FIELD INVESTIGATION OF *BITHYNIA FUNICULATA*, INTERMEDIATE HOST OF *OPISTHORCHIS VIVERRINI* IN NORTHERN THAILAND

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Abstract. A survey of freshwater snail, *Bithynia funiculata*, was conducted in four locations, Doi Saket, Mueang, Saraphi and Mae Rim Districts of Chiang Mai Province, northern Thailand, between June and October, during the rainy season of 2004. A total of 2,240 snails was collected and classified into 7 families and 15 genera; of which 352 *B. funiculata* were obtained. *B. funiculata* was found most abundant in July and September. The infection rate of trematode cercariae in *B. funiculata* was 9.6% (19/352), while that of pleurolophocercous was 0.3% (1/352). Virgulate cercaria was the most common type, followed by lophocercous, monostome and pleurolophocercous. *B. funiculata* prefers habitats with clear water, which was less than 30 cm depth, temperatures between 24.48 and 31.78ºC, dissolved oxygen 2.03-7.66 mg/l, saturated dissolved oxygen 26.70-95.00%, conductivity 0.000-0.2642 mS/cm, turbidity 16.00-288.00 NTU and pH 6.58-7.56. Geographic Information System (GIS) analysis using loose soil mixed with clay revealed that the breeding grounds of this snail species was in the paddy fields and village environs of the Ping, Kuang and Fang river basins.

INTRODUCTION

The liver fluke, *Opisthorchis viverrini*, is a medically important parasite infecting humans in many countries. It has been estimated by the World Health Organization (1995) that infection caused by this parasite affects approximately 9 million people worldwide. In Southeast Asia, endemic areas include Thailand, Cambodia and Lao PDR (Sithithaworn and Haswell-Elkins, 2003). *O. viverrini* has a marked regional variation specifically in Thailand, with a high prevalence occurring in the northeast and northern regions, where raw and/or undercooked fish is frequently consumed (Sadun, 1995). Epidemiological surveys during 1981-1991 indicated an increasing infection rate of *O. viverrini* in the north and central regions of the country, whereas, rates decreased in the northeastern part (Jongsuksuntigul and Imsomboon, 1998). A recent national survey in 2001 revealed that such infection in the northern region was top ranked, with a prevalence of 19.3% (Jongsuksuntigul, 2002).

The life cycle of *O. viverrini* is governed by the existence of freshwater snails as the first intermediate host and freshwater fish as the second. In Thailand, three taxa of *Bithynia* snails act as the first intermediate host; but, the species of *Bithynia* prevails differently de-

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pending upon geographical habitats; *Bithynia funiculata* in the north, *Bithynia siamensis goniomphalos* in the northeast and *Bithynia siamensis siamensis* in the central part (Brandt, 1974). The natural infection of *O. viverrini* surveyed in these snails varied, ranging from 0.05% to 1.6% (Upatham and Sukhapanth, 1980; Brockelman *et al*, 1986; Adam *et al*, 1993). Using either laboratory or field collected snails, *B. funiculata* was significantly more susceptible to *O. viverini* infection than both *B. goniomphalos* and *B. siamensis* (Chanawong and Waikagul, 1991).

Ecological environment has been recorded as having influence on the fluctuation of the snail as an intermediate host in an endemic area (*eg,* Upatham and Sukhapanth, 1981; Brockelman *et al*, 1986; Wullschleger and Jokela, 1999; Kock *et al*, 2004; Rondelaud *et al*, 2004). Nevertheless, information pertaining to water quality as well as the physical environment of the breeding grounds of *B. funiculata* in Chiang Mai, northern Thailand, is very limited. In this study, we surveyed the prevalence of *B. funiculata* and the infection rate of trematode cercariae in this snail species in Chiang Mai during the rainy season of 2004. Other freshwater snails inhabiting the same location with *B. funiculata* were documented. We also investigated the ecological factors, water quality and physical environment, that may entail suitable habitats for *B. funiculata,* while using the application of Geographical Information System (GIS) technology to incorporate snail survey data and environmental factors to predict the distribution of *B. funiculata* in the study areas of Chiang Mai.

MATERIAL AND METHODS

Study areas and land use

Four study areas (Fig 1) were chosen around the downtown area of Chiang Mai (17-21ºN, 98-99ºE) to investigate the *B. funiculata* breeding sites. Area 1 was located at Ban Thawang (Mae Rim District). Areas 2, 3 and 4 were located at Ban Nhongpom (Mueang District), Ban Pakui (Doi Saket District) and Ban Si Boonreang (Saraphi District), respectively (Fig 1A). Descriptive information of such areas is given in Table 1. The coordinates for each site were recorded using the Global Positioning System (GPS) Garmin® GPS 12 Personal Navigator® (Garmin International, Kansas, USA). Rainfall data were obtained from the Meteorological Center, Chiang Mai, Thailand. The depth of water, characteristics of mud, sand and soil substrata of each site was recorded.

Snail sampling method

Routine sampling of snails was conducted monthly from June to October 2004, which represented the rainy season. Snail collection was performed twice a month. The snail sampling method employed was dependent upon the ecology of each study site. Hand scoop sampling was performed in the area having aquatic plants by using the standard wire-mesh scoop with a mesh size of 2 mm. At each sampling location, ten scoops were made along just beneath the mud surface in a set pattern (4x4 m). Thereafter, the mud was rinsed off the scoops and the snails were collected one by one. In areas without aquatic plants, the snails were picked up manually by two people for 10 minutes at each sample site.

After collection, the snails were placed in a plastic bag and transferred to the Department of Parasitology, Faculty of Medicine, Chiang Mai University. All specimens were identified by the methods of Brandt (1974) and Upatham *et al* (1983). Only *B. funiculata* was measured for its size and examined for trematode cercariae shedding.

Cercarial shedding

B. funiculata collected from each site was placed separately in a test tube containing

Table 1
Description of study areas where snail collections were conducted from June to October 2004 in Chiang Mai, northern Thailand.

Area	District	Village	Sub-sites	Soil type	Water supply	Altitude[a]	Georeference coordinates
I	Mae Rim	Ban Thawang	S1P1 (rice paddy) S1P2 (rice paddy) S1C (irrigation canal)	Series-5 (loose soil + clay)	Suthep Mountain and Ping river	319	49.70 E; 20.91 N
II	Mueang	Nong Pom	S2P (rice paddy) S2C (irrigation canal)	Series-7 (sandy soil + clay)	Suthep Mountain and Ping river	317	49.93 E; 20.85 N
III	Doi Saket	Pakui	S3P (rice paddy) S3C (irrigation canal)	Series-59 (clay)	Mae Kuang Udom Thara Dam	315	50.59 E; 20.80 N
IV	Saraphi	Si Boonreang	S4P (rice paddy)	Series-5 (loose soil + clay)	Mae Kuang Udom Thara Dam	314	50.54 E; 20.72 N

[a]Meters above sea level

distilled water. Such tubes were exposed to the illumination of a 25-W light source at a distance of 30 cm for 2 hours at room temperature. Subsequently, the water from each tube was poured into a Petri dish and examined for cercarial shedding under a dissecting microscope. Identification of cercariae was performed by pipetting a few drops of water onto a glass slide, covered with a cover slip, and then examining under a stereomicroscope, as described by Frandsen and Christensen (1984).

Water quality recording

The water quality of each sample site during every snail collection was monitored and recorded with Hydrolab Datasonde® 4a (Hydrolab corporation, Texas, USA). The parameters recorded were temperature, dissolved oxygen (both concentration and percent of saturation), conductivity, turbidity and pH.

GIS database

Base topographical maps of the four locations in this study were digitalized, input and overlaid, with the data gained from each site entered into a GIS database using ArcView 3.3 software. Other thematic maps [soil map, water supply, land-use classes of paddy field (Fig 1C)] were also incorporated. All digital data were displayed in the UTM coordinate system and georeferenced using the Indian 1975 Datum and Everest 1830 (1975 Definition) Ellipsoid Provincial border boundary.

RESULTS

A total count of 2,240 snail specimens was collected from four localities in Chiang Mai Province during the rainy season from June to October 2004. Seven families comprised of fifteen genera were obtained (Table 2). Within the family Bithyniidae, *Hydrobioides nassa* ranked the most prevalent (26.2%), followed by *Wattebledia siamensis* (17.6%), *B. funiculata* (15.7%) and *Bithynia* (*Gabbia*)

Table 2
Collected freshwater snail from four study areas around downtown Chiang Mai, from June to October 2004.

Family	Snail collected		
	Species	N	Percentage
Ampullariidae	*Pomacea* spp	75	3.3
Bithyniidae	*Bithynia* (*Digoniostoma*) *funiculata*	352	15.7
	Bithynia (*Gabbia*) *wykoffi*	59	2.6
	Hydrobioides nassa	586	26.2
	Wattebledia siamensis	394	17.6
Buccinidae	*Clea* (*Anentome*) *helena*	35	1.6
Lymnaeidae	*Austropeplea ollula*	95	4.2
Planorbidae	*Camptoceras jiraponi*	3	0.1
	Indoplanorbis exustus	11	0.5
Thiaridae	*Melanoides tuberculata*	301	13.4
	Tarebia granifera	70	3.1
	Thiara scabra	2	0.1
Viviparidae	*Eyriesia* sp	1	0.0
	Filopaludina spp	135	6.0
	Idiopoma spp	44	2.0
Unidentified		77	3.4
Total		2,240	100.0

wykoffi (2.6%), consecutively.

Of the 352 *B. funiculata* sampled, most of the snails were collected in July (n = 115) and September (n = 102) when rainfall was highest (Table 3). However, there was no correlation between the number of snails and quantity of rainfall (p = 0.391). *B. funiculata* snails were present in 3 Areas of 1, 3 and 4 (Table 3). In the paddy fields of Areas 1 and 4, where soil characteristics were graded as loose soil and clay, *B. funiculata* was found in all five months of the study period. In Area 3, which had a clay type of soil (series 59), *B. funiculata* could be collected only from August to October. No *B. funiculata* was found in the irrigation canals of Area 1 and 3. Likewise, snails could not be detected in the paddy fields of Area 3, which had sandy and clay soil types.

The infection rate of trematode cercariae in *B. funiculata* was 5.4% (19/352) (Table 4). Large *B. funiculata* (ø > 7.0 mm) had an infection rate of 4.8%, while 0.5% was found in smaller ones (ø ≤ 7.0 mm). However, the infection rate in large and small snails was not statistically significant (p = 0.071). Of the nineteen infected *B. funiculata*, virgulate cercaria was the most common type (63.2%), followed by lophocercous (21.0%), monostome (10.5%) and pleurolophocercous cercaria (5.3%) (Table 5). The pleurolophocercous cercaria was probably the cercaria of *O. viverrini*. No mixed infection of trematode cercariae was detected in the same snail specimen. The number of pleurolophocercous cercaria released from a single snail was 286. Morphological features and measurement of these four types of cercaria are shown in Table 6. Monostome cercaria had the biggest body and a distinctive three-eyed spot. Lophocercous and pleurolophocercous cercaria had the same body length; however, the former was broader than the latter (119.26 ± 18.90 *vs* 57.87± 11.76 μm).

Table 3
Number of *B. funiculata* collected from four area of Chiang Mai from June to October 2004.

Month	Area 1			Area 2		Area 3		Area 4	Total	Rainfall[a] (mm)
	S1P1	S1P2	S1C	S2P	S2C	S3P	S3C	S4P		
June	9	6	0	0	0	0	0	15	30	178.8
July	60	45	0	0	0	0	0	10	115	218.0
August	6	16	2	0	0	7	0	1	32	115.7
September	20	8	12	0	0	22	0	40	102	317.4
October	14	5	0	0	0	8	0	46	73	38.3
Total		203		0		37		112	352	

[a] There was no correlation between the number of snails collected and rainfall ($p = 0.391$; $r = 0.50$, Spearman rank correlation coefficients)

Table 4
Infection rate of trematode cercariae found in field collected *B. funiculata* ranked by size.

Length of snails (mm)	Total snails		No. of *B. funiculata*		Infection rate (%)
	No.	%	Uninfected	Infected	
≤ 7.00	108	30.7	106	2	0.6
> 7.00	244	69.3	227	17	4.8
Total	352	100.0	333	19	5.4
p					0.071[a]

[a] No significant difference ($p > 0.05$; chi-square tests)

Table 5
Type of cercariae recovered from *B. funiculata* collected in study areas around downtown Chiang Mai, from June to October 2004.

Type of cercariae	Number of infected snails (%)	Rate of infection (%)	No. shed cercariae/snail (range) (%)
Virgulate	12 (63.2)	3.4	452 (1-1,241)
Lophocercous	4 (21.0)	1.1	37 (7-197)
Monostome	2 (10.5)	0.6	56 (28-84)
Pleurolophocercous	1 (5.3)	0.3	286
Total	19 (100)	5.4	

Table 7 displays information pertaining to water quality recorded from areas where *B. funiculata* was collected. It was found that *B. funiculata* was present in water at temperatures between 24.48 and 31.78ºC, DO ranging from 2.03-7.66 mg/l, DO% of 26.70-95.00, conductivity between 0.000 and 0.264 mS/cm, turbidity between 16.00 and 288.00 NTU, and pH of 6.58-7.56. Moreover, *B. funiculata* preferred water less than 30 cm depth. Areas

Table 6
Characteristics of trematode cercaria recovered from *B. funiculata*.

Type of cercariae	Body (m) (mean ± SD)		Tail (μ) (mean ± SD)		Special characteristics
	Length	Width	Length	Width	
Virgulate	108.07± 11.89	59.59± 12.13	55.8± 11.82	10.79± 3.00	Oral and ventral suckers were large; the ventral were smaller than the oral. Single stylet displayed obvious anteriority. Tail was relatively short with no finfold.
Lophocercous	178.57± 35.93	119.26± 18.90	417.1± 38.12	30.26± 4.5	Body was oval. Oral and ventral suckers, eye spot and transverse array finfold were apparent.
Monostome	323.78± 54.62	159.34± 23.28	499.87± 102.24	30.61	Distinct three-eyed spot and well-developed oral sucker. Tail had no finfold.
Pleurolophocercous	183.67± 23.55	57.87± 11.76	417.1± 38.12	30.26± 4.50	Body was cylindrical. Lateral finfold was prominent.

Table 7
Water quality recorded from sites where *B. funiculata* was collected.

B. funiculata	Median (range)					
	Temperature (ºC)	Dissolved oxygen (%)	Dissolved oxygen (mg/l)	Conductivity (mS/cm)	Turbidity (NTU)	pH
Presence	26.51 (24.48-31.78)	47.45 (26.70-95.00)	3.79 (2.03-7.66)	0.147 (0.000-0.264)	68.55 (16.00-288.00)	7.11 (6.58-7.56)
Absence	27.86 (24.71-30.42)	59.90 (18.30-83.70)	4.71 (1.42-10.44)	0.175 (0.000-0.366)	31.25 (13.00-92.00)	7.38 (6.90-8.30)
p	0.89	0.126	0.154	0.196	0.001[a]	0.012[a]

[a] Significant difference; Mann-Whitney *U* test ($p < 0.05$)

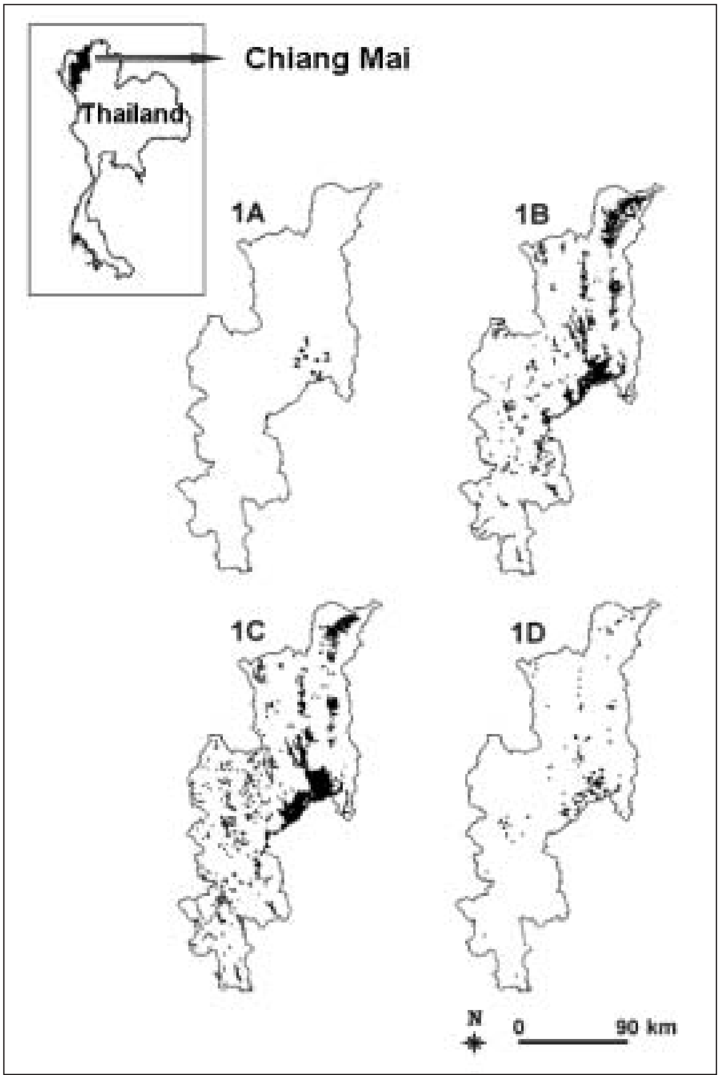

Fig 1–Location of four collection areas in Chiang Mai, northern Thailand, where snails were collected from June to October, 2004. (1A) 1 = Area 1, Ban Thawang, Mae Rim District; 2 = Area 2, Ban Nhongpom, Mueang District; 3 = Area 3, Ban Pakui, Doi Saket District; 4 = Area 4, Ban Si Boonreaung, Saraphi District. (1B) Thematic map showing soil type series 5 and 59. (1C) Thematic map showing paddy field areas. (1D) Predicted area of Chiang Mai where *B. funiculata* is present.

where *B. funiculata* snails were found had significantly higher turbidity and lower pH than those without *B. funiculate* (p = 0.001 and 0.012, respectively).

Using the Arcview 3.3 program to incorporate the snail survey data with soil type (series 5 and 59: Fig 1B), land used (paddy field) and villages of Chiang Mai, the probable breeding grounds of *B. funiculata* were predicted as the Ping, Kuang and Fang river basins and encompassed 165 villages in Chiang Mai. Nevertheless, the expected areas were mainly positioned in the Ping and Kuang river basins (Fig 1D).

DISCUSSION

Many freshwater snail species were present in this study, including *B. funiculata*, the first intermediate host of an important liver fluke in Thailand. The population of *B. funiculata* collected in the months of heavy rain was high, but there was no statistically significant correlation between snail population and rainfall. This phenomenon was unlike the collections of *Bithynia siamensis siamensis* or *Indoplanorbis exustus* investigated in Bangkok, and central Thailand (Upatham and Suthapanth, 1980, 1981). It was interesting to note that heavy rains did not affect the collection of *B. funiculata* in this study. The consistent water depth maintained by the irrigation systems of the paddy fields and the presence of more snails in soil substrate than at the surface level of water may explain the lack of correlation. *B. funiculata* could be found in all months of the rainy season in paddy fields that had loose soil and clay. However, in sub-site S3P of Area 3, which was a paddy field with clay, *B. funiculata* was found at depths of 30 cm in clear water after rice had been planted.

Regarding the natural parasitic infection of *B. funiculata*, snails liberated four types of trematode cercariae: virgulate, lophocercous, monostome and pleurolophocercous cercariae. Ito *et al* (1962) and Wykoff *et al* (1965), classified *O. viverrini* as a type of pleurolophocercous cercariae. With respect to *B. goniomphalos*, the types of cercariae liberated were lophocercous, monostome, furcocercous, xiphidiocercariae (Adam *et al*, 1993), echinostomes, intestinal flukes, xyphidia, furcocercous, *O. viverrini* (Sriaroon *et al*, 2005) or amphistome (Wiwanitkit *et al*, 2002). With respect to *B. siamensis*, the type of cercarial emitted were furcocercous and pleurolophocercous (*O. viverrini*) (Upatham and Sukhapanth, 1980).

The infection rate of pleurolophocercous (*O. viverrini*) cercaria in naturally infected *B. funiculata,* as observed in this study, was relatively low (0.28%) (Table 4), and lower than those of *B. siamensis* in central Thailand (1.6%) (Upatham and Sukhapanth, 1980), *B. goniomphalos* in Lao PDR (0.5-0.9%) (Ditrich *et al*, 1990; Giboda *et al*, 1991). However, a survey of *O. viverrini* cercaria from field collected *B. goniomphalos* in northeast Thailand exhibited no infection, despite the high prevalence of *O. viverrini* infection in the studied area (Nithiuthai *et al*, 2002). This was consistant the 0.11% and 0.05% minimal infection rate of *O. viverrini* in *B. goniomphalos* reported by Brockelman *et al* (1986) and Adam *et al* (1993), respectively.

Larger (>7.0 mm) field-collected *B. funiculata* yielded a higher infection rate of *O. viverrini* than smaller ones (Table 4). Similarly, Upatham and Sukhapanth (1980) observed more infections by this parasite in larger *B. siamensis* (>7.0 mm). Interestingly, a study by Chanawong and Waikagul (1991) found that the immature laboratory bred *Bithynia* (2-4 mm long) was more susceptible to *O. viverrini* infection than mature field-collected specimens (6-10 mm long).

Field investigations in this study indicated that *B. funiculata* inhabit rice paddy fields with clear water of no more than 30 cm in depth. Most *B. funiculata* were sampled from the muddy substrate of rice paddies (S1P, S2P, S4P), which is consistent with previous reports (TROPMED Technical Group, 1986; Chitramvong, 1992). *B. funiculata* was generally observed, as in the collection sites S1P, S2P and S4P, sliding over mud substrate or attached to the bottom of rice, aquatic vegetation and algae. The swampy area of the Bhumibol reservoir (Tak Province, northern Thailand) has also been reported as a habitat of *B. funiculata* infected with *O. viverrini* (Rojanapremsuk *et al*, 1988). Other members belonging to the family Bithyniidae, including *Hydrobioides nassa* and *Wattebledia siamensis*, were found in similar habitats. In general, the ecological niches of *B. funiculata* in this study were similar to those of *B. goniomphalos* investigated in northeast Thailand, in which snails inhabit static water at a depth of no more than 30 cm along the edge of a water reservoir. They were found sliding on aquatic plants growing in red-yellow podzolic soils (Papasarathorn *et al*, 1990). Nevertheless, *B. goniomphalos* have been found in other habitats (Brockelman *et al*, 1986), including mixed sand-mud substrate at pond's edge, where the water is relatively deep and devoid of algae (Chitramvong, 1992).

Determination of water quality in each collection site revealed that the turbidity and pH of the water were the principle parameters which determined the presence and absence of *B. funiculata. B. funicalata* preferred water with high turbidity and a pH of 6.58-7.56. This pH value was broader than the 6.90-7.10 pH value earlier reported for bithyniid snails (TROPMED Technical Group, 1986), or the 6.6-7.2 pH of specific *B. goniomphalos* (Parasarathorn *et al*, 1990). *B. goniomphalos* were also presented in water with high turbidity (Parasarathorn *et al*, 1990).

GIS technology has been applied to investigate and understand the ecology of the intermediate host of medically and/or veterinarily important parasites, such as the snail intermediate hosts of the liver fluke, *Fasciola hepatica* (Zukowski *et al*, 1991, 1993; Yilma and Malone, 1998) and *Fasciola gigantica* (Yilma and Malone, 1998); the blood fluke, *Schistosoma mansoni* (Kristensen *et al*, 2001), *Schistosoma haematobium* (Lwambo *et al*, 1999), and *Schistosoma japonicum* (Maszle *et al*, 1998; Yang *et al*, 2005; Nihei *et al*, 2006); and rumen fluke, *Calicophoron daubneyi* (Cringoli *et al*, 2004). Our report, presented herein, is the first recorded GIS-facilitated monitoring of the snail intermediate host of *O. viverrini* in Chiang Mai. Analysis indicates that the habitat of *B*. *funiculata* in Chiang Mai is characterized by paddy fields with loose soil mixed with clay. Based on our data, we hypothesize that the *B. funiculata* population is distributed in the central part of Chiang Mai Province. The collection of additional data in similar habitats is an ongoing project and is needed to confirm this hypothesis.

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