48	1,757	121	87	20	0	0	0	0	0	0	0	0	0	0	0	2,159
Wattebledia crosseana	0	20	34	0	0	0	0	0	0	0	0	0	50	0	0	0	0	104
W. siamensis	0	20	134	0	0	0	0	0	0	0	0	0	0	0	0	0	0	154
W. baschi	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150	150
Gabbia wykoffi	0	0	120	0	0	0	0	0	120	0	0	0	52	0	150	150	0	592
G. pygmaea	0	0	0	0	0	0	120	0	0	0	0	0	0	0	0	0	0	120
G. erawanensis	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	120	0	120
Hydrobioides nassa	0	0	0	0	0	0	52	0	0	38	20	50	0	0	0	0	0	160
Total	126	88	2,045	121	87	20	609	178	154	38	85	100	404	482	285	520	150	5,492

[a]1, Nong Khai; 2, Nong Bua Lam Phu; 3, Khon Kaen; 4, Maha Sarakham; 5, Yasothon; 6, Roi Et; 7, Chiang Mai; 8, Lamphun; 9, Phitsanulok; 10, Phichit; 11, Nakhon Sawan; 12, Sing Buri; 13, Saraburi; 14, Suphan Buri; 15, Bangkok; 16, Kanchanaburi; 17, Surat Thani.

Table 2
Cercarial infection rates in freshwater snails collected during October 2008 - July 2009.

Species	No. of snails	No. of infected snails (%)	Type of cercaria	No. of snails with cercarial type (%)
B. s. gomiomphalos	2,159	121 (5.60)	Virgulate	62 (2.87)
			O. viverrini	23 (1.07)
			Mutabile	16 (0.74)
			Amartae	12 (0.55)
			Unknown	8 (0.37)
B. s. siamensis	1,813	30 (1.65)	Monostome	20 (1.10)
			Virgulate	10 (0.55)
B. funiculata	120	0	-	0
W. crosseana	104	4 (3.85)	Virgulate	4 (3.85)
W. siamensis	154	8 (5.19)	Virgulate	8 (5.19)
W. baschi	150	0	-	0
G. wykoffi	592	10 (1.69)	Unknown	10 (1.69)
G. pygmaea	120	0	-	0
G. erawanensis	120	0	-	0
H. nassa	160	0	-	0
Total	5,492	173 (3.15)	-	173 (3.15)

O. viverrini cercariae have been found previously in the genus *Bithynia* in Thailand (Brockelman *et al*, 1989; Adam *et al*, 1993; Nithiuthai *et al*, 2002; Sri-aroon *et al*, 2005; Ngern-klun *et al*, 2006; Kiatsopit *et al*, 2012) and not in other genera, which was confirmed by our study. Our results also showed that no trematode infection was detected in *B. funiculata* from the north. Nithiuthai *et al* (2002) and Ngern-klun *et al* (2006) reported that *B. funiculata* in the north of Thailand is infected with the trematode cercariae, with virgulate being the most common type.

The distribution of *B. s. siamensis* snails is only in the central and north of Thailand and is not detected in the northeast using morphological or PCR-based analysis (Kulsanthiwong *et al*, unpublished data). However, previous studies have detected these cercariae only in *Bithynia* snails while our results showed for the first time trematode infection in *G. wykoffi*, *W. crosseana* and *W. siamensis* in Thailand.

The infection rates of trematodes varied and were dependent on snail species, locality, season and cercarial type. Trematode infections in *B. s. gonimophalos*, *B. s. siamensis*, *G. wykoffi* *W. crosseana*, and *W. siamensis* examined by cercarial shedding gave infection prevalence of 5.60%, 1.65%, 1.69%, 3.85%, and 5.19%, respectively. In previous reports, infection of *B. s. siamensis* has a similar rate (0.083% to 1.6%) (Upatham and Sukhapanth, 1980). However, in our study only 3.15% of the snails collected were infected with trematodes as examined by cercarial shedding, with the highest infection rate in *B. s. goniomphalos* (5.60%), which is higher than that previously reported (Nithiuthai *et al*, 2002), but the prevalence of each type of cercarial infection is not different. Kiasopit *et al* (2012) have recently reported that the total prevalence of *O. viverrini* infection in

B. s. goniomphalos is 3.04% in northeastern Thailand, higher than other previous reports (Wykoff *et al*, 1965; Upatham *et al*, 1983; Brockelman *et al*, 1986). In the present study, *O. viverrini* was found only in *B. s. goniomphalos* with a prevalence of 1.07%, similar to previous reports in Yang Talat District, Kalasin Province (1.3%) but higher than in Kamalasai District, Kalasin Province (0.61%) and Khon Kaen Province (0.03%-0.36%) (Lohachit, 2001; Sri-aroon *et al*, 2005). Differences in the overall prevalence of trematode infections may involve many factors, including different collection time, locality, season, type and number of parasite and intermediate host, availability of infected definitive or reservoir hosts. Brockelman *et al* (1986) reported that under conditions of adequate rainfall, two generations of *B. s. goniomphalos* normally are produced per year, occurring in the first summer rains in April or May and after the fall monsoon floods. A number of snails are seen in November and have migrated to the edge of a new environment within a few months. Therefore, the percentage of infected snails may be remarkably low between November and March, which overlaps the sample collection period in our study.

Generally, the populations of snails fluctuate according to rainfall, with snail populations declining as a result of heavy rains in the rainy season, which cause flushing of snail habitats. The snails that survive from flooding take a few months to resettle and subsequently migrate to the edge of reservoirs and begin to reproduce, reaching a carrying capacity of the new environment within a few months (Upatham *et al*, 1983; Nithiuthai *et al*, 2002). Many factors are involved in the prevalence of trematode infection in snail hosts, with prevalence usually varying between different geographical localities, between reservoir and human hosts, density of snail population, water quality, temperature and rainfall (Upatham *et al*, 1983; Nithiuthai *et al*, 2002; Ngern-klun *et al*, 2006). Furthermore, Sri-aroon *et al* (2007) reported that changes in irrigation practice and particularly the development of large-scale irrigation systems are associated with increasing risk of infection, possibly due to the ideal habitat they form for snails.

In summary, ten species/subspecies of bithynid snails were found in this study and the distribution varied depending on the species and ecology of the water bodies. Three snail species/subspecies in this family served as intermediate hosts of human liver fluke. This study revealed that currently *B. siamensis* and especially *B. s. goniomphalos* from northeastern Thailand served as intermediate hosts of *O. viverrini* with varying levels of prevalence of infection. Knowledge of the infection rates and distributions of the host snail populations is essential and must be taken into consideration when developing future control strategies. The results of this study can be used as a data base to examine the diversity of snail hosts and to introduce control strategies regionally.

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REFERENCES

Adam R, Arnold H, Pipitgool V, Sithithaworn P, Hinz E, Storch V. Studies on lophocercous cercariae from *Bithynia siamensis goniomphalus* (Prosobranchia: Bithyniidae). *Southeast Asian J Trop Med Public Health* 1993; 24: 697-700.

Brandt RAM. The non-marine aquatic Mollusca of Thailand. *Arch Mollusken* 1974; 105: 1-423.

Brockelman WY, Upatham ES, Viyanant V, Ardsungnoen S, Chantanawat R. Field studies on the transmission of the human liver fluke, *Opisthorchis viverrini*, in northeast Thailand: population changes of the snail intermediate host. *Int J Parasitol* 1986; 16: 545-52.

Chanawong A, Waikagul J. Laboratory studies on host-parasite relationship of *Bithynia* snails and the liver fluke, *Opisthorchis viverrini*. *Southeast Asian J Trop Med Public Health* 1991; 22: 235-9.

Chitramvong YP. The Bithyniidae (Gastropoda: Prosobanchia) of Thailand: comparative internal anatomy. *Walkerana* 1991; 5: 161-206.

Chitramvong YP. The Bithyniidae (Gastropoda: Prosobanchia) of Thailand: comparative external morphology. *Malacol Rev* 1992; 25: 21-38.

Chitramvong YP, Upatham ES. A new species of freshwater snail for Thailand (Prosobranchia: Bithyniidae). *Walkerana* 1989; 3: 179-86.

Ellen SE, Gargominy O, Ponder WF, Bouchet P. Global diversity of gastropods (Gastropoda; Mollusca) in freshwater. *Hydrobiologia* 2008; 595: 149-66.

Frandsen F, Christensen NO. An introductory 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* 1984; 41: 181-203.

Harinasuta T, Riganti M, Bunnag D. *Opisthorchis viverrini* infection: pathogenesis and clinical features. *Arzneimittelforschung* 1984; 34: 1167-9.

Haswell-Elkins MR, Sithithaworn P, Elkins D. *Opithorchis viverrini* and cholangiocarcinoma in Northeast Thailand. *Parasitol Today* 1992; 8: 86-9.

Honjo S, Srivatanakul P, Sriplung H, Kikukawa H, Hanai S, Uchida K. Genetic and environmental determinants of risk for cholangiocarcinoma via *Opisthorchis viverrini* in a densely infested area in Nakhon Phanom, northeast Thailand. *Int J Cancer* 2005; 117: 854-60.

International Agency for Research on Cancer (IARC). Infection with liver flukes (*Opisthorchis viverrini, Opisthorchis felineus* and *Clonorchis sinensis*). *IARC Monogr Eval Carcinog Risks Hum* 1994; 61: 121-75.

International Agency for Research on Cancer (IARC). A review of human carcinogens: Biological agents, *Opisthorchis viverrini* and *Clonorchis sinensis*. *IARC Monogr Eval Carcinog Risks Hum* 2011; 100B: 351-76.

Jongsuksuntigul P, Imsomboon T. Epidemiology of opisthorchiasis and national control program in Thailand. *Southeast Asian J Trop Med Public Health* 1998; 29: 327-32.

Keiser J, Utzinger J. Emerging foodborne trematodiasis. *Emerg Infect Dis* 2005; 11: 1507-14.

Kiatsopit N, Sithithaworn P, Boonmars T, *et al*. Exceptionally high prevalence of infection of *Bithynia siamensis goniomphalos* with *Opisthorchis viverrini* cercariae in different wetlands in Thailand and Lao PDR. *Am J Trop Med Hyg* 2012; 86: 464-9.

Lohachit C. Ecological studies of *Bithynia siamensis goniomphalos* a snail intermediate host of *Opisthorchis viverrini* in Khon Kaen Province, Northeast Thailand. Bangkok:

Mahidol University 2001. 164 pp. PhD Thesis.

Mills EL, Leach JH, Carlton JT, Secor CL. Exotic species and the integrity of the Great Lakes. *Bioscience* 1994; 44: 666-76.

Ngern-klun R, Sukontason LK, Tesana S, Sripakdee D, Irvine NK, Sukontason K. Field investigation of *Bithynia funiculata*, intermediate host of *Opisthorchis viverrini* in northern Thailand. *Southeast Asian J Trop Med Public Health* 2006; 37: 662-72.

Nithiuthai S, Wiwanitkit V, Suwansaksri J, Chaengphukeaw P. A survey of trematode cercariae in *Bithynia goniomphalos* in northeast Thailand. *Southeast Asian J Trop Med Public Health* 2002; 33: 106-9.

Schell SC. How to know the trematode. Iowa: WMC Brown, 1970: 1-43.

Sithithaworn P, Haswell-Elkins MR, Mairiang P, Satarung S, Mairiang E, Vatanasapt V. Parasite-associated morbidity: liver fluke infection and bile duct cancer in northeast Thailand. *Int J Parasitol* 1994; 24: 833-43.

Sriamporn S, Pisani P, Pipitgool V, Suwanrungruang K, Kamsa-ard S, Parkin DM. Prevalence of *Opisthorchis viverrini* infection and incidence of cholangiocarcinoma in Khon Kaen, Northeast Thailand. *Trop Med Int Health* 2004; 9: 588-94.

Sri-aroon P, Butraporn P, Limsomboon J, *et al*. Freshwater mollusks at designated areas in eleven provinces of Thailand according to the water resource development projects. *Southeast Asian J Trop Med Public Health* 2007; 38: 294-301.

Sri-aroon P, Butraporn P, Limsomboon J, Kerdpuech Y, Kaewpoolsri M, Kiatsiri S. Freshwater mollusks of medical importance in Kalasin Province, Northeast Thailand. *Southeast Asian J Trop Med Public Health* 2005; 36: 653-7.

Thamavit W, Bhamarapravati N, Sahaphong S, Vajrasthira S, Angsubhakom S. Effects of dimethylnitrosamine on induction of cholangiocarcinoma in *Opisthorchis viverrini* infected Syrian golden hamsters. *Cancer Res* 1978; 38: 4634-9.

Upatham ES, Sornmani S, Kitikoon V, Lohachit C, Burch JB. Identification key for the fresh-and brackish-water snails of Thailand. *Malacol Rev* 1983; 16: 107-32.

Upatham ES, Sukhapanth N. Field studies on the bionomics of *Bithynia siamensis siamensis* and the transmission of *Opisthorchis viverrini* in Bangna, Bangkok, Thailand. *Southeast Asian J Trop Med Public Health* 1980; 11: 355-8.

Vatanasapt V, Parkin DM, Sriamporn S. Epidemiology of liver cancer in Thailand. In: Vatanasapt V, Sripa B, eds. Liver cancer in Thailand: Epidemiology, diagnosis and control. Khon Kaen: Siriphan Press, 2000: 3-6.

Waikagul J. *Opisthorchis viverrini* metacercaria in Thai freshwater fish. *Southeast Asian J Trop Med Public Health* 1998; 29: 324-6.

Watanapa P, Watanapa WB. Liver fluke-associated cholangiocarcinoma. *Br J Surg* 2002; 89: 962-70.

Wongratanacheewin S, Pumidonming W, Sermswan RW, Maleewong W. Development of a PCR-based method for the detection of *Opisthorchis viverrini* in experimentally infected hamsters. *J Parasitol* 2001; 122: 175-80.

World Health Organization (WHO). Control of foodborne trematode infections. *World Health Organ Tech Rep Ser* 1995; 849: 1-157.

Wykoff DE, Harinasuta C, Juttijudata P, Winn MM. *Opisthorchis viverrini* in Thailand- the life cycle and comparison with *O. felineus*. *J Parasitol* 1965; 51: 207-14.