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Trematode diversity in the freshwater snail *Bithynia siamensis goniomphalos* sensu lato from Thailand and Lao PDR

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Abstract

In order to obtain a comprehensive understanding of trematode diversity in *Bithynia siamensis goniomphalos* sensu lato, the first intermediate host of the liver fluke *Opisthorchis viverrini* s.l., the prevalence of larval trematode species was investigated in different localities in Thailand and Lao People's Democratic Republic (Lao PDR). In Thailand, snail samples were collected from 29 localities in the nine provinces: Buri Ram, Surin, Chaiya Phum, Maha Sarakham, Khon Kaen, Kalasin, Mukdahan, Sakon Nakhon and Nakhon Phanom. In Lao PDR, snail samples were collected from 21 localities in Vientiane Province and six localities in Savannakhet Province. Snails were identified by standard morphological criteria and then examined for trematode infection using the cercarial shedding method. Twenty different types of cercariae were detected and identified, based on morphological criteria. Virgulate type 1 emerged as the most common cercaria, with an average prevalence of 10.90% (range 0.26–54.22%) in Thailand and 6.58% (range 1.15–89.77%) in Lao PDR. *Opisthorchis viverrini* s.l. cercariae were the fourth most common in Thailand, with an average prevalence of 1.59% (0.15–6.93), while in Lao PDR their prevalence was 0.96% (0.08–8.37). The high diversity of trematode cercariae observed in this study indicates that *B. s. goniomphalos* s.l. is highly susceptible to infection with a variety of trematode species. However, the role of non-opisthorchiid trematodes as fish-borne parasites in human health is not fully known and further molecular identification is required.

Introduction

Opisthorchis viverrini sensu lato is a carcinogenic, food-borne trematode endemic in continental South-East Asian countries, especially in Thailand and Lao PDR (Sithithaworn *et al.*, 2012a). Recent evidence shows that *O. viverrini*

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Table 1. The composition of cercarial types and the number of infected snails in *Bithynia siamensis goniomphalos* samples collected from localities in Thailand and Lao PDR (see fig. 1 for cercarial types and table 2 for prevalence values).

Country	Province	River	Locality (village/district)	Number of collected snails	Number of infected snails for each cercarial type														
					A	B	C	F	G	H	I	K	L	M	N	O	Q	S	T
Thailand	Buri Ram	Mun River	Mueang Buri Ram	380	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Surin		Mueang Surin	110	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
	Chaiya Phum	Chi River	Chaturat	82	2	–	–	–	–	3	–	–	2	–	–	–	–	–	–
	Maha Sarakham		Maha Sarakham University	19	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Kosum Phisai	280	6	–	–	26	–	9	–	–	–	–	–	–	–	–	–
	Khon Kaen		Pon 1	137	29	–	–	–	–	4	–	1	–	–	–	–	–	–	–
			Pon 2	257	30	–	–	–	–	1	–	–	–	–	–	1	–	–	–
			Pon 3	252	22	–	–	–	–	1	–	–	–	–	–	–	–	–	–
			Ban Phai 1	213	2	–	–	–	–	3	–	–	–	–	–	–	–	–	–
			Ban Phai 2	311	2	–	–	–	–	5	–	–	–	–	–	–	–	–	–
			Sa-ard village	335	5	–	–	–	–	1	–	–	–	–	–	–	–	–	–
			Lerngpleuy village 1	164	39	–	–	1	–	–	2	1	–	1	–	–	–	–	–
			Lerngpleuy village 2	83	45	–	–	–	1	–	–	–	–	1	–	–	–	2	–
			Phu Vieng 1	150	2	–	–	–	–	1	1	25	–	–	–	–	2	–	–
			Phu Vieng 2	361	11	–	–	–	–	–	–	4	–	–	–	–	–	–	–
	Kalasin		Lam Pao Dam 1	87	16	–	–	–	–	–	1	–	–	1	–	2	–	–	–
			Lam Pao Dam 2	74	4	–	–	–	–	1	1	–	–	–	–	–	–	–	–
			Lam Pao Dam 3	85	3	–	–	–	–	5	–	–	–	–	–	–	–	–	–
	Mukdahan	Songkram River	Mueang	300	3	–	–	–	–	–	–	–	–	1	–	–	–	–	–
			Dong Luang	239	23	–	–	–	–	4	–	2	–	1	1	12	–	–	–
	Sakon Nakhon		Phang Khon	445	30	–	–	–	1	–	–	3	–	–	–	–	–	–	–
				303	25	–	–	–	21	9	–	1	–	–	–	1	1	–	–
				104	17	–	–	–	–	2	–	1	–	–	–	–	–	–	–
				551	218	–	–	–	19	11	2	2	–	7	–	–	3	9	–
				2,065	106	6	1	–	3	14	–	9	–	3	1	3	–	1	–
				2,127	468	26	37	–	59	38	–	–	–	23	–	–	4	1	–
				966	48	–	20	–	4	–	–	–	–	3	–	–	4	8	1
				808	53	14	8	–	9	2	–	–	–	1	1	–	–	–	–
	Nakhon Phanom		Renu Nakhon	468	40	–	–	–	–	7	9	–	2	6	–	–	–	3	–
Total snails				11,756															
Lao PDR	Savannakhet	Sae Bang Heang River	La Ha Nam, Songkhon	37	3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Na Seng, Khanthabouri	172	9	–	–	–	–	12	–	–	–	–	–	–	–	–	–
			Pon Sa-ard, Khanthabouri	384	26	–	–	–	–	22	–	1	–	1	–	–	–	–	–
			Hau Maung Neang, Khanthabouri	27	1	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Bueng Wa, Khanthabouri	186	21	–	–	–	–	3	–	–	–	–	–	–	–	–	–
			Bueng Wa, Khanthabouri	404	8	–	–	–	9	11	–	–	–	–	–	–	1	–	–

Table 1 – *continued*

Country	Province	River	Locality (village/district)	Number of collected snails	Number of infected snails for each cercarial type														
					A	B	C	F	G	H	I	K	L	M	N	O	Q	S	T
	Vientiane	Num Ngum River	Xaithani	180	11	–	–	–	8	5	–	–	–	–	–	–	–	–	–
				203	29	–	–	–	17	11	–	–	–	1	–	–	5	–	–
				1,031	56	–	–	–	17	15	–	–	–	1	–	2	3	–	–
				155	7	–	–	–	2	–	–	–	–	–	–	–	5	–	–
				469	6	1	2	10	3	2	–	–	–	–	–	–	4	–	–
				1,223	39	53	–	–	1	3	–	1	–	2	–	–	4	–	–
				2,812	98	15	40	–	6	70	1	4	1	35	–	–	1	–	1
			Veiang Jarean, Xaisettha	485	16	–	–	–	5	6	–	–	–	3	–	–	5	–	–
			That Luang	172	20	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Ban Na Hae, Si Khot	261	3	–	–	–	3	3	–	–	–	–	–	–	–	–	–
				302	4	–	–	–	–	5	–	–	–	–	–	–	–	–	–
			Dongnatong, Sikhottabong	219	11	–	–	–	1	8	–	–	–	–	–	–	–	–	–
			Ban Sa Pang Muek, Xaithani	289	65	–	–	–	–	14	–	–	–	–	–	–	–	–	–
			Ban Tanmi Xai, Chanthabouri	68	19	–	–	–	–	3	–	–	–	–	–	–	–	–	–
			Nong Pra Ya, Xaithani	271	10	–	–	–	1	1	–	–	–	–	–	1	–	–	–
			Si Keut, Naxaythong	344	28	–	–	–	–	–	–	–	–	–	–	–	–	–	–
			Ban Thalad, Keo-Oudom	262	72	–	–	–	–	15	–	–	–	–	–	–	–	–	–
			Na Gum, Phonhong	148	26	–	–	–	–	4	–	–	–	–	–	–	–	–	–
			Tha Heur	88	79	–	–	–	2	2	–	–	–	–	–	3	4	–	–
				29	4	–	–	–	–	1	–	–	–	–	–	–	–	–	–
			Vang Vieng	286	17	–	–	–	–	1	–	1	–	1	–	2	1	–	–
Total snails				10,507															

Other cercarial types: D, 1 snail; E, no data; J, 5 snails; P, 2 snails; R, 1 snail (all types were found in Thailand, Sakon Nakhon province, Songkram River).

s.l. is a species complex, with distinct genetic groups in different wetlands (Saijuntha *et al.*, 2007, Andrews *et al.*, 2008; Laoprom *et al.*, 2009, 2010, 2012). Members of this complex cause major medical problems, including bile duct cancer (cholangiocarcinoma), which lead to significant levels of human morbidity and mortality (Saijuntha *et al.*, 2007; Andrews *et al.*, 2008; Sithithaworn *et al.*, 2012b). High prevalences of infection with *O. viverrini* s.l. of up to 50% in human and fish intermediate hosts have been reported (Vichasri *et al.*, 1982; Sithithaworn *et al.*, 1997, 2012a), but the prevalences in snails are much lower, ranging from $<1\%$ to about 9% (Lohachit, 2004–2005; Sri-Aroon *et al.*, 2005, 2007; Kiatsopit *et al.*, 2012; Petney *et al.*, 2012). The flukes have a complex life cycle, including freshwater snails as first intermediate hosts. A wide variety of freshwater cyprinid fishes act as second intermediate hosts, while humans are the most important final hosts, although cats and dogs can harbour adult worms (Sithithaworn *et al.*, 2012a; Petney *et al.*, 2013).

All trematodes use molluscs, in which asexual reproduction occurs, as intermediate hosts. Thus, molluscs play a critical role in the life cycle of the trematodes as multiplying hosts. The freshwater snail *Bithynia siamensis goniomphalos* sensu lato is widely distributed in north-east Thailand and Lao PDR (Petney *et al.*, 2012; Kiatsopit *et al.*, 2013). *Bithynia s. goniomphalos* is a species complex containing two groups with potentially at least nine morphologically similar but genetically distinct cryptic species (taxa) (Kiatsopit *et al.*, 2013). Snails from this complex play a crucial role in the life cycle of *O. viverrini*, being its first intermediate host (Sithithaworn *et al.*, 2012a). A survey of previously published data on trematode cercariae from Thailand and Lao PDR shows that several taxonomic groups infect these snails. In *B. s. goniomphalos* s.l. from Thailand, 15 groups of cercariae have been found: amphistome, echinostome, monostome, strigea, furcocercaria, pleurolophocercous, parapleurolophocercous, lophocercous, virgulate, ubiquita, xiphidiocercaria, ophthalmoxiphidiocercaria, tailless cercariae, cystophorous and gymnocephalous cercariae (Ito *et al.*, 1962; Wykoff *et al.*, 1965; Adam *et al.*, 1993; Nithiuthai *et al.*, 2002; Lohachit, 2004–2005; Kodcharin, 2005; Sri-Aroon *et al.*, 2005, 2007; Tesana *et al.*, 2014). Only *O. viverrini* and one pleurolophocercous species have been reported so far from *B. s. goniomphalos* s.l. from Lao PDR (Ditrich *et al.*, 1990, 1992; Giboda *et al.*, 1991). Cercariae from eight groups were shed from *Bithynia siamensis siamensis* (Upatham & Sukhapanth, 1980; Chontananarth *et al.*, 2013) and four from *Bithynia funiculata* (Ngern-klun *et al.*, 2006), both of which can also act as hosts for *O. viverrini*.

The aim of the present study was to establish a comprehensive understanding of the diversity of larval-stage trematodes occurring in *B. s. goniomphalos* s.l. in Thailand and Lao PDR.

Table 2. Mean prevalences (ranges given in brackets) of type of cercariae (A–T) from field-infected *Bithynia siamensis goniomphalos* from Thailand and Lao PDR (see fig. 1 for cercarial codes).

		Mean prevalence (%)	
Type of cercariae	Code	Thailand	Lao PDR
Xiphidiocercariae			
Virgulate 1	A	10.90 (0.26–54.22)	6.58 (1.15–89.77)
Virgulate 2	B	0.92 (0.29–1.73)	1.53 (0.21–4.33)
Virgulate 3	C	1.11 (0.05–2.07)	1.28 (0.43–1.42)
Virgulate 4	D	0.83	–
Virgulate 5	E	ND	ND
Amphistome cercariae	F	6.08 (0.61–9.28)	2.13
Pleurolophocercous cercariae			
Opisthorchis viverrini cercariae	G	1.59 (0.15–6.93)	0.96 (0.08–8.37)
Monostome cercariae	H	1.40 (0.25–5.88)	2.22 (0.25–6.98)
Parapleurolophocercous cercariae			
Parapleurolophocercous cercariae 1	I	1.15 (0.36–2.45)	0.03
Parapleurolophocercous cercariae 2	J	0.24	–
Mutabile cercariae	K	1.08 (0.33–16.67)	0.15 (0.08-0.35)
Ophthalmoxiphidiocercariae	L	0.89 (0.54–2.44)	0.03
Cystophorous cercariae (Hemiuridae)			
Cystophorous cercariae 1	M	0.62 (0.12–1.63)	0.68 (0.10–1.24)
Cystophorous cercariae 2	N	0.10 (0.05–0.60)	–
Furcocercous cercariae			
Furcocercous cercariae 1	O	0.64 (0.15–6.80)	0.48 (0.19–3.41)
Furcocercous cercariae 2	P	0.25	–
Longifurcate-pharyngeate cercariae 1	Q	0.34 (0.19–1.33)	0.46 (0.04–4.55)
Longifurcate-pharyngeate cercariae 2	R	0.05	–
Echinostome cercariae			
Echinostome cercariae 1	S	0.39 (0.05–2.41)	–
Echinostome cercariae 2	T	0.10	0.03

ND, no data available.

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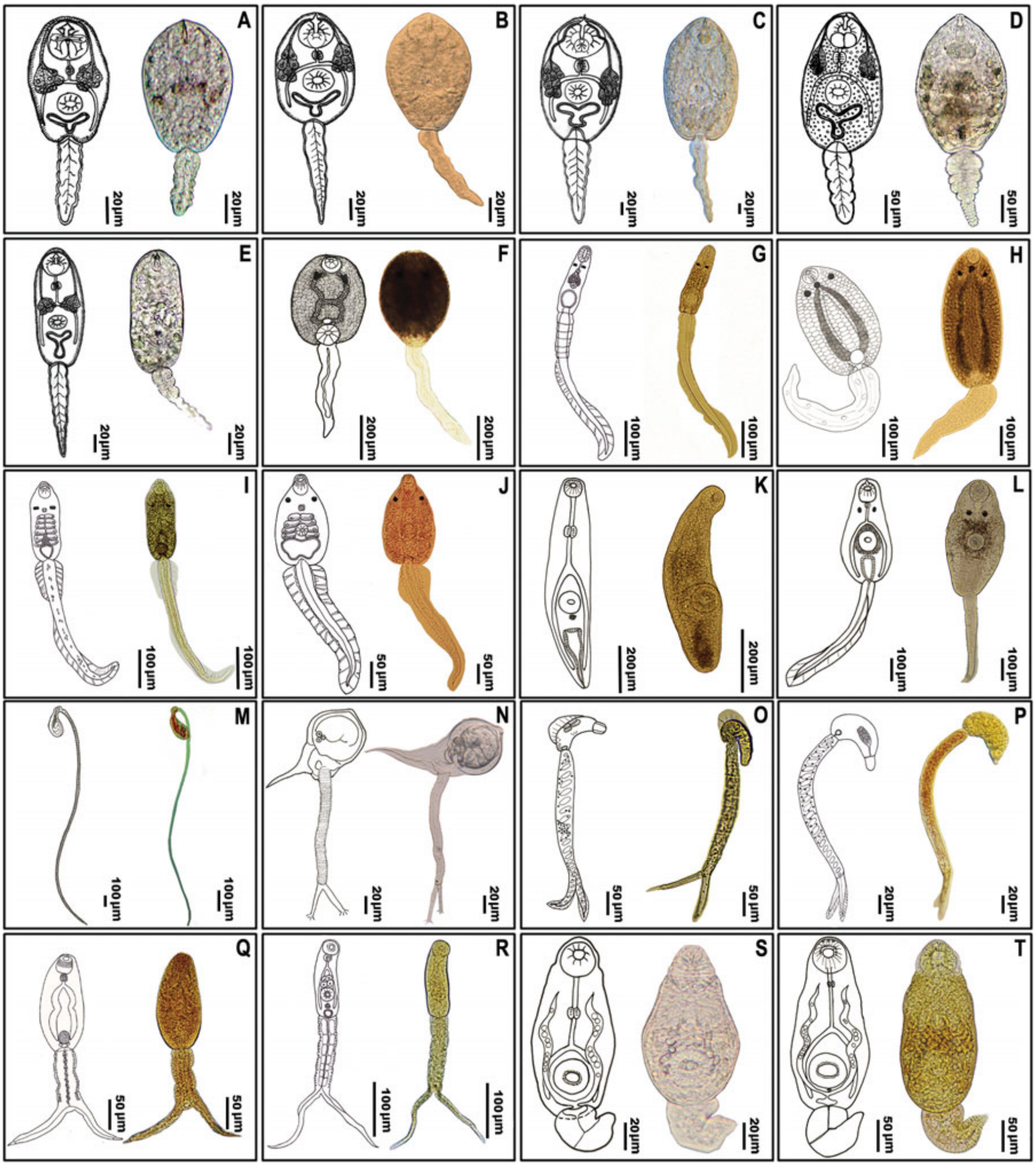

Fig. 1. Type of cercariae infecting *Bithynia siamensis goniomphalos* from Thailand and Lao PDR. (A–E) Xiphidiocercariae, type virgulate 1 to 5; (F) amphistome cercariae; (G) pleurolophocercous cercariae (*Opisthorchis viverrini*); (H) monostome cercariae; (I–J) parapleurolophocercariae 1 and 2; (K) mutabile cercariae; (L) ophthalmoxiphidiocercariae; (M–N) cystophorous cercariae 1 and 2; (O–P) furcocercous cercariae 1 and 2; (Q–R) longifurcate-pharyngeate cercariae 1; (S–T) echinostome cercariae 1 and 2.

Materials and methods

Study localities

Bithynia s. goniomphalos s.l. ($n = 11{,}756$) were collected from 29 Thai localities in nine provinces: Buri Ram, Surin, Chaiya Phum, Maha Sarakham, Khon Kaen, Kalasin, Mukdahan, Sakon Nakhon and Nakhon Phanom. In Lao PDR, snail samples ($n = 10{,}507$) were collected from 21 localities in Vientiane Province and six localities in Savannakhet Province (table 1). Snails were collected from 2009 to 2014 by handpicking and dredging the sediment with a scoop. They were then cleaned, dried and placed into plastic bags in the field before being transported to the laboratory, where they were identified

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using standard morphological criteria (Brandt, 1974; Upatham *et al.*, 1983; Chitramvong, 1992).

Screening of snails for cercariae

Snails collected from each site were examined by the cercarial shedding method (Kiatsopit *et al.*, 2012). Each snail was placed separately into a small plastic container (3 cm in diameter by 2.5 cm high) filled with 5 ml dechlorinated water. The containers were covered with a lid studded with pins to prevent the snail from escaping. The snails were exposed to artificial light (1200 lx) for 5 h during the day at room temperature (25 ± 2°C) during which shedding took place. The containers were checked for the presence of cercariae under a stereomicroscope. Trematode cercariae were identified morphologically under a high-magnification compound microscope. One drop of water containing live, vigorous cercariae was observed carefully on a slide containing 10% formalin or stained with 1% iodine. Cercariae were photographed using a digital camera (Olympus DP 25; Olympus, Tokyo, Japan) fitted to a microscope (Olympus BX 51). They were identified using the taxonomic keys of Ito *et al.* (1962), Schell (1970), Yamaguti (1975) and Ditrich *et al.* (1997). Cercariae were drawn using a light microscope and camera lucida. The different types of cercariae found were coded alphabetically.

Results

The 11,756 *B. s. goniomphalos* collected from Thailand harboured 20 morphologically distinct types of cercariae belonging to 10 groups: virgulate (5 types), amphistome (1), pleurolophocercous (1), monostome (1), parapleurolophocercous (2), mutabile (1), cystophorous (2), ophthalmoxiphidiocercariae (1), furcocercous (4) and echinostome (2) (table 2, fig. 1). Of the 10,507 snails collected from Lao PDR, 10 groups with 13 types of cercariae were found, all of which also occurred in Thailand (table 2).

Virgulate type 1 were the most common (table 1) and had the highest prevalences in snails, with a mean of 10.90% in Thailand and 6.58% in Lao PDR (table 2). This type was found in all samples from Lao PDR and in 27 of 29 samples from Thailand. The second highest prevalence occurred in amphistome cercariae, with a mean of 6.08% in Thailand and 2.13% in Lao PDR. Monostome cercariae had the third highest prevalence, with 1.40% in Thailand and 2.22% in Lao PDR. The pleurolophocercous cercariae of *O. viverrini* s.l. had the fourth highest prevalence, with 1.59% in Thailand and 0.96% in Lao PDR. Lower mean prevalences were noted in the two countries for nine types of cercariae, with percentages ranging from 0.10% (echinostome cercariae type 2) to 1.15% (parapleurolophocercous cercariae type 1) in Thailand, and from 0.03% (echinostome cercariae type 2) to 1.53% (xiphidiocercariae, type virgulate 2) in Lao PDR. Six other types of cercariae – xiphidiocercariae, type virgulate 4 (prevalence, 0.83%); echinostome cercariae type 1 (0.39%); furcocercous cercariae type 2 (0.25%); parapleurolophocercous cercariae type 2 (0.24%); cystophorous cercariae type 2 (0.10%) and longifurcate-pharyngeate cercariae type 2 (0.05%) – were only recorded in snail samples collected from Thailand (table 2 and fig. 1). The virgulate type 5 was found only in Sakon Nakhon Province, Thailand, but prevalence data are not available.

Discussion

Bithynia s. goniomphalos s.l. from Thailand and Lao PDR harbours a wide variety of trematode cercariae. Unfortunately, identification to species level is difficult for most of the taxa involved, as the complete life cycles are known for only a few species. This study confirms the need for molecular identification to species level (Nolan & Cribb, 2005; Olson & Tkach, 2005; Skov *et al.*, 2009) and, if possible, the use of experimental studies to determine the life cycles in the laboratory.

There are relatively few reports of cercariae in *B. s. goniomphalos* s.l. from Thailand or Lao PDR. The 20 morphologically identified larval trematodes that were recorded from the present study contained similar types to those found in other studies; however, we found more cercarial types than were previously known. This might be a result of the larger sampling size and more localities sampled than previous reports. The cystophorous type 2 (furcocystocercous) was discovered for the first time in *B. s. goniomphalos* s.l. This group was found previously in another snail (*Pyrgophorus coronatus*) in Mexico (Ditrich *et al.*, 1997). *Bithynia s. goniomphalos* s.l. shows a particularly high susceptibility to virgulate type 1 infection, the cercariae of which dominate the cercarial fauna.

The occurrence of *O. viverrini* cercariae in *B. s. goniomphalos* s.l. is highly significant, although not unexpected, as this species poses the greatest threat to human health throughout the Mekong area of South-East Asia. The data presented here correspond with those found in previous studies for both Thailand (Sripa *et al.*, 2011; Sithithaworn *et al.*, 2012a) and Lao PDR (Sayasone *et al.*, 2009; Forrer *et al.*, 2012). Natural infection of *O. viverrini* in *B. s. goniomphalos* ranged from 0.22 to 6.93% with an average of 3.04% in Thailand, while in Lao PDR prevalences ranged from 0.37 to 8.37% with an average of 2.01% (Kiatsopit *et al.*, 2012).

Trematodes are a diverse group of parasites requiring molluscs and vertebrates as intermediate and definitive hosts. Trematode parasitism of intermediate hosts is frequently associated with the alteration of a host's growth, fecundity and/or survival, and snail susceptibility to trematodes is highly specific (Kalbe *et al.*, 1997; Sorensen & Minchella, 1998). DNA studies have been carried out to detect the larval stages of trematodes and their taxonomic determination in snails (Chuboon & Wongsawad, 2009; Kraus *et al.*, 2014; Routtu *et al.*, 2014). Recent genetic analyses of *B. s. goniomphalos* s.l. indicate that this taxon represents a species complex containing at least nine cryptic species that have specific associations with defined wetlands in Thailand and Lao PDR (Kiatsopit *et al.*, 2013). Furthermore, the cryptic species of *B. s. goniomphalos* s.l. are associated with cryptic species and/or genetic groups of *O. viverrini* from the corresponding wetlands. How this diversity influences the parasitic trematode community remains to be determined.

Previous studies have shown that individual species of snails can act as intermediate hosts for a number of trematode species (Sousa, 1993; Esch *et al.*, 2001; Loy & Haas, 2001; Faltynkova & Haas, 2006). This varies in space and time depending on environmental conditions, which can be strongly associated with host availability, such as climatic factors including temperature (Koprivnikar *et al.*, 2010; Studer *et al.*, 2013) and drought (Gérard, 2001), the size of the water body (Voutilainen *et al.*, 2009), salinity (Koprivnikar *et al.*, 2010; Lei & Poulin, 2011; Suwannatrai *et al.*, 2011), pH (Koprivnikar *et al.*, 2010; Wang *et al.*, 2015), land use (Koprivnikar *et al.*, 2006; Wang *et al.*, 2015) and habitat complexity (Beasley *et al.*, 2005). In addition to such abiotic/host-related factors, an early meta-analysis by Kuris & Lafferty (1994) showed that the number of multispecies infections in individual snails is on average 10% less than expected, due to interspecific competition.

Recognition of the wide variety of trematode species detected in *B. s. goniomphalos* leads to a number of questions that also bear on the epidemiology of *O. viverrini*. How do the trematode species interact within the snail hosts? How often do mixed infections occur? Do different species inhibit infection with other species? These remain to be answered. Also of significance is the fact the most cercarial species cannot be identified to the species level. Thus molecular identification based on known adults could serve as an effective way to assess the risk posed by the unknown cercariae to human and/or animal health.

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Conflict of interest

None.

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